From: Pham, Co (HC/SC)
To: Lourenco, Celia (HC/SC); Hardy, Stephanie (HC/SC); Bouthillier, Leo (HC/SC); Rosu-Myles, Michael (HC/SC); Tang, Marianne (HC/SC); Fortin, Nathalie (HC/SC); Anoop, Poovadan (HC/SC); HC.F ORA_COVID / BAR_COVID F.SC
Subject: FW: McMaster Inhalable vaccine
Date: 2021-09-10 1:05:04 PM
Attachments: FW Draft Meeting Minutes for COVID-19 Pre-CTA Meeting Control # 250223 with McMaster University for Ad5-triCoVMac and ChAd-triCoVMac Vaccines.msg

---

Hi Team;

No CTA in house or further correspondence regarding this since our pre-CTA last April.

I've attached the email thread with the control # (250223) for the pre-CTA and meeting minutes.

Hope this helps.
C

From: Rosu-Myles, Michael (HC/SC) <michael.rosu-myles@hc-sc.gc.ca>
Sent: 2021-09-10 12:51 PM
To: Lourenco, Celia (HC/SC) <celia.lourenco@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Bouthillier, Leo (HC/SC) <leo.bouthillier@hc-sc.gc.ca>
Cc: Hardy, Stephanie (HC/SC) <stephanie.hardy@hc-sc.gc.ca>; Anoop, Poovadan (HC/SC) <poovadan.anoop@hc-sc.gc.ca>
Subject: RE: McMaster Inhalable vaccine

Yes. This group came in for a pre-CTA meeting earlier this year. Can't remember if the CTA was submitted.
Michael

Sent from my Bell Samsung device over Canada's largest network.

-------- Original message --------
From: "Lourenco, Celia (HC/SC)" <celia.lourenco@hc-sc.gc.ca>
Date: 2021-09-10 12:32 PM (GMT-05:00)
To: "HC.F ORA_COVID / BAR_COVID F.SC" <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>, "Pham, Co (HC/SC)" <co.pham@hc-sc.gc.ca>, "Bouthillier, Leo (HC/SC)" <leo.bouthillier@hc-sc.gc.ca>, "Rosu-Myles, Michael (HC/SC)" <michael.rosu-myles@hc-sc.gc.ca>
Cc: "Hardy, Stephanie (HC/SC)" <stephanie.hardy@hc-sc.gc.ca>, "Anoop, Poovadan (HC/SC)" <poovadan.anoop@hc-sc.gc.ca>
Subject: FW: McMaster Inhalable vaccine

Hi, has anyone heard of this one? An inhalable vaccine for kids from McMaster? The message doesn't specify COVID, so please let me know if you have heard of an inhalable vaccine for other infectious diseases. Thank you, Celia

---

From: Sharma, Supriya (HC/SC) <supriya.sharma@hc-sc.gc.ca>
Sent: 2021-09-10 12:00 PM
To: Lourenco, Celia (HC/SC) <celia.lourenco@hc-sc.gc.ca>; Smith, Melissa (HC/SC) <melissa.smith@hc-sc.gc.ca>; Hardy, Stephanie (HC/SC) <stephanie.hardy@hc-sc.gc.ca>
Subject: Fwd: McMaster Inhalable vaccine

Hello

Any updates on this one? Hadn't heard anything new in our COVID check in

Supriya

Begin forwarded message:

From: [redacted] (MOH)" [redacted]@ontario.ca>
Date: September 10, 2021 at 11:47:01 AM EDT
To: "Sharma, Supriya (HC/SC)" <supriya.sharma@hc-sc.gc.ca>
Cc [redacted] (MOH)" [redacted]@ontario.ca>, " [redacted] (MOH)" [redacted]@ontario.ca>
Subject: McMaster Inhalable vaccine

Hi Supriya,

Hope you are doing well! We've been getting a number of questions lately about the McMaster inhalable vaccine for kids. Last I heard they weren't even entering phase 1 trials until later this year so I'm not sure why the sudden interest but wondering if you have any information you can share about this one?

Thanks so much!

Best,

[redacted]

Mobile: [redacted]
Office Phone [redacted]
e-mail address: [redacted]@ontario.ca

From: Pronovost, Kim (HC/SC)
To: Basta, Patricia (HC/SC)
Subject: FW: Draft Meeting Minutes for COVID-19 Pre-CTA Meeting Control # 250223 with McMaster University for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines
Date: 2021-05-18 12:40:00 PM

Hi Patricia,
No further comments on our end.
Thanks,
Kim

From: Martinez-Arguelles, Daniel (HC/SC) <daniel.martinez-arguelles@canada.ca>
Sent: 2021-05-18 12:13 PM
To: Pronovost, Kim (HC/SC) <kim.pronovost@canada.ca>
Subject: RE: Draft Meeting Minutes for COVID-19 Pre-CTA Meeting Control # 250223 with McMaster University for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines

Hi Kim,

Nothing else to add.

Dan

From: Craig, Constance (HC/SC) <constance.craig@canada.ca>
Sent: 2021-05-18 11:16 AM
To: Taha, Mohamad (HC/SC) <mohamad.taha@canada.ca>; Pronovost, Kim (HC/SC) <kim.pronovost@canada.ca>
Cc: Martinez-Arguelles, Daniel (HC/SC) <daniel.martinez-arguelles@canada.ca>; Rao, Sanath (HC/SC) <sanath.rao@canada.ca>; Johnson, Dustin (HC/SC) <dustin.johnson@canada.ca>
Subject: RE: Draft Meeting Minutes for COVID-19 Pre-CTA Meeting Control # 250223 with McMaster University for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines

Hi Kim – I also have no further comments

Connie

From: Taha, Mohamad (HC/SC) <mohamad.taha@canada.ca>
Sent: 2021-05-18 10:09 AM
To: Pronovost, Kim (HC/SC) <kim.pronovost@canada.ca>
Cc: Craig, Constance (HC/SC) <constance.craig@canada.ca>; Martinez-Arguelles, Daniel (HC/SC) <daniel.martinez-arguelles@canada.ca>; Rao, Sanath (HC/SC) <sanath.rao@canada.ca>; Johnson, Dustin (HC/SC) <dustin.johnson@canada.ca>
Subject: RE: Draft Meeting Minutes for COVID-19 Pre-CTA Meeting Control # 250223 with McMaster University for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines

Hi Kim,
I don't have anything to add to the updated minutes
Thanks
Mohamad

From: Basta, Patricia (HC/SC) <patricia.basta@canada.ca>
Sent: 2021-05-18 9:59 AM
To: Derry, Mélanie (HC/SC) <melanie.derry@canada.ca>; Pronovost, Kim (HC/SC) <kim.pronovost@canada.ca>; Craig, Constance (HC/SC) <constance.craig@canada.ca>; Taha, Mohamad (HC/SC) <mohamad.taha@canada.ca>; Martinez-Arguelles, Daniel (HC/SC) <daniel.martinez-arguelles@canada.ca>; Rao, Sanath (HC/SC) <sanath.rao@canada.ca>; Rosu-Myles, Michael (HC/SC) <michael.rosu-myles@canada.ca>; Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@canada.ca>; Smith, Dean (HC/SC) <dean.smith@canada.ca>; Fernandes, Jason (HC/SC) <jason.fernandes@canada.ca>; Li, Sean (HC/SC) <sean.li@canada.ca>; Pulle, Gayle (HC/SC) <gayle.pulle@canada.ca>; Irwin, Chad (HC/SC) <chad.irwin@canada.ca>; Plamondon, Mireille (HC/SC) <mireille.plamondon@canada.ca>; Gillham-Eisen, Liz Anne (HC/SC) <lizanne.gillham-eisen@canada.ca>; eau / uee (HC/SC) <hc.eau-uee.sc@canada.ca>; Anoop, Valar (HC/SC) <valar.anoop@canada.ca>
Cc: Curtis, Kaitlin (HC/SC) <kaitlin.curtis@canada.ca>; Pham, Co (HC/SC) <co.pham@canada.ca>; Johnson, Dustin (HC/SC) <dustin.johnson@canada.ca>; Tang, Marianne (HC/SC) <marianne.tang@canada.ca>; Siggers, Richard (HC/SC) <richard.siggers@canada.ca>
Subject: RE: Draft Meeting Minutes for COVID-19 Pre-CTA Meeting Control # 250223 with McMaster University for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines

Dear all,

Following Health Canada's comments (HC6-024-c250223 (1.0) Reg Info - Health Product) to the draft meeting minutes from McMaster University in regards to the Pre-CTA Meeting held on April 13, 2021, for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines, the sponsor had made a few revisions to the meeting minutes:

- Clean copy: HC6-024-c250223 (0003) Clinical Trial Dossier
- Annotated copy (shows HC and sponsor comments): HC6-024-c250223 (0004) Clinical Trial Dossier

Please advise of your comments on the revised minutes no later than **Tuesday May 25, 2021**. If feedback/comments are not provided, it will be assumed that the minutes are acceptable from your perspective.

Sincerely,
Patricia

From: Basta, Patricia (HC/SC)
Sent: 2021-04-27 3:28 PM
To: Derry, Mélanie (HC/SC) <melanie.derry@canada.ca>; Pronovost, Kim (HC/SC) <kim.pronovost@canada.ca>; Craig, Constance (HC/SC) <constance.craig@canada.ca>; Taha,

Mohamad (HC/SC) <mohamad.taha@canada.ca>; Martinez-Arguelles, Daniel (HC/SC) <daniel.martinez-arguelles@canada.ca>; Rao, Sanath (HC/SC) <sanath.rao@canada.ca>; Rosu-Myles, Michael (HC/SC) <michael.rosu-myles@canada.ca>; Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@canada.ca>; Smith, Dean (HC/SC) <dean.smith@canada.ca>; Fernandes, Jason (HC/SC) <jason.fernandes@canada.ca>; Li, Sean (HC/SC) <sean.li@canada.ca>; Pulle, Gayle (HC/SC) <gayle.pulle@canada.ca>; Irwin, Chad (HC/SC) <chad.irwin@canada.ca>; Plamondon, Mireille (HC/SC) <mireille.plamondon@canada.ca>; Gillham-Eisen, Liz Anne (HC/SC) <lizanne.gillham-eisen@canada.ca>; eau / uee (HC/SC) <hc.eau-uee.sc@canada.ca>; Anoop, Valar (HC/SC) <valar.anoop@canada.ca>
Cc: Curtis, Kaitlin (HC/SC) <kaitlin.curtis@canada.ca>; Pham, Co (HC/SC) <co.pham@canada.ca>; Johnson, Dustin (HC/SC) <dustin.johnson@canada.ca>; Tang, Marianne (HC/SC) <marianne.tang@canada.ca>; Siggers, Richard (HC/SC) <richard.siggers@canada.ca>
Subject: Draft Meeting Minutes for COVID-19 Pre-CTA Meeting Control # 250223 with McMaster University for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines

Dear all,

The draft meeting minutes from McMaster University in regards to the Pre-CTA Meeting held on April 13, 2021, for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines are available on docuBridge: HC6-024-c250223 (0002) Clinical Trial Dossier

Please advise of your comments no later than **Tuesday May 4, 2021**. If feedback/comments are not provided, it will be assumed that the minutes are acceptable from your perspective.

Sincerely,
Patricia

-----Original Appointment-----
From: Basta, Patricia (HC/SC)
Sent: 2021-04-12 10:06 AM
To: Pham, Co (HC/SC); Derry, Mélanie (HC/SC); Pronovost, Kim (HC/SC); Johnson, Dustin (HC/SC); Craig, Constance (HC/SC); Taha, Mohamad (HC/SC); Curtis, Kaitlin (HC/SC); Rosu-Myles, Michael (HC/SC); Baca-Estrada, Maria (HC/SC); Smith, Dean (HC/SC); Fernandes, Jason (HC/SC); Li, Sean (HC/SC); Pulle, Gayle (HC/SC); Siggers, Richard (HC/SC); Plamondon, Mireille (HC/SC); eau / uee (HC/SC); Anoop, Valar (HC/SC); Maeck, Jessica (HC/SC); Sullivan, Catherine (HC/SC)
Cc: Tang, Marianne (HC/SC); Antonio, Christopher (HC/SC); Wallace, Julie (HC/SC); Gillham-Eisen, Liz Anne (HC/SC); Graham, Brennan (HC/SC); Frappier2, Fiona (HC/SC); Martinez-Arguelles, Daniel (HC/SC); Irwin, Chad (HC/SC)
Subject: CONFIRMED: COVID-19 Pre-CTA Meeting Control # 250223 with McMaster University for Ad5-triCoV/Mac and ChAd-triCoV/Mac Vaccines
When: 2021-04-13 1:00 PM-3:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: Teleconference

Apr 12 Update: Advanced feedback sent to sponsor (link below), Dial-in information added.
Apr 8 Update: Meeting slides attached (now link below).

Please forward this invitation to those from your group who should attend this meeting.

Purpose of meeting:

Pre-CTA Meeting Control # 250223 with McMaster University to discuss their proposed "second generation" COVID-19 vaccine study, funded by CIHR, Protocol # M010, "*Phase 1, open label study to evaluate the safety and immunogenicity of ChAd68 and AdHu5 vector-based trivalent COVID-19 vaccines, using aerosol administration and a novel heterologous prime-boost approach*". McMaster has developed in their GCP-facility two vectors:

- Ad5-triCoV/Mac is a recombinant type 5 human adenovirus vector which has been engineered to express their trivalent SARS-CoV-2 transgene cassette under the control of a MCMV promoter, and is followed by an SV40 polyA signal. The adenovirus construct is E1 and E3 deleted.
- ChAd-triCoV/Mac vector is an E1 and E3 deleted chimpanzee adenovirus serotype 68 where the trivalent SARS-CoV-2 transgene cassette is under the control of an HCMV promoter and is followed by an SV40 polyA signal.
- The trivalent transgene cassette consists of a human tPA signal sequence/propeptide (aa 1-32), the S1 region of SARS-CoV-2 spike protein (aa 47-716), the transmembrane domain of VSV G (aa 443-511) followed by the P2A "self-cleaving" peptide of porcine teschovirus (P2A, sequence: GSGATNFSLLKQAGDVEENPGP), full-length SARS-CoV-2 nucleoprotein (aa 1-419) fused to a highly conserved portion of the SARS-CoV-2 polymerase (aa 4673-4742).

The objectives of this clinical trial are to evaluate the safety and immunogenicity of intramuscular ChAd-triCoV/Mac prime followed by aerosol administration of Ad5triCoV/Mac boost in healthy volunteers without a history of COVID-19 infection or prior vaccination, as well as to evaluate the immunogenicity of a single aerosol dose of ChAd-triCoV/Mac or Ad5triCoV/Mac boost in volunteers with a history of prior COVID infection or vaccination. The Aeroneb Solo Vibrating Mesh Nebulizer (single-patient use) will be used to deliver either Ad5-triCoV/Mac or ChAd-triCoV/Mac vaccine to the lung of human volunteers.

A Letter of Access is provided to cross-reference the information for:

- AdAg85A vector to CTA Control # 126673
- Ad5Ag85A vector to CTA Control # 177700

Dial-in information:

Join Zoom Meeting
https://mcmaster.zoom.us/j/93117736237

Meeting ID: 931 1773 6237
One tap mobile
+17789072071,,93117736237# Canada
+12042727920,,93117736237# Canada

Dial by your location
+1 778 907 2071 Canada
+1 204 272 7920 Canada

+1 438 809 7799 Canada
+1 587 328 1099 Canada
+1 613 209 3054 Canada
+1 647 374 4685 Canada
+1 647 558 0588 Canada

docuBridge Links:

- Meeting Request and Package: HC6-024-c250223 (0000) Clinical Trial Dossier
- Meeting Slides rec'd Apr 8: HC6-024-c250223 (0001) Clinical Trial Dossier
- Advanced Feedback sent to sponsor: HC6-024-c250223 (1.0) Reg Info - Health Product

Sponsor Questions to Health Canada:

Chemistry, Manufacturing and Controls

1) Is the directorate satisfied with our proposed battery of release tests?
2) Please provide guidance on your expected timelines relative to our CTA submission and review regarding release of product.
3) Given our similar experience with the manufacture of AdHu5Ag85A and our ongoing Health Canada approved clinical trial to evaluate the safety and immunogenicity of an adenovirus-based tuberculosis vaccine (AdHu5Ag85A) administered by aerosol, can we leverage any specific components of our experience and manufacturing history to defer any components of the typical regulatory requirements for Ad5-triCoV/Mac and are there components of the regulatory requirements that might be relevant for the approval of ChAd-triCoV/Mac?

Preclinical Toxicity and Efficacy

1) We have described the toxicity tests performed on the vaccines and have identified no safety concerns. Is the Directorate satisfied with this testing and the results?
2) We have a comprehensive series of preclinical experiments completed. Is there further information required?
3) While we expect to obtain preclinical vaccine efficacy data in early April, can we proceed with final CTA submission before this time?

For the clinical trial:

1) The trial design we have chosen enrolls three separate cohorts: a group without a history of COVID-19 infection or vaccination, a group who have received vaccination and a group who have a previous history of COVID-19 infection. Based on the rationale we have provided, do you support this study design and our approach to a prime/heterologous boost?
2) Are the inclusion and exclusion criteria acceptable? We believe we have selected criteria that will ensure the safety of participants, particularly as it relates to the inhaled route of administration. Are our definitions and criteria appropriate?
3) Are the procedures we have described adequate to ensure the safety of our participants? We plan to start with a lower dose of the IM ChAd-triCoV/Mac prime followed by the inhaled aerosol Ad5-triCoV/Mac and not increase the dose and complete enrolment of the cohort until safety criteria (as outlined in the protocol) have been met, with a similar approach for the aerosol administration of vaccines in cohorts 2 and 3.
4) Given the increased confidence now with the overall safety of the adenovirus-vectored

vaccines (Ad5, Ad26 & ChAd) in humans, we have proposed a minimum amount of safety monitoring blood tests. Is this acceptable to the Directorate? When would the final safety visit be required?

5) Do you agree that the measurements we have proposed are appropriate to detect an immune response to vaccine in the blood and bronchiolar lavage collected from participants?
6) Have we adequately addressed recruiting participants who may be/become eligible for vaccination as vaccines are rolled out to the community? Do you have any suggestions as to what advice participants should be provided regarding administration of post-study licensed vaccines?

From: Basta, Patricia (HC/SC)
To: Pronovost, Kim (HC/SC); Baca-Estrada, Maria (HC/SC); BRDD Clinical Trials / Essais Cliniques DMBR (HC/SC)
Cc: Curtis, Kaitlin (HC/SC); Tang, Marianne (HC/SC); Antonio, Christopher (HC/SC); Wallace, Julie (HC/SC); Pham, Co (HC/SC); Fortin, Nathalie (HC/SC); Rosu-Myles, Michael (HC/SC); Lourenco, Celia (HC/SC); Hardy, Stephanie (HC/SC); BRDD.Risk / risque.DMBR (HC/SC); eau / uee (HC/SC); Anoop, Valar (HC/SC); Maeck, Jessica (HC/SC); Sullivan, Catherine (HC/SC)
Subject: New COVID-19 CTA Control No. 257190 – Protocol No. M010
Date: 2021-10-04 1:54:05 PM
Attachments: 257190s1rev.docx

Dear Kim and Maria:

The following submission is ready for review:

(As this is a COVID-19 submission and the docuBridge sequence is not yet available, I have attached my Screening Report and will follow up with an email with the submission. Once it becomes available, I will push it to your workloads and follow up with an email with the sequences.)

Sub. Type:	CTA	**Control No.:**	257190
Brand Name (common name):	ChAd-CoV/Mac Ad5-CoV/Mac		
Manufacturer/Sponsor Name:	McMaster University		
Target Date:	*November 3, 2021* **Expedited 14-day target: October 18, 2021**		

Review Type:		**Previous Reviewer:**
☒ **Clinical**	CREST-OCT-E	N/A
☒ **Chemistry**	CBE-VQD1	N/A
☐ **Joint Review**	N/A	

Investigational Status Assessment (ISA):	Yes ☐ No ☒
Summary of Additional Drug (SOAD):	Yes ☐ No ☒
Notes to Review:	- **COVID-19 CTA(-A) (Division 5) – Expedited Review** - **First Entry for:** o **ChAd-CoV/Mac** o **Ad5-CoV/Mac** - Pre-CTA Meeting Control # 250223, held on April 13, 2021. - Letter of Authorization for sharing information for the below previous submissions is provided: o CTA Control # 126673 for AdAg85A Vector o Pre-CTA Meeting Control # 163439 for AdAg85A o CTA Control # 177700 for AdAg85A Vector o Pre-CTA Meeting Control # 250223 for Ad5-triCoV/Mac and ChAd-triCoV/Mac vectors

Link to sequence on docuBridge:	- Original submission, Seq. 0005: *Pending upload to docuBridge* - Response to Screening IR, Seq. 0006: *Response to be received post-acceptance into review*
Link to screening report:	*Pending upload to docuBridge*

Sincerely,

Patricia Basta

Senior Regulatory Affairs Officer
Office of Regulatory Affairs
Biologic and Radiopharmaceutical Drugs Directorate (new name)
Health Products and Food Branch / Health Canada
Patricia.Basta@hc-sc.gc.ca / Tel: 613-299-2669 / Fax: 613-946-9520

Agente principale des affaires réglementaires
Bureau des affaires réglementaires
Direction des médicaments biologiques et radiopharmaceutiques (nouveau nom)
Direction générale des produits de santé et des aliments / Santé Canada
Patricia.Basta@hc-sc.gc.ca / Tél. : 613-299-2669 / Fax : 613-946-9520

Health Canada / Santé Canada

MEMORANDUM
NOTE DE SERVICE

BIOLOGIC AND RADIOPHARMACEUTICAL DRUGS DIRECTORATE
DIRECTION DES MÉDICAMENTS BIOLOGIQUES ET RADIOPHARMACEUTIQUES

To/À: FILE	Security Classification/Classification de sécurité: **HC Protected B (when completed)**
	Our File/Notre référence: **Dossier ID :** HC6-024-c250223
From/De: Patricia Basta Senior Regulatory Affairs Officer Office of Regulatory Affairs Biologic and Radiopharmaceutical Drugs Directorate	Your File/Votre référence: **Control No:** 257190 **Document No:** 1724308 **Version(s):** 0005 **Date:** October 4, 2021

Sub. Type:	CTA	**Control No.:**	257190
Brand Name (common name):	ChAd-CoV/Mac Ad5-CoV/Mac		
Manufacturer/Sponsor Name:	McMaster University		
Target Date:	*November 3, 2021* **Expedited 14-day target: October 18, 2021**		

Review Type:		**Previous Reviewer:**
☒ **Clinical**	CREST-OCT-E	N/A
☒ **Chemistry**	CBE-VQD1	N/A
☐ **Joint Review**	N/A	

Investigational Status Assessment (ISA):	Yes ☐ No ☒
Summary of Additional Drug (SOAD):	Yes ☐ No ☒
Notes to Review:	- **COVID-19 CTA(-A) (Division 5) – Expedited Review** - **First Entry for:** o **ChAd-CoV/Mac** o **Ad5-CoV/Mac** - Pre-CTA Meeting Control # 250223, held on April 13, 2021. - Letter of Authorization for sharing information for the below previous submissions is provided: o CTA Control # 126673 for AdAg85A Vector o Pre-CTA Meeting Control # 163439 for AdAg85A o CTA Control # 177700 for AdAg85A Vector o Pre-CTA Meeting Control # 250223 for Ad5-triCoV/Mac and ChAd-triCoV/Mac vectors
Link to sequence on docuBridge:	- Original submission, Seq. 0005: *Pending upload to docuBridge* - Response to Screening IR, Seq. 0006: *Response to be received post-acceptance into review*
Link to screening report:	*Pending upload to docuBridge*

Phase:	Phase 1	**Protocol No:**	M010
Protocol Title: Phase 1, open label study to evaluate the safety and immunogenicity of ChAd68 and AdHu5 vector-based trivalent COVID-19 vaccines delivered via inhaled aerosol Protocol Version # 1.0, dated September 30, 2021			

Product Information:			
Medicinal ingredient:	**Strength:**	**Form:**	**Route:**
1) ChAd-triCoV/Mac	1) 5.61e9 TCID50/mL	1) Aerosol	1) Inhalation
2) Ad5-triCoV/Mac	2) 3.55e9 TCID50/mL	2) Aerosol	2) Inhalation
Description:	1) 1 mL polypropylene Nalgene cryovials 2) 1 mL polypropylene Nalgene cryovials		

Study Summary: This is an open-labeled study, using an adaptive trial design to manage uncertainty, evaluating the administration of recombinant replication-defective adenoviral vector-based trivalent COVID-19 vaccines (ChAd-triCoV/Mac and Ad5-triCoV/Mac) using aerosol administration in healthy human subjects with a history of a completed vaccine series (two doses) with a mRNA COVID-19 vaccine.

The sponsor has developed in their GMP-facility at McMaster two vectors:

- Ad5-triCoV/Mac is a recombinant type 5 human adenovirus vector which has been engineered to express their trivalent SARS-CoV-2 transgene cassette under the control of a MCMV promoter, and is followed by an SV40 polyA signal. The adenovirus construct is E1 and E3 deleted.
- ChAd-triCoV/Mac vector is an E1 and E3 deleted chimpanzee adenovirus serotype 68 where the trivalent SARS-CoV-2 transgene cassette is under the control of an HCMV promoter and is followed by an SV40 polyA signal.
- The trivalent transgene cassette consists of a human tPA signal sequence/propeptide (aa 1-32), the S1 region of SARS-CoV-2 spike protein (aa 47-716), the transmembrane domain of VSV G (aa 443-511) followed by the P2A "self-cleaving" peptide of porcine teschovirus (P2A, sequence: GSGATNFSLLKQAGDVEENPGP), full-length SARS-CoV-2 nucleoprotein (aa 1-419) fused to a highly conserved portion of the SARS-CoV-2 polymerase (aa 4673-4742).

Given the changing landscape in Canada's and global COVID-19 vaccine roll-out, the trial design has changed since that discussed at the Pre-CTA meeting to focus on a single-dose inhaled aerosol delivery to participants who have completed 2-dose mRNA vaccine series.

The aerosol COVID-19 vaccine clinical trial builds on the sponsor's experience with McMaster's adenovirus-vectored TB vaccine (Ad5Ag85A), where they have characterized the aerosol delivery of adenovirus vectors using the Aeroneb® Solo Vibrating Mesh Nebulizer and demonstrated the safety and enhanced immunogencity of the aerosol route of administration in their recently completed phase 1 study in humans. A Letter of Authorization to the Ad5Ag85A studies is provided in Module 1.2.6. Further details in *Module 2 and 3 Screening Comments* below. The same Aeroneb nebulizer will be used in this study. The sponsor will be requested, via Screening IR post-acceptance into review, to confirm the License No. of the nebulizer.

The primary objective of this study is to evaluate and compare the safety and tolerability of a single inhaled aerosol dose of ChAdtriCoV/Mac or Ad5-triCoV/Mac in healthy volunteers with a history of prior COVID-19 vaccination.

The secondary objectives are:

- To evaluate and compare the local respiratory and systemic immunogenicity of a single inhaled aerosol dose of ChAd-triCoV/Mac or Ad5-triCoV/Mac in healthy volunteers with a history of prior COVID-19 vaccination.
- To evaluate the effect of pre-existing anti-adenoviral antibodies on the safety and immunogenicity of ChAd-triCoV/Mac and Ad5-triCoV/Mac.

Synopsis of Proposed Change(s): N/A

Joint Review: N/A

SUBMISSION SCREENING ASSESSMENT

Module 1 Administrative Information and Prescribing Information

Module	Contents of Submission Package	Select Answer
1.0	**Correspondence**	
1.0.1	Cover Letter CTA-As should indicate the original CTA(s) and any relevant CTA-A(s) with the file	Yes ☒ No ☐

	number and control number (s) on the cover page. **For COVID-19 studies filed under the Interim Order**: While the application process is the same, applicants must clearly indicate in their CTA cover letter that they are applying for authorization under the Interim Order. They must also indicate how the drug meets the definition of a COVID-19 drug given its direct use in relation to COVID-19, including the active role the drug is intended to play in the diagnosis, treatment, mitigation or prevention of COVID-19.	
1.0.5	Meeting Information A copy of the finalized pre-CTA meeting minutes and any other relevant correspondence between Health Canada and the clinical trial sponsor.	Yes ☒ No ☐ N/A ☐
1.2	**Administrative Information**	
1.2.1	Submission Application Form (HC/SC 3011) **Required** If there is more than one investigational drug, a separate HC-SC 3011 form (alternately, separate Part 2 only) will need to be filled out for each. See guidance for instructions.	Yes ☒ No ☐
	Appendix 1: CTA Third Party Authorization for Importation A separate Appendix 1 should be provided for each importer authorised to import the new drug into Canada by the clinical trial sponsor.	Yes ☐ No ☐ N/A ☒
	Appendix 2: Third Party Authorization for Filing Required only if the party signing the HC/SC 3011 is a third party acting on behalf of the manufacturer/sponsor company identified in Section 11of the form. A separate authorisation is required for each application.	Yes ☐ No ☐ N/A ☒
	Appendix 3: CTA information – **Required** A separate HC/SC 3011 and Appendix 3 should be provided for each protocol. For an institution/investigator – initiated clinical trial, Appendix 3 may be signed by the Qualified Investigator in lieu of the Senior Medical Officer and the appropriate Department Head in lieu of the Senior Executive Officer (Box 91) The SRAO should verify if the correct version of the Appendix 3 has been provided. • **For COVID-19 CTAs filed under the Interim Order**, the sponsor must use the Appendix 3 template that includes reference to the Interim Order.	Yes ☒ No ☐
	Appendix 4 : Drug Product formulation information - Animal and/or Human sourced ingredients/materials - **Not Required for CTA (-As).**	Yes ☐ No ☐ N/A ☒
1.2.3	Certification and Attestation Forms A Summary of Additional Drugs (SOAD) Form for additional drugs (e.g., comparator, concomitant and rescue medications) imported for the purpose of the clinical trial.	Yes ☐ No ☐ N/A ☒
1.2.6	Authorization for Sharing Information (see Quality Information below for MF/SMF information)	
1.2.7	International Information – Information regarding refusals by regulatory authorities outside Canada and foreign Research Ethics Boards, if applicable. **Note:** Select Yes if any international information/documents are provided. Select No only if there are foreign refusals. Select N/A if no international information/documents are provided.	Yes ☐ No ☐ N/A ☒
1.2.9	Other Application-related Information – This section is for any administrative information that does not have a designated location in the CTD format. This section should not contain any scientific information.	Yes ☐ No ☐ N/A ☒
1.3	**Product Information**	
1.3.4	Investigator's Brochure (IB) • A copy of the current IB for each investigational product, supplemented as appropriate with up-to-date safety, non-clinical and clinical data. The IB should be prepared in accordance with the Health Canada / ICH Guidance Document *E6: Good Clinical Practice: Consolidated Guideline*, and reviewed at least annually and revised as necessary. • If the IB has been updated relative to a version contained within a previously approved CTA/CTA-A, a summary of the changes should be provided. For products marketed in Canada, a reference to the most recent Canadian Product Monograph (PM) may be submitted in lieu of the IB.	Yes ☒ No ☐ N/A ☐
1.4	**Health Canada Summaries**	
1.4.1	PSEAT or Submission Rationale - **Required for CTA only** The **P**rotocol **S**afety and **E**fficacy **A**ssessment **T**emplate-Clinical Trial Application (PSEAT- CTA) summarizes the safety and efficacy information in clinical trial	Yes ☒ No ☐ N/A ☐

	protocols.	
1.4.1	Investigational Status Assessment (ISA) Form Completed forms can only be provided for Canadian marketed products, considered to be used off-label, but not investigational. Any supporting literature references should be provided in Module 5.4.	Yes ☐ No ☐ N/A ☒
1.7	**Clinical Trial Information**	
1.7.1	Study Protocol - final study protocol, including version number For CTA-A, a copy of the **amended or working protocol** with a clear description of the changes that are being proposed (i.e. original wording vs. revised wording), a rationale for ***each*** proposed change, and a copy of the **most recently approved protocol.**	Yes ☒ No ☐ N/A ☐
1.7.2	Informed Consent Documents For CTA-A, the ICF with changes clearly indicated (i.e. annotated)	Yes ☒ No ☐ N/A ☐
1.7.3	Canadian Research Ethics Board (REB) refusals, if applicable **Note:** Select Yes if any REB information/documents are provided. Select No only if there are REB refusals. Select N/A if no REB information/documents are provided.	Yes ☐ No ☐ N/A ☒
1.7.4	Information on prior related submissions A list of ongoing clinical trials in Canada for which approval has been granted by Health Canada, if applicable. Pay particular attention to parent CTA and applicable amendments to the same protocol.	Yes ☐ No ☐ N/A ☒

Module 1 Screening Comment

1.0.5 Meeting Information:
A Pre-CTA Meeting, Control # 250223, was held on April 13, 2021. From Health Canada, the following groups were in attendance: CREST (A/Director, OCT-E, ORA), CBE (Director, CVQD), OPIC and HECSB.
The final meeting minutes were provided in Seq. 0003 as well as in this CTA: HC6-024-c250223 (250223 - Revised Minutes of Meeting dated 2021-04-13 (Basta)) - BRDD pre-CTA Minutes_Final_13May2021.

Since the Pre-CTA meeting, the sponsor has completed the additional pre-clinical studies requested and addressed the comments and questions raised by BRDD. For a summary of the meeting response, refer to the *Cover Letter*, pages 4-9. The sponsor has a detailed section in the protocol describing the procedures they have implemented to minimize, manage and assess risk to participants in the study. They have modified the trial design to reflect the rapidly changing landscape of COVID-19 infections and the rapid and effective roll-out of licensed vaccines. They no longer plan to evaluate an IM prime and aerosol boost in an unimmunized cohort, but now intend to evaluate an aerosol boost following a completed mRNA vaccine series.

1.3.4 Investigator's Brochure:
The newest IB for Ad5-triCoV/Mac and ChAd-triCoV/Mac is Edition #1.0, dated September 30, 2021, is now uploaded on docuBridge under this Control # 257190: *pending upload to docuBridge*.
The sponsor explained in the *Cover Letter* that both adenovirus vectors (Ad5-triCov/Mac and ChAd-triCoV/Mac) are described in the same IB given the overlapping details.

1.7.1 Study Protocol:
The following document is provided:
- Protocol, Version 1.0, dated September 30, 2021

1.7.2 Informed Consent Forms:
The following document is provided:
- ICF, Version 1.0, dated September 20, 2021

1.7.4 Information on prior related submissions:

Submission Type	Control No	Notes (NOL, Withdrawn, Reclassified, etc.)	Decision Date
Pre-CTA Meeting	250223		April 13, 2021

Conclusion: All supporting data requirements are met.

Module 2 & 3 **Quality Information**

Module	Contents of Submission Package	Select Answer
1.2.6	Authorization for Sharing Information (see Mod. 3 for MF/SMF information) Reference to a Master File (MF) and/or Site Master File (SMF) A letter by the holder of the MF permitting Health Canada to reference information in the MF and/or SMF, if applicable. Ensure that the supporting MF (including the letter of access and fees) has been submitted to and accepted by the MF Unit. If not, the CTA must be placed on Processing Hold.	Yes ☐ No ☐ N/A ☒
	Reference to an application previously submitted by a different sponsor A letter by the sponsor of the referenced application authorizing Health Canada to access their information in support of the CTA.	Yes ☒ No ☐ N/A ☐
2.3	Quality Overall Summary (QOS) - Prepared in accordance with the Quality Guidances: Common Technical Document for Biological Products • Alternate formats (i.e. Investigational Medicinal Product Dossier) are acceptable For CTA-A, a clear description of the changes that are being proposed (i.e. original wording vs. revised wording) with rationale for the changes	Yes ☒ No ☐ N/A ☐
3.2	Body of Data – Additional supporting quality data Submission of Module 3 is not necessary if sufficient information is provided in the QOS/QIS-R/QIS-PER	Yes ☐ No ☐ N/A ☒

Module 2 and 3 Screening Comment

1.2.6 Reference to an application previously submitted:
A Letter of Authorization, dated September 20, 2021, is provided from McMaster University to authorize Health Canada access to any of their files pertaining to the below previous submissions relating to this current application:

- CTA Control # 126673 for AdAg85A Vector, Protocol # M001, NOL issued on January 9, 2009
- Pre-CTA Meeting Control # 163439 for AdAg85A
- CTA Control # 177700 for AdAg85A Vector, Protocol # M002, NOL issued on October 3, 2014
- Pre-CTA Meeting Control # 250223 for Ad5-triCoV/Mac and ChAd-triCoV/Mac vectors

Module 2.3:
The following documents are provided:

- Ad5-triCoV/Mac QOS
- ChAd-triCoV/Mac QOS

Ad5-triCoV/Mac
A single lot of clinical-grade Ad5-triCoV / Mac with batch number FP-06, Lot 10 was prepared. Three sequential lots (FP-06 Lot 07, FP-06 Lot 08, FP-06 Lot 09) each consisting of 10L, were prepared from the same cell train. Additionally, the Master Virus Bank is Batch # MVB-09, Lot 02, and the Master Cell Bank is Batch # MCB-04.

Drug Substance Container Closure System
The desalted, filtered purified bulk (28 mL) was filled in a sterile 50 mL polypropylene tube, placed inside a sterile bag and stored at -70°C for 8 days prior to final product vial filling.

Drug Substance Stability
Based on the titers of previous purified bulk that have been manufactured in their facility and the titers of the corresponding final vialled product, purified bulk stored in 50 mL Falcon tubes at -70°C is stable for at least two months as there was no reduction in titer observed with the final vialled product.

Drug Product Container Closure System
Ad5-triCoV / Mac was filled into sterile, non-cytotoxic and non-pyrogenic polypropylene Nalgene cryovials at 1.0 mL per vial. The vials have external threads and polyethylene screw caps with silicone gaskets. Prior to dosing, Ad5-triCoV / Mac will be thawed to room temperature and diluted to the appropriate concentration.

Drug Product Stability
The sponsor expects their Adenovirus-based vaccines, when stored under the stated conditions, to be extremely stable. Stability data from other clinical-grade vector have demonstrated vector titer to be maintained for at least 17 years (AdHER2.1) and 13 years (AdAg85A) after manufacturing when stored at -70°C. Based on this, the expiry date for the vaccines is set for ten years from the manufacturing date.
Data will be accumulated to document the stability of the Adenovirus-based vaccines over the duration of the

trial. The vector will be titered on 293 cells every year at the Robert E. Fitzhenry Vector Laboratory using an established SOP. They expect a less than 1 log reduction in titer over a one-year period. Health Canada will be notified of any stability failures.

ChAd-triCoV/Mac

A single lot of clinical-grade ChAd-triCoV / Mac with batch number FP-07, Lot 14 was prepared. Three sequential lots (FP-07 Lot 11, FP-07 Lot 12, FP-07 Lot 13) each consisting of 10L, were prepared from the same cell train. Additionally, the Master Virus Bank is Batch # MVB-11, Lot 01, and the Master Cell Bank is Batch # MCB-04.

Drug Substance Container Closure System

The desalted, filtered purified bulk (24 mL to 36 mL) was filled in sterile 50 mL polypropylene tubes, placed inside sterile bags and stored at -70°C for 3 to 20 days prior to final product vial filling.

Drug Substance Stability

Based on the titers of previous purified bulk that have been manufactured in their facility and the titers of the corresponding final vialled product, purified bulk stored in 50 mL Falcon tubes at -70°C is stable for at least two months as there was no reduction in titer observed with the final vialled product.

Drug Product Container Closure System

ChAd-triCoV / Mac was filled into sterile, non-cytotoxic and non-pyrogenic polypropylene Nalgene cryovials at 0.8 mL per vial. The vials have external threads and polyethylene screw caps with silicone gaskets. Prior to dosing, ChAd-triCoV / Mac will be thawed to room temperature and diluted to the appropriate concentration.

Drug Product Stability

The sponsor expects their Adenovirus-based vaccines, when stored under the stated conditions, to be extremely stable. Stability data from other clinical-grade vector have demonstrated vector titer to be maintained for at least 17 years (AdHER2.1) and 13 years (AdAg85A) after manufacturing when stored at -70°C. Based on this, the expiry date for the vaccines is set for ten years from the manufacturing date.
Data will be accumulated to document the stability of the Adenovirus-based vaccines over the duration of the trial. The vector will be titered on 293 cells every year at the Robert E. Fitzhenry Vector Laboratory using an established SOP. They expect a less than 1 log reduction in titer over a one-year period. Health Canada will be notified of any stability failures.

Nebulizer

For both products, a single dose diluted in 0.5 mL sterile buffered saline will be aerosolized using the AeroNeb Solo Vibrating Mesh Nebulizer and inhaled via mouthpiece using tidal breathing. The sponsor will be requested, via Screening IR post-acceptance into review, to confirm the License No. of the nebulizer.

Conclusion: All supporting data requirements are met, however further clarification will be requested post-acceptance.

SCREENING RECOMMENDATION

- All supporting data requirements have been met. This submission is considered acceptable for review as a CTA. Additional clarification of the following is required, an Information Request was issued post-acceptance into review:

  1) Confirm the License No. of the Aeroneb Solo Vibrating Mesh Nebulizer used in this study.

Name and Title	Date
Patricia Basta Senior Regulatory Affairs Officer BRDD, ORA	Date Review Commenced: October 4, 2021 Date Review Completed: October 4, 2021
Signature	***This document has been signed electronically using the Health Canada docuBridge system.***

REFERENCES:

- Interim Order respecting clinical trials for medical devices and drugs relating to COVID-19
- Order Respecting Certain Time Limits Under the Food and Drug Regulations (COVID-19)

Submission Format:

- Applications for Drug Clinical Trials under the Interim Order Respecting Clinical Trials for Medical Devices and Drugs Relating to COVID-19: Guidance Document
- Guidance Document for Clinical Trial Sponsors: Clinical Trial Applications
- Guidance Document: Master Files (MFs) – Procedures and Administrative Requirements
- Guidance Document: Preparation of Clinical Trial Applications for use of Cell Therapy Products in Humans

Electronic Format:

- Guidance Document: Preparation of Drug Regulatory Activities in the Electronic Common Technical Document (eCTD) Format
- Guidance Document: Preparation of Regulatory Activities in the "Non-eCTD Electronic-Only" Format
- Guidance Document: The Management of Drug Submissions and Applications
- Organisation and Document Placement for Canadian Module 1 of the Common Technical Document (CTD) Structure

Quality:

- Guidance for Industry: Preparation of the Quality Information for Drug Submissions in the CTD Format: Biotechnological/Biological (Biotech) Products
- Guidance for Industry: Preparation of the Quality Information for Drug Submissions in the CTD Format: Blood Products
- Guidance for Industry: Preparation of the Quality Information for Drug Submissions in the CTD Format: Conventional Biotherapeutic Products
- Guidance Document: Harmonized Requirements for the Licensing of Vaccines and Guidelines for the Preparation of an Application

ORA Screening Documents:

- 3011 Screening Guide
- CTA/CTA-A Screening Reminders
- Investigational Status Assessments (ISA) Work Instruction
- Issue Sheet Foreign Comparator
- Issue Sheet 18FDG Off-Label

From: The Pan American Network for Drug Regulatory Harmonization on behalf of [redacted]
To: PANDRH@LISTSERV.PAHO.ORG
Subject: PUBLIC CONSULTATION - RECOMMENDATIONS TO ASSURE THE QUALITY, SAFET AND EFFICACY OF POLIOMYELITIS VACCINE (ORAL, LIVE, ATTENUATED)
Date: 2022-06-10 2:19:53 PM
Attachments: BS 2423_OPV_TZ_7 June 2022.pdf
Comment_Form_OPV BS2423_TZ_7 June 2022.doc
Importance: High

FYI from WHO

World Health Organization

The 2nd round of public consultation on **Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccine (oral, live, attenuated) – Proposed replacement of Annex 2 of WHO Technical Report Series, No. 980**, (**WHO/BS/2022.2423)**, is now open for **inviting public comments** on the WHO website at the link below:

https://www.who.int/groups/expert-committee-on-biological-standardization

Direct link to the document WHO/BS/2022.2423 is here:
https://cdn.who.int/media/docs/default-source/biologicals/bs-2423_opv_tz_7-june-2022.pdf?sfvrsn=5c535068_1

Please use the WHO Comment Form to provide your comments:
https://cdn.who.int[redacted]

For your easy use, the two documents are also attached to this email.

DEADLINE for submission of comment: **15 August 2022. Please send comments to Dr** [redacted] **at:** [redacted]@who.int.

You are strongly encouraged to share this information with your colleagues or expert groups who may be interested in the subject.

Comments received by the published deadline (shown above) will be considered in the preparation of the discussion at the ECBS meeting to be held from 24- 28 October 2022.

We are looking forward to receiving your valuable feedback.

Thank you!

World Health Organization

WHO/BS/2022.2423
ENGLISH ONLY

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccine (oral, live, attenuated)

Proposed replacement of Annex 2 of WHO Technical Report Series, No. 980

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed document- *Recommendations to assure the quality, safety and efficacy Of poliomyelitis vaccine (oral, live, attenuated)- Proposed replacement of Annex 2 of WHO Technical Report Series, No. 980,* to a broad audience and to ensure the transparency of the consultation process.

The text in its present form does not necessarily represent the agreed formulation of the ECBS. Written comments proposing modifications to this text MUST be received by 15 August 2022 using the Comment Form available separately and should be addressed to the Department of Health Products Policy and Standards, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland. Comments may also be submitted electronically to the Responsible Officer: **Dr Tiequn Zhou at email: zhout@who.int**.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide, second edition" (KMS/WHP/13.1).

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccine (oral, live, attenuated)

Proposed replacement of Annex 2 of WHO Technical Report Series, No. 980

Recommendations published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Recommendations be made only on condition that such modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the Recommendations set out below. The parts of each section printed in small type are comments or examples intended to provide additional guidance to manufacturers and NRAs.

Introduction

Requirements for oral poliomyelitis vaccine (OPV) were first formulated in 1962 (1) and revised in 1966 (2) and 1972 (3) when an appendix describing the production of OPV in human diploid cells was added. The requirements were further updated in 1982 (4) following an accumulation of data, particularly on the performance and evaluation of the monkey neurovirulence test (MNVT) and tests on the karyology of human diploid cells. The Requirements for poliomyelitis vaccine (oral) were updated in full in 1989 (5) to take account of the general requirements for the characterization of continuous cell lines for the preparation of biologicals which were adopted in 1985 (6), and after a WHO Study Group concluded that, in principle, such cell lines are acceptable as substrates for the production of biologicals (7). An addendum was subsequently adopted (8) that introduced changes in tests for freedom from detectable DNA sequences of Simian virus 40 (SV40), introduced the mutant analysis by polymerase chain reaction (PCR) and restriction enzyme cleavage (MAPREC) assay as an optional additional in vitro test for poliovirus type 3, increased levels of laboratory containment for wild polioviruses (WPVs); and provided guidance on additional antibody screening tests (for foamy viruses) for animals from closed primate colonies used as a source for primary monkey kidney cells.

The Requirements (now Recommendations) were subsequently revised in full in 1999 (9) when the use of transgenic mice expressing the human poliovirus receptor (TgPVR21 mice) (10) as an alternative to the MNVT for type 3 virus was included in the revision and MAPREC test was introduced as the in vitro test of preference for the evaluation of filtered bulk suspensions for poliovirus type 3 (11). The previously mandated reproductive capacity at elevated temperature (rct40) test then became an optional, additional test if MAPREC test was performed. The studies with poliovirus types 1 and 2 in TgPVR21 mice were completed by June 2000, and an addendum to the Recommendations for the production and control of poliomyelitis vaccine (oral) was adopted in 2000 (12) that included the neurovirulence test in TgPVR21 mice (TgmNVT) as an alternative to the MNVT for all three poliovirus serotypes.

In 2012 the Recommendations were last revised in full to update on the origin of different virus strains for OPV production with inclusion of a new Appendix 1 and include consideration of new vaccine formulations (monovalent OPV- mOPV and bivalent OPV-bOPV) (13). The new Recommendations contained updated sections on international standards and reference preparations, general manufacturing recommendations and control tests, and the WHO standard operating procedures (SOPs) for TgmNVT and MAPREC in light of current developments in techniques. The document also included new sections on nonclinical and clinical evaluation of OPV, an update on terminology, and the introduction of the “virus sub-master seed lot” concept applicable only to the virus master seed supplied by WHO. Finally, it updated information on neurovirulence tests (MNVT and TgmNVT) and the MAPREC test which is extended to all three types of virus seeds and vaccine bulks; and inclusion of a new Appendix 2 giving the rationale for the choice of monkey or mouse neurovirulence tests.

Since then, there has been significant progress in global polio eradication as well as advances in scientific knowledge, novel laboratory techniques, including high-throughput sequencing (HTS, also known as next generation sequencing, massively parallel, or deep sequencing), and new non-pathogenic strains of polioviruses for quality control tests have become available. Wild-type polioviruses of serotypes 2 and 3 were declared eradicated globally by WHO in 2015 and 2019, respectively. Consequently, in April-May of 2016 the global use of trivalent OPV formulation (tOPV) for routine immunizations was replaced by the exclusive use of bOPV containing only serotypes 1 and 3. Therefore, manufacture of tOPV was discontinued, and now bOPV is being used for routine and supplemental immunization. Monovalent OPV of serotype 2 (mOPV2) is used to control outbreaks of type 2 circulating vaccine-derived poliovirus (cVDPV2). In addition, tOPV was approved by the Strategic Advisory Group of Experts on Immunization (SAGE) for use in control of type 2 cVDPV outbreaks occurring alongside WPV1 circulation. Rationally designed, more-genetically-stable strains of Sabin 2 virus were developed to minimise reversion of the vaccine strain to virulence and have been used to manufacture novel OPV2 (nOPV2). At the end of 2020 nOPV2 was introduced for cVDPV2 outbreak control under the WHO Emergency Use Listing (EUL) (14).

Other new WHO guidance documents were issued since the last revision of WHO Recommendations for OPV in 2012. A WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) was adopted in 2014 (15). It tightened biosafety and biosecurity requirements for handling live polioviruses and led to the adoption of new Guidelines for the safe production and quality control of poliomyelitis vaccines in 2018 (16), and a subsequent amendment in 2020 (17). In 2020, the WHO Expert Committee on Biological Standardization (ECBS) concluded that the 2012 Recommendations for OPV should be revised. WHO convened a drafting group composed of regulators from several countries to prepare the draft of revision of TRS 980, Annex 2. A virtual informal consultation meeting was held by WHO on 15-17 November 2021. It was attended by experts and representatives from academia, national regulatory authorities (NRAs)/national control laboratories (NCLs), industry and other international health organizations and institutions involved in the research, manufacture, authorization, and testing/release of OPV from countries around the world to discuss and reach consensus on the issues in the revision (18).

Major issues addressed during this revision include:

- the use of HTS in quality control of OPV as an alternative to MAPREC test as a preferred in vitro test.
- analysis of whole genome mutational profiles generated by HTS as a possible future replacement of MNVT and TgmNVT for routine lot release after the manufacture consistency has been established. Practical experience in these areas is currently limited. Further guidance will be provided in due course.
- removal of the rct40 test because it is insufficiently sensitive and requires WPVs as control strains, which complicates GAPIII compliance.
- considerations of the design, manufacture, and quality control of nOPV strains.

- use of new non-pathogenic strains for measurement of neutralizing antibodies to polioviruses.
- updates on international reference materials relevant to OPV manufacture and control and inclusion of a new appendix on that.
- updates on Terminology.
- introduction of "virus sub-master seed" concept for nOPV strains in addition to Sabin OPV.
- guidance for clinical evaluation of new OPV vaccine strains that may be developed following OPV cessation.

Additional changes have been made to refer to and align the document with other WHO recommendations published since the last revision.

Purpose and scope

These WHO Recommendations provide guidance to NRAs and manufacturers on the manufacturing processes, quality control and nonclinical and clinical evaluations needed to assure the quality, safety and efficacy of live attenuated polio vaccines (oral).

The scope of these recommendations encompasses live attenuated polio vaccines (oral) derived from the original Sabin strains, some by simple passage and others by more complex routes, including plaque purification. This document is intended to apply to all OPV products prepared from Sabin poliovirus strains and their derivatives.

The recommendations also include consideration of issues raised in the manufacture and control of nOPV made from rationally designed strains created by targeted genetic manipulation of Sabin viruses and the introduction of HTS as a quality control method in a regulatory setting for both nOPV and Sabin OPV.

In the current document, OPV refers to oral polio vaccines made from any attenuated poliovirus, both the original Sabin strain and novel, genetically modified strains. In some cases, Sabin OPV and nOPV are used intentionally to distinguish between classical OPV and novel OPV.

These WHO Recommendations should be read in conjunction with other relevant WHO guidance documents such as that on nonclinical (19) and clinical (20) evaluation of vaccines, good manufacturing practices for biological products (21), characterization of cell banks (22), lot release (23), and guidelines for the safe production and quality control of poliomyelitis vaccines (16, 17).

Terminology

The definitions given below apply to the terms as used in these WHO Recommendations. These terms may have different meanings in other contexts.

Adventitious agents: contaminating microorganisms of the cell substrate or source materials used in their cultures, that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced.

Cell-culture infective dose 50% ($CCID_{50}$): the quantity of a virus suspension that will infect 50% of cell cultures.

Cell seed: a quantity of vials containing well-characterized cells derived from a single tissue or cell of human or animal origin stored frozen in liquid nitrogen in aliquots of uniform composition, one or more of which would be used for the production of a master cell bank.

Comparator vaccine: an approved vaccine with established efficacy or with traceability to a vaccine with established efficacy that is tested in parallel with an experimental vaccine and serves as an active control in nonclinical or clinical testing.

Final bulk: the finished vaccine from which the final containers are filled. The final bulk may be prepared from one or more monovalent bulks and may contain more than one virus type.

Final lot: a collection of sealed final containers of finished vaccine that is homogeneous with respect to the risk of contamination during the filling process. All the final containers must therefore have been filled from a single vessel of final bulk in one working session.

Master cell bank (MCB): a quantity of fully characterized cells of human or animal origin frozen at □70 °C or below in aliquots of uniform composition, derived from the cell seed. The MCB is itself an aliquot of a single pool of cells, dispensed into multiple containers and stored under defined conditions. The MCB is used to derive all working cell banks. The testing performed on a replacement MCB – derived from the same cell clone, or from an existing master or working cell bank (WCB) – is the same as that for the initial master cell bank unless a justified exception is made.

Monovalent bulk: a pool of a number of single harvests of the same virus type processed at the same time.

High throughput sequencing (HTS): a next-generation sequencing technology based on sequencing of individual nucleic acid molecules that allows each nucleotide to be sequenced multiple times (massively parallel or deep sequencing), thereby enabling the detection and quantitation of sequence heterogeneities including single nucleotide polymorphisms (SNP).

Novel OPV (nOPV): rationally designed genetically modified derivatives of the live-attenuated Sabin vaccine strain. nOPV has enhanced genetic stability and lower risk of reversion to neurovirulence compared to the original Sabin strain.

Production cell culture: a cell culture derived from one or more ampoules of the WCB or primary tissue used for the production of vaccines.

RSO: RNA-plaque-derived Sabin Original type 3 virus (24). All subsequent passages are designated by an additional number – e.g., RSO1 (master seed) is one passage on from RSO. It is distributed to vaccine manufacturers that create their own master/sub-master and working seed stocks used for manufacture of monovalent bulks of OPV3.

Sabin strains: preparations of attenuated polioviruses of types 1, 2 and 3 derived by limited number of passages from stocks developed by Dr Albert Sabin (25) which retain attenuated properties as measured by biological and molecular markers.

Single harvest: a quantity of virus suspension of one virus type harvested from cell cultures derived from the same WCB and prepared from a single production run.

SO: Sabin Original virus as described in Sabin and Boulger 1983 (25). All subsequent passages are designated by an additional number – e.g. SO+1 is one passage on from Sabin Original.

Virus master seed lot: a quantity of virus suspension that has been processed at the same time to assure a uniform composition and has been characterized to the extent necessary to support development of the virus working seed lot. The characterized virus master seed lot is used for the preparation of virus working seed lots or a virus sub-master seed (if applicable).

Virus sub-master seed lot: a quantity of virus suspension produced by a single passage from the virus master seed and made at a multiplicity of infection that ensures the development of cytopathic effect within an appropriate timeframe, and that has been processed at the same time to assure a uniform composition. Sub-master seeds should be made by the manufacturer when the supply of well characterised master seed of Sabin OPV supplied by WHO is insufficient to meet production needs. They may also be produced from qualified nOPV master seeds if it is necessary. The virus sub-master seed lot should be characterized as extensively as the virus master seed lot to support the development of the virus working seed lot. The characterized virus sub-master seed lot is used for the preparation of virus working seed lots (see section A.3.2.2 and Part B).

Virus working seed lot: a quantity of virus of uniform composition, fully characterized, derived by only one passage from master or sub-master virus seed lot made at the multiplicity of infection, ensuring that cytopathic effect develops within an appropriate timeframe (e.g. three days) from a virus master seed lot or sub-master seed lot approved by the NRA for the manufacturing of vaccine.

Working cell bank (WCB): a quantity of cells of uniform composition derived from one or more ampoules of the MCB at a finite passage level, stored frozen at –70 °C or below in

aliquots, one or more of which would be used for vaccine production. All containers are treated identically and once removed from storage are not returned to the stock.

General considerations

Poliomyelitis is an acute communicable disease of humans caused by three distinct poliovirus serotypes called types 1, 2 and 3 distinguished by neutralization with type specific antibodies (26). Poliovirus is a species C human enterovirus of the Picornaviridae family and consists of a single-stranded, positive-sense RNA genome and a protein capsid.

Where sanitation is poor, these viruses are believed to spread mainly by faecal-to-oral transmission, whereas the oral-to-oral mode of transmission probably dominates in areas with a higher standard of sanitation. Mixed patterns of transmission are likely to occur in most settings. In the pre-vaccine era, roughly one out of 200 susceptible individuals infected by polioviruses developed paralytic poliomyelitis, while the rest were asymptomatic or had mild symptoms (26).

Progress in polio control (and, since 1988, polio eradication) has been mainly due to widespread use of vaccines. An inactivated poliovirus vaccine (IPV Salk vaccine) was licensed in 1955. The use of live, attenuated OPV (Sabin vaccine) for mass immunizations started in the Soviet Union and few other countries in 1959. It was licensed in the United States and some European countries as monovalent OPV in 1961, and as trivalent OPV (tOPV) in 1963. The Sabin strains of poliovirus used in the production of Sabin OPV were shown to be both immunogenic and highly attenuated when administered orally to susceptible children and adults. Most countries that initially introduced vaccination with IPV later changed to OPV because of ease of administration, suitability for mass vaccination campaigns, induction of superior intestinal mucosal immunity, and lower production costs. In 1974, OPV was recommended as part of the Expanded Programme on Immunization (EPI), and OPV was again the vaccine of choice in 1988 when the World Health Assembly resolved to eradicate polio globally by the year 2000. The last cases of poliomyelitis caused by WPV type 2 (WPV2) and 3 (WPV3) were reported in October 1999 in India and November 2012 in Nigeria respectively. Subsequently, the global eradication of WPV2 and WPV3 was certified on 20 September 2015 and 24 October 2019, respectively (26). By the end of 2021, WPV1 only remained endemic in two countries – Afghanistan and Pakistan.

Although OPV is a safe vaccine, adverse events may occur on rare occasions (26). Vaccine-associated paralytic poliomyelitis (VAPP) is the most important of these rare adverse events. and is clinically indistinguishable from poliomyelitis caused by WPV. Identification of VAPP requires laboratory analysis of the virus isolated from the case. The incidence of VAPP has been estimated at 2-4 cases per million annual birth cohort in countries using OPV (26). Sabin viruses can spread in populations where the coverage of OPV is low. In such situations, Sabin viruses can acquire the neurovirulence and transmissibility characteristics of WPV, thus becoming cVDPV that can cause outbreaks of the disease (27), presenting a significant challenge to the global eradication campaign. cVDPV2 is the predominant type, and its continued circulation is

fuelled by inadequate population immunity. To prevent gaps in population immunity the switch from tOPV to bOPV (containing only vaccine viruses of serotypes 1 and 3) was supposed to be accompanied by the introduction of supplemental immunization with trivalent IPV. However, the shortage of IPV in some countries led to the decline in population immunity to type 2 poliovirus and the increase of cVDPV2 cases from 2 in 2016 to over 1,000 in 2020. Control of cVDPV2 outbreaks has been performed with targeted use of mOPV2, but where the campaigns have been poor because of difficulties in delivery they have triggered the emergence of new cVDPV2 outbreaks. Outbreaks due to type 1 and 3 cVDPVs have also occurred but to a lesser extent and continue to occur in recent years.

cVDPVs will continue to emerge as long as classical Sabin OPV is used and gaps in population immunity exist. To overcome this problem, in 2011 an international consortium of scientists sponsored by the Bill and Melinda Gates Foundation set out to develop novel vaccine strains having reduced risk of losing attenuated phenotype and evolving to neurovirulent cVDPVs. One of the resulting viruses has been used to produce an nOPV2 which has been granted EUL by WHO for use in type 2 cVDPV outbreaks (28-31). Additional strains could be developed in the future, including similar genetically stabilized strains of serotypes 1 and 3. The design of the novel strains is based on understanding of the molecular biology of polio viruses and vaccines that has been gained over the years. The attenuation of the Sabin strains is associated in part with a highly base-paired hairpin structure in the 5' non-coding region of the virus, called domain V, which is involved in the initiation of protein synthesis. The three Sabin strains have less thermally stable structures of this domain compared to respective wild strains, as a result of the introduction of a single base change in this section of the RNA which is different for each serotype, but which changes the strength of a base pair. As it is a single base change, all three serotypes can readily revert by a single mutational event to the wild type sequence at this position and this is observed in vaccine recipients. Viruses have therefore been constructed which make it harder for the hairpin structure of domain V become stronger by mutation. This was done by replacing stronger GC pairs and weaker GU pairs with intermediately strong AU pairs, so that the overall thermostability of the hairpin and therefore the virus neurovirulence remained unchanged. However, this made the attenuated phenotype more stable because in this re-designed structure two simultaneous mutations at any given position are required to revert to the wild type base pair strength. The nOPV strains should therefore be at least as attenuated as the Sabin strains and genetically more stable. This was demonstrated to be the case both in vitro, in animal models and in human trials. Modifications were also introduced into the viral polymerase to increase the virus genetic stability by reducing the mutation and recombination rates. In addition, an essential cis-acting replicative element has been moved from the centre of the genome to the 5'-UTR to minimise the risk of removing the genetically modified domain V region by recombination.

The key to nOPV safety lies in the low level of reversion at key known sites. Consistency has been monitored by molecular means rather than animal tests, although animal tests are retained as a final check. The nOPV strains have different properties to the classical Sabin strains with respect to optimal growth conditions, therefore production and quality control of vaccines made from the new strains may differ in detail from those used for the classical Sabin strains. This

includes growth and titration properties, optimal temperature of growth, dose required, thermal stability and other parameters. The nucleotide sequence of nOPV2 strain is available in GenBank (accession number MZ245455) and the graphic representation of its structure is shown in Figure 3 in Appendix 1. Similar nOPV1 and nOPV3 strains are currently in early clinical development and in the future may be used in trivalent formulations. The novel strains are of great importance to the eradication programme and are considered in this revision.

Trivalent formulations of conventional (Sabin) OPV were created in the early 1960s to ensure that the immune response against all three poliovirus serotypes was adequate. Subsequent studies demonstrated that the Sabin 2 virus had higher fitness and interfered with the immunogenicity of serotypes 1 and 3, leading to lower seroconversion (32). In 2008, a clinical trial to evaluate the immunogenicity of alternative OPV formulations (mOPV1, mOPV3 and bOPV) compared to tOPV was conducted by WHO in the Indian population. The seroconversion rates to poliovirus type 1 and type 3 following immunization with bOPV were significantly higher than that induced by tOPV and were not lower than those induced by immunization with either mOPV1 or mOPV3 alone (33). The introduction and widespread use of mOPV1 and mOPV3 in supplementary immunization activities in 2005 resulted in substantial reductions of cases caused by the respective serotypes. This led to the stopping of WPV1 circulation in India, and WPV3 eradication worldwide, which was declared in 2019. However, continued circulation of WPV1 in two remaining polio-endemic countries requires huge quantities of bOPV to be given in routine and mass campaigns conducted in 140 countries throughout the world.

In addition to bOPV, which is used in most countries for routine or supplementary vaccination, monovalent OPVs of all three serotypes are used by the Global Polio Eradication Initiative (GPEI)[1] and have been licensed for use in endemic countries or for outbreak control in situations where one or two types can re-emerge. In 2020 SAGE recommended that tOPV be made available to countries for cVDPV2 outbreak response in subnational areas where there is co-circulation or high risk of co-circulation of cVDPV2 with cVDPV1, cVDPV3 or WPV1 instead of dual mOPV2 and bOPV campaigns (34). Therefore, at the moment there is a need for all known formulations of OPV.

Live vaccines prepared from Sabin poliovirus strains of types 1, 2, and 3 were introduced for large-scale immunization in 1959. In 1972, Sabin proposed that WHO should be the custodian of his poliovirus seed strains. The Director-General of WHO agreed to assume responsibility for ensuring the proper use of the strains and established a scientific committee, the Consultative Group on Poliomyelitis Vaccines, to advise WHO on all matters pertaining to their use. Detailed information on the work of the Consultative Group and the preparation of the seed stocks made by Behringwerke has been published by Cockburn (35). NRAs should decide on the use of virus strains and on the detailed procedures applicable to the preparation of virus seed lots for the production of OPV in their own countries.

[1] https://polioeradication.org/

The original poliovirus seeds produced by Sabin (SO) (25) were sent to Merck which generated seeds from them that were designated as SOM (Sabin Original Merck). Aliquots of SOM were supplied to a number of other manufacturers to enable them to develop their own seeds. Some seed lots were contaminated with SV40 which was present in the primary Rhesus kidney cells, the preferred cell culture system at that time for virus propagation. OPV manufacturers followed various strategies to reduce the contamination, including passage in the presence of specific antibody or treatment with toluidine blue, or thermal inactivation of SV40 in the presence of 1M $MgCl_2$ that stabilizes poliovirus. In 1974 Behringwerke AG of Marburg/Lahn, Germany, generously agreed to produce SO+1 seeds for WHO free of charge. The Behringwerke type 1 and type 2 seeds have been particularly widely used from the 1970s to date. In the 1950s, it was established that, particularly for the type 3 strain, increase in the passage number correlated with an increase in the reactivity in the MNVT. This finding led to the establishment of rigorous limits on the passage level for vaccine production for all types.

In order to develop a more stable type 3 strain, a new seed was prepared by Pfizer from a single plaque after transfecting susceptible cells with viral RNA extracted from poliovirus at the SO+2 level. This also reduced any residual risk of SV40 contamination. One plaque, which was designated 457-III, was identified with particularly favourable properties (24). Theoretically, vaccine derived from this stock was at passage SO+7. However, the purpose of tracking passage history of seed viruses is to reduce the accumulation of mutations that takes place during their serial propagation. Since plaque purification represents the cloning of a single infectious particle, it eliminates the heterogeneity of viral population, and the passage level is effectively reset to zero. Thus, the cloned stock 457-III was renamed RSO (for RNA-derived Sabin Original). Two additional passages were used to prepare virus master (RSO1) and working seeds (RSO2), and vaccines produced from this virus are at RSO3 level. Retrospectively, the consensus sequence of RSO has been shown to be the same as the consensus of SO (36), but it was more homogeneous and contained lower quantities of viruses with sequence polymorphisms. Consensus sequences of all three Sabin strains are available in GenBank[1] under accession numbers AY184219, AY184220, and AY184221.

The RSO seed was not used for the production of type 3 vaccine until the 1980s when it became clear that the virus stocks passaged from the SOM and other SO+1 seeds were inadequate. Since then, however, it has been widely used by European and American manufacturers as it is of lower virulence in laboratory tests than the SO+1 type 3 seed. The RSO seeds were bought from Pfizer by Sanofi Pasteur (formerly Institut Mérieux, Pasteur Mérieux Connaught and subsequently Aventis Pasteur) which has donated them to WHO.

The virus seeds available from WHO (designated “the WHO master seeds”) are therefore types 1 and 2 at SO+1 level produced by Behringwerke from SO seeds and the type 3 RSO “Pfizer” seed donated by Sanofi Pasteur. The seeds are kept at the National Institute for Biological Standards and Control in the United Kingdom and FDA/CBER in the United States and include a

[1] https://www.ncbi.nlm.nih.gov/nucleotide/

proportion of the stocks of the SO+1 seeds formerly held at Istituto Superiore di Sanità in Italy which kindly transferred them (24, 35). The virus seed stocks are available to vaccine manufacturers upon request to the WHO.

In addition to the RSO type 3 seed, a number of manufacturers in China, Japan and Russia have used their own purified seed stocks of Sabin 3 strain that were derived by a combination of passage and plaque purification (cloning). Sequencing of these seed viruses demonstrated that, while they contained low quantities of neurovirulent mutants, there were differences at other genomic sites between these strains and the consensus sequence of Sabin Original virus in the form of SNPs (35). However, there are no reports of any differences in clinical safety or immunogenicity between OPV produced from Pfizer stocks and the alternative seeds of Sabin 3 virus. An overview of virus seeds used in OPV production is given in Appendix 1.

The MNVT, as described in the 1989 requirements (5), has been used as a quality control test and is based on the level and the distribution of virus-specific lesions within the central nervous system produced by vaccine virus upon intraspinal inoculation into the anterior horns of Rhesus or Cynomolgus monkeys as compared to an appropriate reference preparation (37). Because nonhuman primates are used, efforts to complement and eventually replace the test are of considerable importance. WHO has encouraged and supported research on various aspects of poliovirus biology, including the development of alternative animal models, as part of the WHO initiative to promote the development of new norms and standards for vaccines. Two groups of scientists developed transgenic (TgPVR) mice by introducing into the mouse genome the human gene encoding the cellular receptor for poliovirus (38, 39). This receptor, known as CD155, makes TgPVR mice susceptible to poliovirus infection with clinical signs of flaccid paralysis and with histological lesions in the central nervous system similar to those observed in monkeys.

In 1992, WHO initiated a project to evaluate the suitability of transgenic mice for testing the neurovirulence of OPV with the aim of replacing monkeys with mice. The advantages of a neurovirulence test in transgenic mice are:

- a reduction in the number of primates used in quality control of OPV;
- the use of animals of highly-defined genetic and microbiological quality standards;
- a reduction in hazards to laboratory personnel through a reduced need to handle primates;
- a reduction in the time and cost of quality control tests for OPV.

Studies were carried out initially on type 3 monovalent polio vaccines using the TgPVR21 mouse line, generously provided free of charge for the study by the Central Institute for Experimental Animals in Kawasaki, Japan. Researchers at the FDA Centre for Biologics Evaluation and Research (CBER) in Rockville, MD, USA developed an intraspinal inoculation method suitable for tests of vaccine lots. This was evaluated in an international collaborative study on the establishment of a standardized mouse neurovirulence test (TgmNVT) for OPV (40). Several laboratories participated in the collaborative study and results were assessed by WHO at meetings held in 1995, 1997 and 1999 in Geneva, Switzerland, in 1997 in Ottawa, Canada, and in 1998 in Rockville, MD, USA. As a result of these studies, the revised

Recommendations for the production and control of poliomyelitis vaccine (oral) (9) introduced the murine model as an alternative to the MNVT for type 3 poliovirus and further studies demonstrated that this test was also suitable as an alternative to the MNVT for poliovirus types 1 and 2 (12). Laboratories must comply with specifications for containment of the transgenic animals (41). The MNVT and TgmNVT can provide evidence of consistency of production.

The molecular mechanisms and genetic determinants of attenuation and reversion to virulence of all three types of Sabin polioviruses used for the manufacture of OPV have been studied in several laboratories. As discussed above in the context of nOPV, evidence strongly suggests that mutations in the domain V of the internal ribosome entry site (IRES) in the 5' untranslated region (5'-UTR) of poliovirus genome are critical in determination of the attenuated phenotype (42). A molecular biological test, MAPREC assay, was developed by researchers at CBER to quantify reversion at the molecular level (43). Studies showed that all batches of type 3 OPV contained measurable amounts of revertants with C instead of U at nucleotide 472. Batches that failed the MNVT contained significantly higher quantities of 472-C than batches that passed the test. Studies with coded samples at CBER identified 100% of lots that failed the MNVT (44).

In 1991, WHO initiated a series of international collaborative studies to evaluate MAPREC assay for all three types of polioviruses and to validate appropriate reference materials. Several laboratories participated in the collaborative studies and results were assessed by WHO at meetings held in 1995 and 1997 in Geneva, Switzerland. It was concluded that MAPREC assay was a sensitive, robust, and standardized molecular biological assay suitable for use by manufacturers and NRAs for monitoring the consistency of production of type 3 OPV. The revised Recommendations for the production and control of poliomyelitis vaccine (oral) (9) introduced, for type 3 poliovirus, the use of MAPREC as the in vitro test of preference in place of the rct40 test. Reference materials for MAPREC were established for all three serotypes. For type 3 the International Standard used in MAPREC test defines the threshold of 472-C content above which vaccine lots have a high chance of failing MNVT. Reference materials for comparable positions in type 1 and type 2 are used to provide a measure of production consistency, but they do not define the pass/fail threshold because the amount of domain V mutants that make these vaccine preparations fail MNVT is much higher than their content in production lots.

High Throughput Sequencing (HTS) also known as deep sequencing or next generation sequencing is a powerful methodology that could be applied in many areas of the regulation of biological products. Classical (Sanger) sequencing determines the consensus or average sequence of a population of nucleic acid molecules, whereas HTS determines the sequence of individual molecules in a population. HTS generates multiple reads of each base position and produces large amounts of sequence data very rapidly. Determining the sequence of complete viral genomes is relatively straightforward. While the technology is still evolving rapidly this usually involves amplifying sequences by PCR, using primers which may be either specific for a given sequence or random to pick up any nucleic acid sequence present. HTS could therefore be used in principle to detect adventitious agents whose presence is not even suspected. Given that

HTS determines the sequence of individual molecules it will also detect minority populations and polymorphisms so that revertants can be accurately quantitated. HTS has applications in the quality control of live vaccines and could reduce the need for in vivo testing by demonstrating consistency of production on a previously impossible scale.

The bioinformatic analysis required for HTS is significant and the validation of the method for a specific purpose remains a major issue. It would be possible to determine that the frequency of a particular mutation (single nucleotide polymorphism or SNP) varies from production run to production run. It remains to be determined to what degree this happens and what limits are allowed for the runs to be acceptable. In the context of OPV, HTS could be a replacement for MAPREC when used to monitor the frequency of one or two particular mutations and studies are underway to validate this application. Early evidence showed that HTS can accurately measure the 472-C content of type 3 OPV lots and that it could be an alternative to the MAPREC test (45, 46). Whole genome HTS could be a unique tool in determining product consistency. It has been applied very extensively to nOPV, where it is arguably a more sensitive procedure for monitoring product consistency than animal neurovirulence tests.

Significant developmental work needs to be completed before HTS can be introduced for general regulatory purposes. At its meeting in 2019, ECBS recommended that a study be performed to explore the utility of HTS technology for quality control of OPV made from Sabin strains. The resulting study showed that HTS could accurately quantify 472-C mutants in monovalent bulks of OPV3 and the final product (47). A second phase of this study showed that HTS could also accurately quantify mutations of 480-A/525-C and 481-G for OPV1 and OPV2, respectively (48). The results generated by HTS and MAPREC methods were very well correlated (47-49) so that HTS could in principle be used as an alternative to MAPREC, providing an appropriate test format and analytical process to establish assay validity and pass/fail decisions were agreed with the NRA.

HTS makes it possible to conduct whole-genome sequencing on a routine basis. The degree of sequence heterogeneity expressed in terms of the number of single nucleotide polymorphisms (SNP) at other nucleotide positions in the genome not necessarily linked to any tangible biological properties provides a unique molecular “fingerprint” characterizing a particular virus preparation. HTS is ideally suited for generating quantitative whole-genome SNP profiles of individual vaccine lots that can be used to identify types of polio seed virus and to monitor consistency of manufacture. After appropriate validation and establishing manufacture consistency, quantitative whole-genome SNP profiles of OPV lots it is possible that they could be used for routine lot release instead of MNVT or TgmNVT. If this is the case, appropriate acceptance criteria should be approved by the NRA.

The manufacturer of the final lot must be responsible for ensuring conformity with all the recommendations applicable to the final vaccine (Part A, sections A.5–A.11) even where manufacturing involves only the filling of final containers with vaccine obtained in bulk form from another manufacturing establishment. The manufacturer of the final lot must also be

responsible for any production and control tests performed by an external contract laboratory, if applicable, with the approval of the NRA.

OPV has been in worldwide use since the 1960s and experience has indicated that human diploid cells, primary monkey kidney cells, and continuous cell lines derived from them (Vero cells) can produce safe and effective vaccines.

In 1986, a WHO study group (7) stated that the risks for residual cellular DNA (rcDNA) for vaccines produced in continuous cell lines should be considered negligible for preparations given orally. This conclusion was based on the finding that polyoma virus DNA was not infectious when administered orally (50). For such products, the principal requirement is the elimination of potentially contaminating viruses. Additional data on the uptake of DNA via the oral route have been published (51). These studies demonstrated that the efficiency of uptake of DNA introduced orally was significantly lower than that of DNA introduced intramuscularly. Nevertheless, the specifics of the manufacturing process and the formulation of a given product should be considered by NRAs (22) and, where possible, data should be accumulated on the levels of rcDNA in OPV produced in Vero cells.

International reference materials

WHO International Standards and International Reference Preparations are available to ensure manufacture and quality control testing of the different versions of OPV meet appropriate regulatory requirements.

International Standards for the potency testing of tOPV have been available since 1995. More recently, new International Standards have been established for bOPV, mOPV1, mOPV2 and mOPV3, with compositions and potencies similar to vaccines needed for the final phase of the GPEI. Additionally, low titre monovalent type 1, 2 and 3 poliovirus WHO reference reagents are available for use in reference laboratories to measure the sensitivity of cell cultures for poliovirus infection.

An International Standard for anti-poliovirus types 1, 2 and 3 antibodies (human) is also available for the standardization of neutralizing antibody tests for poliovirus.

In addition, International Standards for MAPREC analysis of poliovirus types 1, 2 and 3 (Sabin) and International Reference Reagents for control of MAPREC assays of poliovirus type 1, 2 and 3 (Sabin) are available. Some of these references might be useful for HTS assays for Sabin OPV upon suitable validation. Alternatively, new reference materials might be needed for this purpose.

Reference preparations at the SO+2 passage level, designated WHO/I for type 1 virus, WHO/II for type 2 virus and WHO/III for type 3 virus, are available upon request through WHO. These reference preparations are for use in *in vivo* neurovirulence tests for OPV, both in monkeys and

transgenic mice. The relevant reference materials should be included in each vaccine test (see section A.4.4.7.2). Virus panels for validation and implementation of the transgenic mouse neurovirulence test, as specified in the WHO SOP, are also available.

New non-pathogenic, hyper-attenuated S19 strains of all three serotypes of poliovirus, are available for conducting neutralization assays. S19 strains are polioviruses that replicate in tissue culture but are unlikely to replicate at all in humans should they be exposed even to large amounts and for this reason they can be used outside GAPIII containment requirements.

Some of the references developed for Sabin OPV might be suitable for nOPV assays after suitable validation but establishment of nOPV-specific references might be required.

The above reference materials are available from the National Institute for Biological Standards and Control, Potters Bar, United Kingdom[1]. Full details of these materials, including literature references, are given in Appendix 8.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be poliomyelitis vaccine (oral, live, attenuated) with additions to indicate the virus serotype or serotypes of the vaccine and if the vaccine is a novel or Sabin OPV. The proper name should be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the recommendations formulated below.

A.1.2 Descriptive definition

Poliomyelitis vaccine (oral, live, attenuated) is a preparation of live-attenuated poliovirus type 1, 2 or 3 grown in in vitro cultures of suitable cells containing any one type or any combination of the three types of the Sabin strains or novel genetically stabilized attenuated strains, presented in a form suitable for oral administration and satisfying all the recommendations formulated in this document, as applicable.

A.2 General manufacturing recommendations

The general guidance provided in WHO Good manufacturing practices for pharmaceutical products: main principles (52) and WHO Good manufacturing practices for biological products (21) should apply to establishments where OPV is manufactured, with the addition of the following recommendations:

- The production of OPV should be conducted by staff who are healthy and who are examined medically at regular intervals. Steps should be taken to ensure that all

[1] https://www.nibsc.org/

persons in the production areas are immune to poliomyelitis. Personnel working in monkey quarters should also be examined for tuberculosis as outlined in Part A, section 2 of Recommendations to assure the quality, safety, and efficacy of BCG vaccine (53).

- The establishment should be in compliance with the current global recommendations for poliovirus containment (15-17, 54).

A.3 Control of source materials

General production precautions, as formulated in Good manufacturing practices for biological products (21) should apply to the manufacture of OPV, with the additional recommendation that during production only one type of cell should be introduced or handled in the production area at any given time.

A.3.1 Cell lines

Some of the licensed OPV products are produced in primary monkey kidney cells (PMKC) (see Part E). However, new OPV manufacturers are encouraged to use cell lines, such as MRC-5 and Vero cells (see section A.3.1), for vaccine production (22).

A.3.1.1 Master cell bank (MCB) and working cell bank (WCB)

The use of a cell line for the manufacture of OPVs should be based on the cell bank system. The cell seed and cell banks should conform with the Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (22). The cell bank should be approved by the NRA. The maximum number of passages (or population doublings) allowed between the cell seed, the MCB, the WCB and the production passage level should be established by the manufacturer and approved by the NRA. Additional tests may include but are not limited to: propagation of the MCB or WCB cells to or beyond the maximum in vitro age for production, and examination for the presence of retrovirus and tumorigenicity in an animal test system (22).

It is important to show that the cell banks (cell seed, MCB and WCB) are free of adventitious agents relevant to the species used in their derivation. Cell banks should be assessed for the absence of adventitious agents that may have been present during production.

> The WHO Vero reference cell bank 10-87 is considered suitable for use as a cell seed for generating an MCB (22) and is available to manufacturers on application to the Group Lead, Norms and Standards for Biologicals, Technical Specifications and Standards, Department of Health Product Policy and Standards, Access to Medicines and Health Products Division, World Health Organization, Geneva, Switzerland.

A.3.1.2 Identity test

Identity tests on the master (MCB) and working cell banks (WCB) should be performed in accordance with WHO's Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (22) and should be approved by the NRA.

The cell banks should be identified by means of tests such as biochemical tests, immunological tests, cytogenetic marker tests and DNA fingerprinting or sequencing (22). The tests should be approved by the NRA.

A.3.1.3 Cell culture medium

Serum used for the propagation of cells should be tested to demonstrate freedom from bacterial, fungal and mycoplasma contamination by appropriate tests- as specified in Part A, sections 5.2 (55) and 5.3 (56) of the WHO General requirements for the sterility of biological substances - as well as freedom from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of the WHO's Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (22).

Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera if approved by the NRA. As an additional monitor of quality, sera may be examined for freedom from bacteriophage and endotoxin. Gamma irradiation may be used to inactivate potential contaminant viruses, while recognizing that some viruses are relatively resistant to gamma irradiation.

The source(s) of animal components used in the culture medium should be approved by the NRA. These components should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (57).

Human serum should not be used. If human serum albumin derived from human plasma is used at any stage of product manufacture, the NRA should be consulted regarding the relevant requirements, as these may differ from country to country. At a minimum, it should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (58). In addition, human albumin and materials of animal origin should comply with current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (57).

Penicillin and other beta-lactams should not be used at any stage of manufacture, as they are highly sensitizing substances. If well justified, other antibiotics may be used during early stages of production, and should be cleared during the downstream manufacturing process. Clearance should be demonstrated through a residual removal study (or studies) and acceptable residual levels should be approved by the NRA (21).

Nontoxic pH indicators may be added (for example, phenol red at a concentration of 0.002%).

Only substances that have been approved by the NRA may be added.

Bovine or porcine trypsin used for preparing cell cultures should be tested and found to be free of cultivable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate (22). The methods used to ensure this should be approved by the NRA.

In some countries, irradiation is used to inactivate potential contaminant viruses in trypsin. If irradiation is used, it is important to ensure that a reproducible dose is delivered to all batches and to the component units of each batch. The irradiation dose must be low enough so that the biological properties of the reagents are retained while being high enough to reduce viral contamination. Therefore, irradiation cannot be considered a sterilizing process (22). The irradiation method should be validated by the manufacturer and approved by the NRA.

Recombinant trypsin is available and OPV manufacturers are encouraged to use the recombinant trypsin due to reduced risk of contamination compared to animal sourced trypsin; however, it should not be assumed to be free of the risk of contamination and should be subject to the usual considerations for any reagent of biological origin (22).

The source(s) of trypsin of bovine origin, if used, should be approved by the NRA and should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (57).

A.3.2 Virus seeds

A.3.2.1 Virus strains

Strains of poliovirus used in the production of OPV should be identified by historical records. This should include information on their origin and subsequent manipulation or passage, including steps of recombinant DNA technology used to modify viral genome when applicable.

In addition, it is recommended that the presence of sequence heterogeneities across the entire genome of OPV is determined by HTS and documented as a reference for future characterization of the virus seed lots.

Producers of Sabin OPV can obtain virus master seeds from WHO. Manufacturers receiving this virus master seed may prepare a sub-master seed by a single passage and then prepare their working seed from the characterized sub-master seed. At the time of writing this document the nOPV strains are only available from the developers.

Only virus strains that are approved by the NRA should be used (see General considerations).

A.3.2.2 Virus seed lot system

Vaccine production should be based on the seed lot system. Virus seed lots should not be purified. The virus working seed lot used for the production of vaccine batches should be prepared by a single passage from the virus master seed lot or the virus sub-master seed lot (if used), by a method and at a passage level from the original seed virus approved by the NRA.

Virus master, sub-master and working seed lots should be stored as recommended in WHO Good manufacturing practices for biological products (21), e.g. in temperature-monitored freezers at a temperature of ≤ −60 ºC that ensures stability on storage. Guidance on additional characterization of master and sub-master seeds is provided in Part B.

A.3.2.3 Tests on virus master, sub-master and working seed lots

The Sabin virus master seeds provided by WHO are well-characterized, and can be used to prepare sub-master seed using the approved process. All other virus seed lots used for the production of OPV batches, including sub-master seed derived from WHO master seed, should be subjected to the tests listed in this section and should conform to the recommendations set out in Part A, sections A.4.3 (single harvests) and A.4.4.1–A.4.4.4 (monovalent bulks). The control cell cultures for virus seeds production should conform to section A.4.1 (control of cell cultures).

A.3.2.3.1 Tests for adventitious viruses and freedom from detectable SV40 sequences

The virus seed lots should be shown to be free from detectable adventitious viruses and from detectable SV40 DNA when applicable as determined by a validated nucleic acid amplification test. The need for testing SV40 DNA, and other human, simian, bovine or porcine adventitious agents should be based on risk assessment of potential contamination of the cell substrates used to propagate the virus, as well as the adventitious agents that may be inadvertently introduced through the use of raw materials, e.g. animal-derived culture medium components. If necessary, viruses such as bovine polyomavirus, porcine parvovirus or porcine circovirus may be screened for using specific assays, such as molecular assays based on nucleic acid amplification techniques (NAT) (22).

> DNA of SV40 is widely used as molecular biological reagent, and contamination of PCR assays is potentially a major problem. One approach is to identify separate genomic regions of SV40 for amplification, and to use one region for screening purposes and the other for the confirmation of repeatedly positive samples. It is useful if the second genomic region used for confirmation varies between isolates from different sources, as it is then possible to show that it has a unique sequence and that positive results are not due to contamination with laboratory strains of SV40. The sensitivity of the PCR assays for the genomic regions used should be established.
>
> New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: (a) degenerate NAT for whole virus families, with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (b) NAT with random primers followed by analysis of the amplicons on large oligonucleotide micro-arrays of conserved viral sequencing, or digital subtraction of expressed sequences; and (c) HTS. These methods might be used in the future to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and with the approval of the NRA (22).

The testing strategy for adventitious virus(es) on seed lots should be based on risk assessment. However, sterility testing for bacteria, fungi and mycoplasmas should be conducted.

A.3.2.3.2 Tests to monitor virus molecular characteristics

A.3.2.3.2.1 Tests in vitro

New virus seed lots used for OPV production should be evaluated for molecular consistency using a suitable test, such as HTS, and should meet the acceptance criteria approved by the NRA. Virus seeds prepared from Sabin strain may be evaluated using MAPREC test and should meet the acceptance criteria described in section A.4.4.7.1. In addition, at least three consecutive monovalent bulks prepared from the new seed virus should meet the acceptance criteria of the applicable in vitro test described in section A.4.4.7.1. Where HTS method is used it should be validated using appropriate standards and materials, and acceptance criteria approved by the NRA. At this point the use of HTS remains a work in progress and is a subject of international collaborative study that may result in the establishment and availability of appropriate reference materials with defined acceptance criteria.

> The acceptance criteria for percentage of mutations at positions that are not examined by MAPREC but found to be variable under the conditions used by the manufacture should be based on the molecular characteristics of vaccine batches shown to be safe and immunogenic in clinical studies. The acceptance criteria of HTS should be updated periodically based on manufacturing experience. Acceptance criteria should be approved by the NRA.

nOPV seeds and at least three consecutive monovalent bulks prepared from each new working seed should be characterised by HTS, with particular attention to the regions of the genome that are modified in the parental nOPV strain compared to the Sabin OPV strain. The genetic modifications introduced in domain V of the 5'-untranslated region (UTR) of nOPV include changes in specific base pairs of the hairpin structure where GC and GU pairs were replaced by AU base pairs. Strengthening of the hairpin structure that leads to neurovirulent reversion requires two simultaneous mutations, and the frequency of such double reversions should be minimal. Therefore, HTS analysis should be conducted to ensure that there are no undesirable modifications in the 5'-UTR, with particular attention to changes in base-pairing in domain V.

A.3.2.3.2.2 Neurovirulence tests

A.3.2.3.2.2.1 Neurovirulence tests for virus seeds prepared from Sabin strain

New virus seeds prepared from Sabin strains, except the well characterized WHO master seed, should be evaluated for neurovirulence using MNVT or TgmNVT. Summaries of the MNVT and TgmNVT, including pass/fail criteria, are given in Appendix 2 along with considerations on the choice of assay. The test should be approved by the NRA for the specific product.

The test for neurovirulence in nonhuman primates should be carried out as summarized in Appendix 2 and following the SOP Neurovirulence test of types 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in monkeys, available from WHO.[1]

[1] Available on WHO website: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/poliomyelitis.

Under normal circumstances, a new virus working seed will be prepared using the same production protocol and from the same virus master seed or sub-master seed as the currently approved virus working seed. If the TgmNVT has been approved by the NRA for the release of vaccine batches, and if the virus working seed is generated by the same production process, the new seed can be qualified by use of the transgenic mouse test and supporting in vitro data alone. TgmNVT should be carried out as summarized in Appendix 2 and described in detail in the SOP Neurovirulence test of type 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus, available from WHO (see above).

In case there are any major changes in the production process for a new virus working seed or virus sub-master seed, full characterization using an in vivo neurovirulence test and HTS will be required (See Part B).

The neurovirulence of the virus working seeds and at least three consecutive monovalent bulks prepared from it should meet the criteria for acceptability given in section A.4.4.7.2 and the appropriate SOP before the virus working seed can be considered suitable for use for the production of OPV, with agreement of the NRA.

A.3.2.3.2.2.2 Neurovirulence tests for nOPV virus seeds

The virus seed lot used for nOPV production should be evaluated for neurovirulence. The testing strategy (e.g. MVS and/or WVS) and the selection of method (MNVT and/or TgmNVT) should be approved by the NRA. The in vivo neurovirulence test should be carried out as summarized in Appendix 2 and the applicable SOPs available from WHO. The current WHO reference preparation for MNVT derived from Sabin strain is suitable for evaluating neurovirulence of virus seeds and vaccine batches of nOPV.

It is likely that the molecular assays will be more sensitive than the animal tests used to justify the limits chosen. All nOPV producers should generate data in support of replacing in vivo neurovirulence tests with HTS for the evaluation of neurovirulence of nOPV seeds and vaccine batches by examining the entire genome. The acceptance criteria for percentage of mutations should be set in the first instance based on molecular characteristics of vaccine batches shown to be safe in clinical studies that have met the acceptance criteria when tested using an in vivo neurovirulence test. Specifications are likely to change with experience. The data generated will be used to demonstrate consistency and limits should be set on this basis in the longer term.

A.4 Control of vaccine production

Part E contains additional or alternative recommendations for OPV prepared in cultures of primary monkey kidney cells and concerns the testing of the cell substrate used for the production of the vaccine.

A.4.1 *Control cell cultures*

When human diploid or continuous cell lines are used to prepare cultures for the production of vaccine, a fraction equivalent to at least 5% of the total or 500 ml of cell suspension, or 100 million cells, at the concentration and cell passage level employed for seeding vaccine production cultures, should be used to prepare control cultures. (See Appendix 3 for an example of a flowsheet of tests in cell cultures).

If bioreactor technology is used, the NRA should determine the size and treatment of the cell sample to be examined.

A.4.1.1 Tests of control cell cultures

The treatment of the cells set aside as control material should be similar to that of the production cell cultures, but they should remain uninoculated for use as control cultures for the detection of adventitious agents.

These control cell cultures should be incubated under conditions as similar as possible to the inoculated cultures for at least two weeks and should be tested for the presence of adventitious agents as described below. For the test to be valid, not more than 20% of the control cell cultures should have been discarded for nonspecific, accidental reasons.

At the end of the observation period, the control cell cultures should be examined for evidence of degeneration caused by an adventitious agent. If this examination, or any of the tests specified in this section, shows evidence of the presence of any adventitious agent in the control culture, the poliovirus grown in the corresponding inoculated cultures should not be used for vaccine production.

If not tested immediately, samples should be stored at −60 °C or below.

A.4.1.2 Tests for haemadsorbing viruses

At the end of the observation period, at least 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter cells have been stored, the duration of storage should not have exceeded seven days and the storage temperature should have been in the range of 2–8 °C. In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

> Some NRAs require, as an additional test for haemadsorbing viruses, that other types of red cells, including cells from humans (blood group IV O), monkeys and chickens (or other avian species), should be used in addition to guinea-pig cells.

A reading should be taken after incubation at 2–8 °C for 30 minutes, and again after a further incubation for 30 minutes at 20–25 °C.

If a test with monkey red blood cells is performed, readings should also be taken after a final incubation for 30 minutes at 34–37 °C.

In some countries the sensitivity of each new lot of red blood cells is demonstrated by titration against a haemagglutinin antigen before use in the test for haemadsorbing viruses.

A.4.1.3 Tests for other adventitious agents in cell supernatant fluids

At the end of the observation period, a sample of the pooled supernatant fluid from each group of control cultures should be tested for adventitious agents. For this purpose, 10 ml of each pool should be tested in the same cells, but not the same batch of cells, as those used for the production of vaccine.

A second indicator cell line should be used to test an additional 10 ml sample of each pool. When a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line. When a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line (22).

The pooled fluid should be inoculated into culture vessels of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 part in 4. The area of the cell monolayer should be at least 3 cm^2 per ml of pooled fluid. At least one culture vessel of each kind of cell culture should remain uninoculated and should serve as a control.

The inoculated cultures should be incubated at a temperature of 35–37 °C and should be observed for a period of at least 14 days.

Some NRAs require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least an additional 14 days. Furthermore, some NRAs require that these cells should be tested for the presence of haemadsorbing viruses.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvests produced from the batch of cells from which the control cells were taken should be discarded.

Some selected viruses may be screened by using specific validated assays which are approved by the NRA, such as molecular techniques (e.g. nucleic acid amplification or HTS) (22).

If these tests are not performed immediately, the samples should be kept at a temperature of −60 °C or below.

A.4.1.4 Identity test

At the production level, the cells should be identified by means of tests approved by the NRA. Suitable methods include, but are not limited to, biochemical tests (e.g. isoenzyme analyses), immunological tests, cytogenetic tests (e.g. for chromosomal markers) and tests for genetic markers (e.g. DNA fingerprinting or sequencing).

A.4.2 Cell cultures for vaccine production

A.4.2.1 Observation of cultures for adventitious agents
On the day of inoculation with the virus working seed lot, each cell culture or a sample from each culture vessel should be examined visually for degeneration caused by infective agents. If such examination shows evidence of the presence of any adventitious agent the culture should not be used for vaccine production (see section A.4.1.3).

If animal serum is used for cell cultures before the inoculation of virus, the medium should be removed and replaced with serum-free maintenance medium after the cells have been washed with serum-free medium, if appropriate.

A.4.3 Control of single harvests

A.4.3.1 Single harvest
After inoculation of the production cells with the virus working seed lot, inoculated and control cell cultures should be held at a fixed temperature that is shown to be suitable, e.g. within the range 33–35 °C for Sabin strains.

The range required to produce a consistent satisfactory product for nOPV may be different and should be validated.

The temperature should be controlled within a narrow range (e.g. not vary by more than 0.5 °C from the set temperature). The optimal range for pH, multiplicity of infection, cell density, virus recovery and time of incubation should be established by each manufacturer and should be approved by the NRA.

The incubation time of the viral culture should be established and validated for each OPV product during product development. The virus suspension of Sabin strain should be harvested not later than four days after virus inoculation to limit the number of replication cycles.

Similar appropriate limits should be investigated and set for nOPV harvests.

The inoculated cell cultures should be processed in such a manner that each virus suspension harvested remains identifiable as a single harvest and is kept separate from other harvests until the results of all the tests described in Part A sections A.4.1.2, A.4.1.3, A.4.1.4, A.4.3.3.1, A.4.3.3.2, A.4.3.3.3, A.4.3.3.4 and A.4.3.3.5 have been obtained.

A 4.3.2 Sampling

Samples required for the testing of single harvests should be taken immediately on harvesting. If the tests for adventitious agents as described in Part A, section A.4.3.3.3 are not performed immediately, the samples taken for these tests should be kept at a temperature of −60 °C or lower and subjected to no more than one freeze–thaw cycle.

A.4.3.3 Tests on single harvest

A.4.3.3.1 Identity

Each single harvest should be identified using a suitable method, such as an immunological assay on cell culture using specific antibodies or by a molecular method which has been validated and approved by the NRA. If the virus seeds used for production and other poliovirus strains are manipulated or stored at the same production facilities, the identity test should have the ability to distinguish between these strains as well as the ability to distinguish different serotypes of poliovirus.

> Neutralization tests can distinguish the serotypes of poliovirus. Molecular method such as sequencing, HTS, or qPCR, can distinguish different strains and serotypes of poliovirus.
>
> Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be useful in this test.

A.4.3.3.2 Titration for virus content

The virus titre per ml of single harvest should be determined in cell cultures in comparison with an existing reference preparation (see Appendix 4).

A.4.3.3.3 Tests of neutralized single harvests for adventitious agents

For the purposes of the recommendations set out in this section of Part A, the volume of each single harvest taken for neutralization and testing should be at least 10 ml and should be such that a total of at least 50 ml or the equivalent of 500 doses of final vaccine, whichever is the greater, has been withheld from the corresponding single harvest.

The antisera used for neutralization should be of nonhuman origin and should have been prepared in animals other than monkeys, using virus cultured in cells from a species different from that used in the production of the vaccine. Samples of each virus harvest should be tested in human cells and at least one other sensitive cell system.

The neutralized suspensions should be inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of neutralized suspension. At least one bottle of each kind of cell culture should remain uninoculated and should serve as a control; it should be maintained using nutrient medium containing the same concentration of the specific antiserum used for neutralization.

Animal serum may be used in the propagation of the cells, but the maintenance medium used after inoculation of the test material should contain no added serum other than the poliovirus neutralizing antiserum or foetal calf serum of controlled origin.

The inoculated cultures should be incubated at a temperature of 35-37 °C and should be observed for a period of at least 14 days.

If adequately justified and validated, lower temperatures may be used.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvest should be discarded.

New molecular methods with broad detection capabilities are being developed for detection of adventitious agents. These methods include degenerate NAT for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; NAT with random primers followed by analysis of the amplicons on large oligonucleotide micro-arrays of conserved viral sequencing or digital subtraction of expressed sequences; and high throughput sequencing. These methods might be used to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and approval of the NRA (22).

A.4.3.3.4 Sterility tests for bacteria, fungi and mycoplasmas

A volume of at least 10 ml of each single harvest should be tested for bacterial, fungal, and mycoplasmal contamination by appropriate tests, as specified in Part A, sections 5.2 and 5.3 of the WHO General requirements for the sterility of biological substances (55, 56*),* or by methods approved by the NRA.

Molecular assays, e.g. NAT alone or in combination with cell culture, may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and agreement from the NRA (22).

A.4.3.3.5 Test for mycobacteria

The virus harvest should be shown to be free from mycobacteria by an appropriate method approved by the NRA.

Molecular assays (e.g. NAT) may be used as an alternative to mycobacteria microbiological culture method tests for the detection of mycobacteria following suitable validation and agreement from the NRA (22).

Some manufacturers test for mycobacteria only at the monovalent bulk stage with the agreement of the NRA

A.4.3.3.6 Tests for molecular consistency of production

OPV producers may monitor the molecular characteristics of single harvests or monovalent bulks using an in vitro test as described in A.3.2.3.2.1. These data may further demonstrate manufacturing consistency.

A.4.4 Control of monovalent bulk

A.4.4.1 Preparation of monovalent bulk

The monovalent bulk may be prepared by pooling a number of single harvests of the same virus serotype into a single vessel. This bulk should be filtered through a filter that is able to retain cell debris.

The NRA may require further purification of harvests derived from continuous cell lines. If the harvests are derived from human diploid or monkey kidney cells, further purification is not required.

A.4.4.2 Sampling

Samples of the monovalent bulk prepared as described in section A.4.4.1 should be taken immediately and, if not tested immediately, should be kept at a temperature of −60 °C or below until the tests described in the following sections are performed.

A.4.4.3 Identity test

Each monovalent bulk should be identified using a suitable method, as described in Section A.4.3.3.1.

A.4.4.4 Titration for virus content

The virus titre per ml of filtered monovalent bulk should be determined in cell cultures in comparison with an existing reference preparation (see Appendix 4).

The virus titre as determined by this test should be the basis for the quantity of virus used in the neurovirulence tests in monkeys or in transgenic mice (see Part A, section A.4.4.7.2) and for formulation of the final bulk (see Part A, section A.4.5).

The detailed procedures for carrying out this test and for interpreting the results should be approved by the NRA.

A.4.4.5 Sterility tests for bacteria and fungi

The final vaccine bulk should be tested for bacterial and fungal sterility as specified in Part A, section 5.2 of the General requirements for the sterility of biological substances (55).

A.4.4.6 Test for mycobacteria

The virus harvest should be shown to be free from mycobacteria by an appropriate method approved by the NRA.

Molecular assays (e.g. NAT) may be used as an alternative to mycobacteria microbiological culture method tests for the detection of mycobacteria after suitable validation and agreement from NRA (34).

A.4.4.7 Tests to monitor virus molecular characteristics (consistency)

The poliovirus in the filtered monovalent bulk, prepared as described in section A.4.4.1, should be tested in comparison with the seed lot or a reference virus preparation (see Part A, section A.1.3) to ensure that the vaccine virus has not undergone changes during its multiplication in the production cell culture.

A.4.4.7.1 Tests in vitro

The virus in the monovalent bulk should be tested by at least one in vitro test as described in Section A.3.2.3.2.1.

A.4.4.7.1.1 MAPREC

The MAPREC assay is suitable for all three serotypes of Sabin OPV, but not nOPV which should be evaluated for molecular consistency using a suitable test, such as whole genome HTS. Implementation of the assay should be fully validated by each manufacturer and performed according to the WHO SOP Mutant analysis by PCR and restriction enzyme cleavage (MAPREC) for oral poliovirus (Sabin) vaccine, developed from WHO collaborative studies and available from WHO,[1] or according to a validated alternative procedure.

The MAPREC assay should be used to establish the consistency of production once the test has been validated and normal values for the standards have been established. For all Sabin OPV preparations, and depending on a laboratory's experience with the MAPREC test, an approach using "warning limits" of ±2 standard deviations and "rejection limits" of ±3 standard deviations from the historical mean may be appropriate. Acceptance and rejection criteria should be specific to each manufacturer and each working seed and should be continually updated as each new bulk is prepared. An investigation of consistency should take place if a batch gives results that are inconsistent with previous production batches.

Results should be expressed as ratios relative to the relevant type-specific International Standard for MAPREC analysis of poliovirus (Sabin). The acceptable variation of mutant content from batch to batch should be agreed with the NRA in the light of production and testing experience.

For type 3 (472-C), a batch should be rejected if the level of mutations is above 1.0% when normalized against the International Standard. The limits for types 1 and 2 should be approved by the NRA.

Levels of mutations obtained by manufacturers who have implemented the test for types 1 and 2 virus have been less than 2.0% for type 1 Sabin (for the sum of both mutations 480-A, 525-C) and 1.5% for type 2 Sabin (481-G) (59).

[1] Available on WHO website: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/poliomyelitis.

If a filtered monovalent bulk fails in a MAPREC assay, it cannot be used in the manufacturing of finished product, and an evaluation of the manufacturing process, including the suitability of the virus working seed, should be undertaken and discussed with the NRA. Filtered monovalent bulks that pass the MAPREC assay should be tested subsequently for in vivo neurovirulence.

The assay for type 3 is highly predictive of in vivo neurovirulence in animal models. No such correlation exists for types 1 and 2 at the level of revertants present in vaccine bulks. For these types the assay results provide a measure of consistency (59).

Non-radioactive methods for MAPREC are available and may be introduced after validation and approval by the NRA.

A.4.4.7.1.2 HTS

Alternative molecular biology methods, such as HTS, that demonstrate an equivalent or better level of discrimination may be used after validation and approval by the NRA. The MAPREC reference materials might be useful for HTS assays for Sabin OPV upon suitable validation. Alternatively, new reference materials might be needed for this purpose.

A.4.4.7.2 Neurovirulence tests

A.4.4.7.2.1 Neurovirulence tests for Sabin OPV

An appropriate *in vivo* test should be used to evaluate virus monovalent bulks. Summaries of the MNVT and TgmNVT, including pass and fail criteria, are given in Appendix 2 along with considerations on the choice of assay.

The test should be approved by the NRA for the specific product and may use transgenic mice or nonhuman primates or both. The test for neurovirulence in nonhuman primates should be carried out as summarized in Appendix 2 and described in the SOP Neurovirulence test of types 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in monkeys, available from WHO.

Where the TgmNVT has been approved by the NRA, it should be carried out as summarized in Appendix 2 and described in detail in the SOP Neurovirulence test of type 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus, available from WHO (see above). Its use for batch release purposes should follow the appropriate validation and implementation processes according to national and international regulations. This SOP has been validated for vaccines made from Behringwerke SO-derived seeds (types 1 and 2) and RSO-derived seeds (type 3).

To qualify as competent to perform the TgmNVT, there is a requirement for laboratories to complete a standard implementation process as detailed in the WHO SOP. Once qualified as competent, each laboratory should continue to monitor its performance on a routine basis.

The WHO collaborative study demonstrated that MNVT and TgmNVT are equivalent for testing vaccines prepared from RSO seeds but that TgmNVT may fail otherwise acceptable (by MNVT) lots prepared from derivative strains containing additional mutations (40). Therefore, TgmNVT

can be used as a replacement of MNVT for vaccines made from RSO Sabin 3 strain, while the TgmNVT may require further validation for other derivative strains. This may include the development of an appropriate homologous reference.

It is possible that the in vivo neurovirulence test could be omitted in the future when manufacturing consistency has been established based on the results of both in vivo and whole genome HTS. However, additional experience and data are required to establish suitable acceptance criteria for whole genome HTS performed for the control of Sabin OPV.

A.4.4.7.2.2 Neurovirulence tests for nOPV

Where the results of manufacturing, preclinical and clinical studies have demonstrated the genetic stability of the attenuation to the satisfaction of the NRA, the in vivo MNVT may be omitted for routine manufacturing control of nOPV with the agreement of NRA.

Only monovalent bulks that meet the acceptance criteria using a validated HTS are used to formulate the final product.

The acceptance criteria for percentage of mutations at positions found to be variable under the conditions used by the manufacture should be based on the molecular characteristics of vaccine batches shown to be safe and immunogenic in clinical studies, or vaccine batches that have met the acceptance criteria of an in vivo NVT. When mutations arise at additional positions, a risk assessment should be performed to assess the potential impact on neurovirulence based on current understanding of the genetic basis for the attenuation (60, 61). An in vivo NVT should be performed to assess the suitability of the monovalent bulk when required by the risk assessment. The acceptance criteria of HTS should be updated periodically based on manufacturing experience, and approved by the NRA.

A.4.5 Final bulk

Final bulk may contain one or more serotypes of poliovirus of the same type of strain, Sabin or nOPV. The operations necessary for preparing the final bulk should be conducted in such a manner as to avoid contamination of the product.

> The dilution and mixing procedures involved in preparing the final vaccine bulk should be approved by the NRA.

A.4.5.1 Stabilizers

Any stabilizers that may be included in the final bulk should have been shown, to the satisfaction of the NRA, to improve the stability of the vaccine in the concentrations used and not to impair the safety of the vaccine.

All the tests described in Part A, sections A.4.3.3 and A.4.4, should be performed on samples taken before any stabilizers are added where possible.

A.4.5.2 Sterility tests for bacteria and fungi
The final vaccine bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the General requirements for the sterility of biological substances (55).

A.5 Filling and containers

The requirements concerning filling and containers given in WHO good manufacturing practices for pharmaceutical products: main principles (35) and WHO good manufacturing practices for biological products (21) should apply to OPV filled in the final form. Single- and multi-dose containers may be used.

A final filtration may be included just before the filling operations.

The conditions for storage and shipping, as well as the shelf-life should be supported by adequate stability data and approved by the NRA.

A.6 Control tests on the final lot

Samples should be taken from each final lot for the tests described in the following sections. The following tests should be performed on each final lot of vaccine (that is, in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers from each final lot by means of validated methods approved by the NRA. In general, the specification for each test of OPV final lot should be supported by quality attributes of the clinical lots shown to be safe and sufficiently immunogenic in clinical studies and should be approved by the NRA.

A.6.1 Inspection of final containers

Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities should be discarded and recorded for each relevant abnormality.

A.6.1.1 Appearance
The appearance of the vaccine should meet the specification approved by the NRA with respect to its form and colour.

A.6.2 Extractable volume

Unless otherwise justified and authorized, the extractable volume (ml) and the number of drops, using the approved dropper, should be determined in a minimum of five individual final containers, and should meet the specification approved by the NRA.

A.6.3 pH

The pH of the final lot should be tested in a pool of final containers and the result should be within the range shown to be adequate for preserving virus stability.

A.6.4 Identity

An identity test should be performed on at least one labelled container from each final lot using a suitable method as described in Section A.4.3.3.1.

A.6.5 Sterility tests for bacteria and fungi

Each final lot should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the General requirements for the sterility of biological substances (55), or by methods approved by the NRA.

A.6.6 Potency

At least three final containers should be selected at random from each final lot and should be individually tested in a single assay. When the vaccine contains more than one poliovirus type, each type should be titrated separately by using appropriate type-specific antiserum to neutralize each of the other types present. The poliovirus content of each serotype, and the total virus content, should be determined. The assay should include a reference preparation as described in Appendix 4 of these recommendations. The minimum virus titre per human dose should be shown to induce adequate immune response in clinical studies.

> An upper limit may be established by each manufacturer to ensure lot-to-lot consistency (e.g. based on mean titre $CCID_{50}$ +3 standard deviations). The upper limit should be approved by the NRA.
>
> Based on available data, it is recommended that the estimated mean virus titres for a single human dose of tOPV prepared from Sabin strain should be not less than $10^{6.0}$ $CCID_{50}$ for type 1, $10^{5.0}$ $CCID_{50}$ for type 2, and $10^{5.5}$ $CCID_{50}$ for type 3, as determined in an assay described in Appendix 4. The 95% confidence intervals of the assays should not differ by a factor of more than 0.3 $\log_{10}$ of the estimated number of infectious units in the vaccine. Different potency limit may be acceptable if supported by clinical data.
>
> In 1986 the WHO Region of the Americas began to use a trivalent formulation with $10^{5.8}$ $CCID_{50}$ of poliovirus type 3 (62), following a study in Brazil which demonstrated improved immunogenicity when the amount of type 3 virus in the trivalent vaccine was increased (63). The subsequent success in controlling poliomyelitis in the Americas using this formulation led the EPI Global Advisory Group to recommend a formulation of trivalent OPV with $10^{6.0}$, $10^{5.0}$, $10^{5.8}$ $CCID_{50}$ per dose for types 1, 2 and 3 respectively, on a global basis (34, 64).

The potency specifications for nOPV should be set based on the potency of clinical lots shown to induce adequate protective immunity in clinical trials plus human immunogenicity data. An upper limit should also be defined based on available human safety data.

A.6.7 Thermal stability

Thermal stability should be considered as a vaccine characteristic that provides an indicator of consistency of production. The thermal stability test is not designed to provide a predictive value of real-time stability but to evaluate whether the product complies with a defined specification.

Additional guidance on the evaluation of vaccine stability is provided in WHO's Guidelines on stability evaluation of vaccines (65).

Three final containers of each final lot should be incubated at 37 °C for 48 hours. The total virus content in both exposed and unexposed containers should be determined concurrently with that of a suitable validated reference preparation. The loss of potency on exposure should be within the limit approved by the NRA.

For trivalent vaccine prepared from Sabin strain, the vaccine passes the test when the loss on exposure is not greater than a factor of 0.5 $\log_{10}$ $CCID_{50}$ per human dose. Several OPV manufacturers have recently demonstrated that the thermal stability test specification applied to tOPV formulations (loss on exposure is not greater than a factor of 0.5 $\log_{10}$ $CCID_{50}$ per human dose) is not applicable to some monovalent and bivalent OPVs. Some manufacturers have shown that mOPV formulations that failed the current specification of 0.5 $\log_{10}$ have an acceptable stability profile throughout the product shelf-life. Therefore, a specification of 0.6 $\log_{10}$ has been accepted by the NRAs and by the WHO Prequalification Programme on the basis of documented evidence that the mOPV1 was stable over two years when stored at −20 °C or below and six months when stored at 2–8 °C.

Suitable thermal stability test for nOPV should be established and validated.

A.6.8 Residual antibiotics (if applicable)

If any antibiotics are added during vaccine production, the content of the residual antibiotics should be determined and should be within limits approved by the NRA. This test may be omitted for routine lot release once consistency of production has been established to the satisfaction of the NRA.

A.6.9 Stabilizer (if applicable)

If a stabilizer is added during vaccine production, the content of the stabilizer should be determined and should be within limits approved by the NRA.

A.7 Records

The recommendations given in section 17 of Good manufacturing practices for biological products (21) should apply.

A.8 Retained samples

The requirements given in section 16 of Good manufacturing practices for biological products (21) should apply.

A.9 Labelling

The requirements given in section 14 of Good manufacturing practices for biological products (21) should apply.

The label on the carton, the container or the leaflet accompanying each container should include the following information:

- the designation(s) of the strain(s) of poliovirus contained in the vaccine;
- the minimum amount of virus of each type contained in one recommended human dose;
- the cell substrate used for the preparation of the vaccine, and the nature and amount of any stabilizer present in the vaccine;
- a statement that the vaccine is not to be injected;
- the number of doses in each vial;
- the volume of the dose.

It is desirable for the label to carry the names both of the producer and of the source of the bulk material if the producer of the final vaccine did not prepare it. The nature and amount of the antibiotics present in the vaccine, if any, may be included.

A.10 Distribution and shipping

The requirements given in WHO Good manufacturing practices for biological products (21) should apply. Further guidance is provided in WHO's Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (66).

A.11 Stability testing, storage and expiry date

A.11.1 Stability testing

Adequate stability studies form an essential part of vaccine development. These studies should follow the general principles outlined in the WHO Guidelines on stability evaluation of vaccines (65) and WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (67). The shelf-life of the final product and the hold time of each process intermediate (such as single harvests, monovalent bulk, final bulk) should be established based on the results of real-time, real-condition stability studies, and approved by the NRA.

The stability of the vaccine in its final container, maintained at the recommended storage temperatures up to the expiry date, should be demonstrated to the satisfaction of the NRA on at least three consecutive lots of final product. Accelerated thermal stability tests may be undertaken to provide additional information on the overall characteristics of the vaccine and may also aid in assessing comparability should the manufacturer decide to change any aspect of manufacturing.

The formulation of the vaccine should be shown to minimize potency loss throughout its shelf-life. In case of potency loss (e.g., when stored at 2-8°C for 6 months), the manufacturer should implement a higher potency limit at release to ensure that all vaccine lots released will meet the minimum potency specification at the end of shelf-life as described in WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (67). Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing

monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (65). The on-going stability testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection of stability data, and criteria for the rejection of vaccine(s). Data should be provided to the NRA in accordance with local regulatory requirements.

Where the vaccine is to be stockpiled, manufacturers should conduct real-time stability studies on monovalent bulks at –40 °C or below, or on finished monovalent, bivalent and trivalent composition at –20 °C.

Any extension of the shelf-life should be based on stability data and approved by the NRA.

A.11.2 Storage conditions

Before being released by the manufacturing establishment, all vaccines in final containers should be kept continuously at a temperature that minimizes potency loss, e.g., in the frozen state at a temperature below –20 °C.

To facilitate vaccine distribution, OPV may be stored at a higher temperature for a specified period during shipping and distribution in the field, such as at 2–8ºC for six months. In addition, during manufacturing, shipment or in the field, the vaccine may be thawed and refrozen. Manufacturers should conduct real-time and real-condition stability studies to support the storage conditions at different temperatures as well as the number of freeze-thaw cycles. The stability data should demonstrate that the vaccine conforms to the requirements of potency until the expiry date stated on the label, as approved by the NRA.

A.11.3 Expiry date

The expiry date should be based on the shelf-life as supported by the stability studies and approved by the NRA. The start of the dating period should be specified, e.g. based on the date of filling or the date of the first valid potency test on the final lot, and should be approved by the NRA.

Part B. Nonclinical evaluation of poliomyelitis vaccines (oral, live, attenuated)

The nonclinical evaluation of candidate poliomyelitis vaccines (oral, live, attenuated) should be based on the principles outlined in the WHO guidelines on nonclinical evaluation of vaccines (19) which provide details on the design, conduct, analysis, and evaluation of nonclinical studies. In principle, all changes made to a product post-approval should follow the requirements listed in the WHO general Guidelines (68). The following specific issues should be considered in addition to the tests described in section A.3.2.3 in the context of a change in virus seed.

B.1 Characterization of a new Sabin virus sub-master seed

In the event that a new Sabin virus sub-master seed is prepared by a single passage from a well characterized master seed, including the WHO master seed, it should be subjected to extensive characterization which should include evaluation of the virus working seeds and at least three monovalent bulks derived from it, as described in section A.4.4.7. Characterization studies must include the evaluation of identity by complete nucleotide sequencing to prove that the new sub-master seed consensus sequence is identical to conventional Sabin master seeds and that the mutational composition (e.g., in MAPREC) is consistent. HTS should be undertaken to evaluate heterogeneity of the virus sequence. These approaches have not yet been formally validated, other than the MAPREC tests for base positions in the 5' non-coding region of type 3 OPV, as described in section A.4.4.7.1.1. A new virus sub-master seed should be tested for neurovirulence using the MNVT or the TgmNVT, subject to the approval of NRA. Summaries of the MNVT and TgmNVT are given in Appendix 2 along with considerations on the choice of assay.

B.2 Characterization of virus seeds for the production of nOPV

Virus strains used for the production of nOPV were constructed using recombinant DNA technology and are genetically stabilized attenuated strains designed based on current knowledge concerning the molecular mechanisms of attenuation and reversion of poliovirus. The virus master, sub-master (if applicable) and working seed lots used to manufacture a candidate nOPV should be subjected to extensive characterization as described in section A.3.2.3. In addition, genetic stability of the strains used for nOPV production should be confirmed at least at the passage level (or beyond) used to prepare the vaccine using a molecular method approved by the NRA, such as whole-genome HTS analysis.

B.3 Evaluation of immunogenicity of nOPV in suitable models

The genome of nOPV production strains are rationally designed to stabilize the attenuation. However, variation can arise in the viral genome of the nOPV production strains on passage in cell cultures. Whether these genome changes (introduced or cumulated) have any impact on the immunogenicity of the candidate nOPV should be studied using suitable methods, such as the antigenicity and/or the ability to grow in in vitro cell culture of the production strain. If required based on the outcomes of the in vitro testing, transgenic mice with interferon-receptor knock-out and expression of human poliovirus receptor are also available to study vaccine induced neutralizing antibodies. Proof-of-concept nonclinical studies based on type-specific serum neutralizing antibody titres may also assist in the selection of doses to be tested in the dose-finding studies in humans.

Part C. Clinical evaluation of poliomyelitis vaccines (oral, live, attenuated)

Clinical trials should adhere to the principles described in WHO's Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (68) and Guidelines on clinical evaluation of vaccines: regulatory expectations (20). All clinical trials should be approved by the relevant NRAs.

Some of the issues that are specific to the clinical evaluation of OPVs are discussed in the following sections. These sections should be read in conjunction with the general guidance mentioned above. It is also recommended that manufacturers should consult with relevant NRAs regarding the overall clinical development programme.

Part C considers the provision of clinical data required for:

- nOPV prepared from genetically stabilized attenuated strains;
- new formulations based on licensed OPVs that are derived from Sabin poliovirus strains, including monovalent, bivalent, and trivalent vaccines;
- situations where there have been major changes to the manufacturing process of an established vaccine (e.g., changing from primary monkey kidney cells to a cell line).

Clinical evaluation is not required for a vaccine manufactured using a new virus working seed lot, provided that the passage level is not more than one from the master/sub-master seed lot, the working seed has been characterized, and consistency of the manufacturing process has been demonstrated (see sections A.3.2.3). Generating a new sub-master seed requires extensive characterization but not clinical trials (see Part B).

Vaccine formulations containing one or two poliovirus serotypes have been licensed based on clinical trials in endemic countries. The results of clinical trials in Egypt and northern India have demonstrated that the efficacy of mOPV1 is superior to that of trivalent OPV in terms of inducing immunity against poliovirus type 1 (34, 70). Health authorities have recommended widespread use of this vaccine to eliminate poliovirus type 1 transmission in India. In addition, studies on bOPV containing type 1 and type 3 have demonstrated that bOPV is noninferior to mOPV1 and mOPV3 individually, and superior to tOPV.

C.1 General considerations

The Global Polio Eradication Initiative was prompted by World Health Assembly resolution WHA41.28in 1988, and has led to a dramatic decrease in poliomyelitis cases globally (*26*). As a result, efficacy studies for poliovirus vaccines are not feasible, and clinical evaluations and seroprevalence studies should compare the safety and immunogenicity of candidate vaccines with a licensed vaccine (comparator vaccine). The assessment of seroconversion should be based on the elicitation of neutralizing antibodies, which are the basis of protection (*26*). The approval of a candidate OPV should be based on a clear demonstration of noninferiority compared with a licensed OPV or an OPV used under WHO EUL, as described in section C.2.2 of this document. The relative risk of VAPP for a new candidate vaccine versus approved vaccines cannot be estimated from pre-approval studies but should be addressed as part of post-marketing surveillance. The genetic stability of the novel strain should be monitored.

C.2 Safety and immunogenicity studies

C.2.1 Assessment of the immune response

The presence of neutralizing antibodies against polioviruses is considered a reliable correlate of protection against poliomyelitis. However, immunity induced by one serotype does not provide protection against the other two serotypes. A serum neutralizing antibody titre of ≥ 8 is considered to be a marker of clinical protection against poliomyelitis (71). The demonstration of an immune response to OPV vaccination should be based on the measurement of neutralizing antibody titres at pre- and post-vaccination time points. Seroconversion for polio antigen is defined as:

- for subjects seronegative at the pre-vaccination time point, postvaccination antibody titres of ≥ 8;
- for subjects seropositive at the pre-vaccination time point, a ≥ 4-fold rise in post-vaccination antibody titres. If the pre-vaccination titre is due to maternal antibodies, a 4-fold rise above the expected titre of maternal antibodies based on the pre-vaccination titre declining with a half-life of 28 days indicates seroconversion, or post-vaccination antibody titres of ≥ 8, whichever is higher.

The assay used to assess serum neutralizing antibodies in the clinical samples should follow the key parameters described in the WHO Manual for the virological investigation of poliomyelitis (72), with the exception of the challenge poliovirus strains. OPV developers are encouraged to use genetically modified poliovirus strains that can be manipulated outside of containment facilities (for example, S19 strains) as challenge viruses. The level of neutralizing antibody present in a serum sample is expressed as a titre, which is the reciprocal of the highest serum dilution that inhibits the viral cytopathic effect in 50% of cell cultures. A reference serum calibrated against or traceable to the appropriate International Standard (see section A.1.3 of this document) should be used to control assay performance.

Geometric mean titres (GMTs), seroconversion rates and reverse cumulative distributions should be provided.

C.2.2 Immunogenicity studies

New candidate OPVs manufactured using different vaccine compositions (e.g. monovalent, bivalent, or trivalent) or from genetically stabilized attenuated strains should be compared with a licensed OPV or an OPV used under WHO EUL. The comparator vaccine(s) selected should have been in use for some years so that some data on effectiveness are available in addition to a reliable description of the safety profile. When no licensed type-matched OPV is available for use in clinical trials, one or more licensed OPV (or nOPV used under WHO EUL) may be used as comparator (s) to cover all serotypes included in the candidate vaccine. For example, a tOPV candidate may be compared to two suitable comparators, one bivalent and the other monovalent, in a noninferiority immunogenicity study. In this case, any potential impact on immunogenicity outcomes (e.g. a negative immune interference) due to different compositions/serotypes between the comparators and candidate vaccines should be considered in study design. Further details on

selection of comparators are described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20), and the study design should be discussed with and approved by the NRA.

C.2.3 Population

The immunogenicity data provided to support the licensure of a candidate OPV as primary series should include data generated in a naive target population, such as infants. The evaluation of new OPV formulations prepared from Sabin strains may be conducted directly in infants and newborns since safety profiles in these populations have already been established. However, the first clinical study (Phase I) of a candidate nOPV should be performed in healthy adults to assess vaccine safety.

The study exclusion criteria should reflect the current contraindications to administration of OPVs.

C.2.4 Endpoints and analyses

The clinical study protocol should state the primary objective(s) of the study. The neutralizing antibody response to the candidate vaccine should be demonstrated to be non-inferior versus an appropriate licensed OPV, an OPV used under WHO EUL (as described in C.2.2) based primarily on GMTs and/or seroconversion rates. The primary endpoint should be selected according to the study population and the anticipated immune response. For example, very high seroprevalence rates are expected in highly immunized populations, thus having implications for the selection of the non-inferiority margin and therefore the sample size calculation. Further details on demonstrating non-inferiority are described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20).

Other immunological parameters should be compared in planned secondary analyses (e.g. percentages reaching predefined titres).

C.2.5 Dose-ranging studies

At the time of preparation of this document, all licensed Sabin OPV formulations (e.g. monovalent, bivalent and trivalent) contained the recommended dose for each poliovirus type (not less than $10^{6.0}$ $CCID_{50}$ for type 1, $10^{5.0}$ $CCID_{50}$ for type 2, and $10^{5.5}$ $CCID_{50}$ for type 3). However, development of nOPV or novel formulations with improved stability (addition of stabilizers/excipients) or immunogenicity (used in combination with an adjuvant) may require dose-ranging studies to determine the minimum dose of virus required in $CCID_{50}$ to provide adequate immune responses (*20*). These data could also be used to support the minimum viral titre that should be present in the vaccine at the end of its shelf-life.

C.2.6 Vaccine virus shedding and transmission

Changes in the viral genome of nOPV candidates or changes in vaccine composition may impact virus replication in the intestinal tract and may influence the ability to induce immune responses and the potential for VAPP or spread to non-target populations. Manufacturers should undertake

studies to determine the profile of the vaccine virus (if applicable, by serotype) excreted in the stools of vaccinees and the duration of shedding. Virus excretion of nOPV candidates or new vaccine formulations should be evaluated alongside a licensed OPV product or an OPV product used under WHO EUL (*34*). For nOPV, virus recovered from stool samples collected from the vaccinees should be evaluated by HTS to verify the genetic stability of the vaccine candidate. Regions including key attenuating mutations should be examined, but drift in the whole genome should monitored.

C.2.7 Challenge studies with attenuated Sabin poliovirus

Induction of mucosal immunity by the candidate and the comparator vaccines should be determined by the assessment of virus excretion after the administration of a challenge dose of OPV, such as nOPV. Excretion of poliovirus in stool specimens is determined at various intervals immediately before the challenge (day 0) and on days 7, 14, 21 and 28 thereafter (70).

C.2.8 Concomitant administration with other vaccines

An evaluation of the effects of co-administration of an OPV with other vaccines should be considered, taking into account which vaccines are most likely to be given concomitantly in different age groups and populations.

When OPVs are used in an EPI programme simultaneously with other vaccines, it is particularly important that the effects of co-administration should be evaluated (e.g. co-administration studies with rotavirus vaccines which are also administered by the oral route).

Immune responses to all other antigens co-administered with the new OPV should be measured at least in subsets. While the study will usually be powered only to demonstrate non-inferiority with respect to neutralizing antibody against the different poliovirus types used in the vaccine, the protocols should at least include planned secondary analyses of antigen-specific responses. If these analyses indicate that immune responses are lower on co-administration with a new OPV compared to the licensed vaccine(s), NRAs will need to consider the potential clinical consequences on a case-by-case basis.

C.2.9 Pre-licensure safety data

The general approach to the assessment of safety of a new OPV during clinical studies should be in accordance with WHO's Guidelines on clinical evaluation of vaccines: regulatory expectations (20). Planned safety studies should be supported by a clear scientific rationale. Given the long history of the use of vaccines based on Sabin strains, the NRA may decide that additional pre-licensure safety studies are not required. Where a new vaccine formulation, which has not been used previously, is investigated, larger-scale studies will be needed.

An appropriate pharmacovigilance plan should be developed and approved by the NRA prior to licensure.

C.3 Post-marketing studies and surveillance

Enhanced safety surveillance, particularly for detection of VAPP, should be undertaken during the initial post-approval years in collaboration with NRAs. Environmental surveillance should be conducted. The risk and benefit of using monovalent and bivalent OPV derived from Sabin strains should be carefully considered, as this practice in areas with sub-optimal polio vaccine coverage may lead to the emergence of circulating cVDPVs. Manufacturers and health authorities should work in collaboration with the global polio surveillance laboratory network to monitor new vaccines once they are introduced into immunization programmes. These laboratories have extensive experience in poliovirus surveillance and may provide excellent surveillance and post-marketing support.

The total duration of enhanced surveillance should be regularly reviewed by the NRA. If particular issues arise during pre-licensure studies or during post-licensure safety surveillance, it may be necessary to conduct specific post-licensure safety studies.

Part D. Recommendations for NRAs

D.1 General recommendations

The guidance for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (73) and Guidelines for independent lot release of vaccines by regulatory authorities (23) should be followed. These guidelines specify that no new biological product should be released until consistency of manufacturing and product quality has been established and demonstrated by the manufacturer.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of live attenuated OPV should be discussed with and approved by the NRA.

For control purposes, the relevant international reference preparations currently in force should be obtained for the purpose of calibrating national, regional and working standards as appropriate (74). The NRA may obtain the product-specific or working reference from the manufacturer to be used for lot release until the international/national standard preparation is established.

Only a monovalent bulk approved by the NRA can be used by the manufacturer for the formulation of a final bulk.

Where the monkey neurovirulence test (MNVT) is performed for the control of the monovalent bulk and the national control laboratory does not perform this test itself, the NCL should carry out a second reading of the histological sections provided by the manufacturer for each monovalent bulk. In addition, the national control laboratory or a contract organization certified by the NRA for proficiency to conduct NVT should perform a second reading of at least four neurovirulence tests on the reference preparations using the monkey neurovirulence test in order to obtain the necessary baseline data for comparison with the neurovirulence of test vaccines.

The national control laboratory should encourage the use of the standard form for the reporting of data on virus activity in the sections taken from histopathological examination.

Where the transgenic mouse neurovirulence test (TgmNVT) is performed for the control of the monovalent bulk and the national control laboratory performs this test itself, it should complete the standard implementation process.

If the national control laboratory does not perform the transgenic mouse neurovirulence test, it should carry out a clinical scoring of mice in parallel with the manufacturer at least at day 3 or day 4. Whether a clinical scoring at day 14 is needed should be justified for each monovalent bulk. Moreover, once a year, the injection of mice should be observed by the national control laboratory. Only appropriately trained staff from a competent national control laboratory can carry out a clinical scoring of mice in parallel with the manufacturer.

> In one region of the world, 1 in 10 bulks is also independently tested by a national control laboratory competent in carrying out the test. Other regions that implement the transgenic mouse neurovirulence test may wish to follow this approach.

Consistency of production has been recognized as an essential component in the quality assurance of live attenuated OPV. In particular, the NRA should carefully monitor production records and quality control test results for clinical lots, as well as a series of consecutive lots of the vaccine.

D.2 Official release and certification

A vaccine lot should be released only if it fulfils the national requirements and/or satisfies Part A of these WHO recommendations (23).

A summary protocol for the manufacturing and control of OPV, based on the model provided in Appendix 5, signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA/NCL in support of a request for release of the vaccine for use.

A lot release certificate signed by the appropriate official of the NRA/NCL should then be provided to the manufacturing establishment if requested and should certify that the lot of vaccine in question meets all national requirements, and/or Part A of these WHO recommendations. The certificate should provide sufficient information on the vaccine lot, including the basis of the release decision (by summary protocol review and/or independent laboratory testing). The purpose of this official national lot release process is to assess independently the quality and safety of the batches, to facilitate the exchange of vaccines between countries and should be provided to importers of the vaccines.

A model NRA/NCL lot release certificate for OPV is provided in Appendix 6.

Part E. Recommendations for poliomyelitis vaccines (oral, live, attenuated) prepared in primary monkey kidney cells

The following additional or alternative recommendations are for Sabin OPV prepared in cultures of primary monkey kidney cells and concern the testing of the cell substrate used for the production of the vaccine. They should therefore be added to, or used as an alternative to - the appropriate sections in Part A.4 as follows:

- sections E.1.1.1, E.1.3.1, E.1.4.1 and E.1.4.2 are additions to the corresponding Part A.4 sections as indicated below;
- sections E.1.2.1, E.1.2.2 and E.1.2.3 are replacements for the corresponding Part A.4 sections as indicated below.

All the other recommendations given in Parts A and B of this document are also applicable to this type of vaccine.

E.1 Control of vaccine production

E.1.1 Control of source materials

E.1.1.1 Monkeys used for preparation of kidney-cell cultures and for testing of virus

Addition to section A.4.1.

If vaccine is prepared in monkey kidney-cell cultures, animals should be from a species approved by the NRA, and the animals should be in good health and not previously have been used for experimental purposes.

Manufacturers should use animals from closed or intensively monitored colonies.

The monkeys should be kept in well-constructed and adequately ventilated animal rooms in cages separated in such a way as to prevent cross-infection between cages, together with other adequate precautionary measures. Cage-mates should not be interchanged. The monkeys should be kept in the country of manufacture of the vaccine in quarantine groups[1] for a period of not less than six weeks before use. If at any time during the quarantine period the overall death rate of a shipment consisting of one or more groups reaches 5% (excluding deaths from accidents or where the cause was specifically determined not to be an infectious disease), monkeys from that entire shipment should continue in quarantine for a further period of not less than six weeks. The monkeys used should be free of infection. At the end of the extended quarantine period, and following thorough investigations, if any additional monkeys die of the same infectious disease, the entire group is discarded from production.

The groups should be kept continuously in isolation, as in quarantine, even after completion of the quarantine period, until the monkeys are used. After the last monkey of a group has been

[1] A quarantine group is a colony of selected healthy monkeys kept in one room, with separated feeding and cleaning facilities, and having no contact with other monkeys during the quarantine period.

taken, the room that housed the group should be thoroughly cleaned and decontaminated before being used for a fresh group.

> In countries in which the kidneys from near-term monkeys are used, the mother should be quarantined for the term of pregnancy.

All actions taken by working personnel should be based on the assumption that a great potential hazard exists at all times in the quarantine area. Personnel should be provided with protective clothing, including gloves, footwear and masks or visors. Street clothes should not be permitted in the animal rooms. Smoking, eating and drinking should be forbidden while personnel are in the animal rooms.

A supervisor should be made responsible for reporting unusual illness among employees and for ensuring that all injuries are properly treated. No worker who has cuts or abrasions on exposed areas of the body should enter the animal area. Any unexplained febrile illness, even while off duty, should be considered as potentially related to the employee's occupation.

Monkeys from which kidneys are to be removed should be anaesthetized and thoroughly examined, particularly for evidence of tuberculosis and herpes B virus infection.

If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of a seed lot or vaccine, it should not be used, nor should any of the remaining monkeys of the quarantine group concerned be used unless it is evident that their use will not impair the safety of the product.

All the operations described in this section should be conducted outside the areas where vaccine is made.

The monkeys should be shown to be free from antibodies to SV40 virus and simian immunodeficiency virus.

> It is desirable that kidney-cell cultures are derived from monkeys shown to be free from antibodies to foamy viruses. In some countries, monkeys are tested for antibodies to herpes B virus.

E.1.2 Production precautions

The general production precautions called for by the Good manufacturing practices for biological products (21) should apply to the manufacture of vaccine, with the addition of the following tests.

E.1.2.1 Monkey kidney-cell cultures for vaccine production

Replacement of section A.4.2.1 – in conjunction with section E.1.2.2 (below).

Cultures of monkey kidney cells should be prepared from kidneys that have shown no pathological signs. Virus for the preparation of vaccine should be grown by aseptic methods in such cultures. If animal serum is used in the propagation of the cells, the maintenance medium used after virus inoculation should contain no added serum.

> To reduce animal use, the virus may be grown in serially passaged monkey kidney-cell cultures from primary monkey kidney cells.

Each group of cell cultures derived from a single monkey, or from no more than 10 near-term monkeys, should be prepared and tested as an individual group.

E.1.2.2 Tests of cell cultures used for vaccine production (see Appendix 7)
Replacement of section A.4.2.1 – in conjunction with section E.1.2.1 (above).

On the day of inoculation with virus working seed lot, each cell culture should be examined for degeneration caused by an infective agent. If, in this examination, evidence is found of the presence in a cell culture of any adventitious agent, the entire group of cultures concerned should not be used for vaccine production.

On the day of inoculation with the virus working seed lot, a sample of at least 30 ml of the pooled fluid removed from the cell cultures of the kidneys of each single monkey, or from no more than 10 near-term monkeys, should be divided into two equal portions. One portion of the pooled fluid should be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other portion of the pooled fluid should be tested in kidney-cell cultures from another species of monkey, provided that the tests are done in cell cultures from at least one species known to be sensitive to SV40 virus. The pooled fluid should be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of pooled fluid. At least one bottle of each kind of cell culture should remain uninoculated and should serve as a control.

> When the monkey species used for vaccine production is known to be sensitive to SV40 virus, a test in a second species may be omitted with the approval of the NRA.
>
> Animal serum may be used in the propagation of the cells, provided that it does not contain SV40 antibody or other inhibitors, but the maintenance medium used after inoculation of the test material should contain no added serum except as described below.

The cultures should be incubated at a temperature of 35–37 °C and should be observed for a total period of at least four weeks. During this observation period, and after not less than two weeks' incubation, from each of these cultures at least one subculture of fluid should be made in the same tissue culture system. The subculture should also be observed for at least two weeks.

Serum may be added to the original culture at the time of subculturing, provided that the serum does not contain SV40 antibody or other inhibitors. Immunochemical techniques may be useful for detecting SV40 and other viruses in the cells.

A further sample of at least l0 ml of the pooled fluid should be tested for the presence of herpes B virus and other viruses in rabbit kidney-cell cultures. Serum used in the nutrient medium of these cultures should have been shown to be free from inhibitors.[1] The sample should be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of pooled fluid. At least one bottle of the cell cultures should remain uninoculated and should serve as a control.

The cultures should be incubated at a temperature of 35–37 °C and should be observed for a period of at least two weeks.

It is suggested that, in addition to these tests, a further sample of l0 ml of pooled fluid removed from the cell cultures on the day of inoculation with the seed lot virus should be tested for the presence of adventitious agents by inoculation into cell cultures sensitive to measles virus.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the respective test periods.

If, in these tests, evidence is found of the presence of an adventitious agent, the single harvest from the whole group of cell cultures concerned should not be used for vaccine production.

If the presence of the herpes B virus is demonstrated, the manufacture of vaccine should be discontinued and the NRA should be informed. Manufacturing should not be resumed until a thorough investigation has been completed and precautions have been taken against any reappearance of the infection, and then only with the approval of the NRA.

If these tests are not carried out immediately, the samples of pooled cell-culture fluid should be kept at a temperature of –60 °C or below, with the exception of the sample for the test for B virus, which may be held at 4 °C provided that the test is done not more than seven days after the sample has been taken.

E.1.2.3 Test of control cell cultures

Replacement of section A.4.1.

Cultures prepared on the day of inoculation with the virus working seed lot from 25%, but not more than 2.5 litres, of the cell suspension obtained from the kidneys of each single monkey, or

[1] Human herpesvirus (herpes simplex) has been used as an indicator for freedom from B virus inhibitors because of the danger of handling herpes B virus.

from not more than 10 near-term monkeys, should remain uninoculated and should serve as controls. These control cell cultures should be incubated under the same conditions as the inoculated cultures for at least two weeks, and should be examined during this period for evidence of cytopathic changes. For the tests to be valid, not more than 20% of the control cell cultures should have been discarded for nonspecific, accidental reasons. At the end of the observation period, the control cell cultures should be examined for degeneration caused by an infectious agent. If this examination, or any of the tests required in this section, shows evidence of the presence in a control culture of any adventitious agent, the poliovirus grown in the corresponding inoculated cultures from the same group should not be used for vaccine production.

E.1.2.3.1 Tests for haemadsorbing viruses

At the time of harvest, or not more than four days after the day of inoculation of the production cultures with the virus working seed lot, a sample of 4% of the control cell cultures should be taken and should be tested for haemadsorbing viruses. At the end of the observation period, the remaining control cell cultures should be similarly tested. The tests should be carried out as described in Part A, section A.4.1.2.

E.1.2.3.2 Tests for other adventitious agents

At the time of harvest, or no more than seven days after the day of inoculation of the production cultures with the virus working seed lot, a sample of at least 20 ml of the pooled fluid from each group of control cultures should be taken and tested in two kinds of monkey kidney-cell culture, as described in Part E, section E.1.2.2.

At the end of the observation period for the original control cell cultures, similar samples of the pooled fluid should be taken and the tests referred to in this section in the two kinds of monkey kidney-cell culture and in the rabbit-cell culture should be repeated, as described in Part E, section E.1.2.2.

If the presence of herpes B virus is demonstrated, the production cell cultures should not be used and the measures concerning vaccine production described in Part E, section E.1.2.2, should be taken.

> In some countries, fluids are collected from the control cell cultures at the time of virus harvest and at the end of the observation period. Such fluids may then be pooled before testing for adventitious agents.

E.1.3 Control of single harvests

Addition to section A.4.3.

E.1.3.1 Tests for neutralized single harvests in monkey kidney-cell cultures

A sample of at least 10 ml of each single harvest should be neutralized by type-specific poliomyelitis antiserum prepared in animals other than monkeys. In preparing antisera for this purpose, the immunizing antigens used should be prepared in non-simian cells.

Care should be taken to ensure that the antiserum used is monospecific. This may be demonstrated by titration of the antiserum against homotypic and heterotypic virus of known virus titre using the same dilution of the antiserum as that used for neutralization.

Half (corresponding to at least 5 m1 of single harvest) of the neutralized suspension should be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other half of the neutralized suspension should be tested in monkey kidney-cell cultures from another species, provided that the tests are done in cell cultures from at least one species known to be sensitive to SV40 virus.

The neutralized suspensions should be inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of neutralized suspension. At least one bottle of each kind of cell culture should remain uninoculated to serve as a control and should be maintained using nutrient medium containing the same concentration of the specific antiserum used for neutralization.

Animal serum may be used in the propagation of the cells provided that it does not contain inhibitors, but the maintenance medium used after the inoculation of the test material should contain no added serum other than the poliovirus neutralizing antiserum, except as described below.

The cultures should be incubated at a temperature of 35–37 °C and should be observed for a total period of at least four weeks. During this observation period, and after no less than two weeks' incubation, at least one subculture of fluid should be made from each of these cultures in the same tissue culture system. The subcultures should also be observed for at least two weeks.

Serum may be added to the original cultures at the time of subculturing provided that the serum does not contain inhibitors. Immunohistochemical techniques may be useful for detecting SV40 and other viruses in the cells.

It is suggested that, in addition to these tests, a further sample of the neutralized single harvest is tested by inoculation of 10 ml into human cell cultures sensitive to measles virus.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the respective test periods.

If any cytopathic changes occur in any of the cultures, the causes of these changes should be investigated. If the cytopathic changes are shown to be due to unneutralized poliovirus, the test should be repeated. If there is evidence of the presence of SV40 virus or other adventitious agents attributable to the single harvest, that single harvest should not be used for vaccine production.

E.1.4 Control of monovalent bulk

Addition to section A.4.

E.1.4.1 Monovalent bulk (before filtration)

E.1.4.1.1 Tests in rabbits

A sample of the monovalent bulk should be tested for the presence of herpes B virus and other viruses by injection in at least 10 healthy rabbits each weighing between 1.5 and 2.5 kg. The sample should consist of at least 100 ml. Each rabbit should receive not less than 10 ml and not more than 20 ml, of which 1 ml is given intradermally at multiple sites, and the remainder subcutaneously. The rabbits should be observed for between three and five weeks for death or signs of illness.

It is suggested that the sample should consist of at least 1% of monovalent bulk, provided that this is not less than 100 ml, up to a maximum of 500 ml.

All rabbits that die after the first 24 hours of the test should be examined by autopsy, the brain and organs being removed for detailed examination to establish the cause of death. Animals showing signs of illness should be humanely killed and subjected to a similar autopsy.

The monovalent bulk passes the test if no more than 20% of the inoculated rabbits show signs of intercurrent infection during the observation period and if none of the rabbits shows evidence of infection with B virus or other adventitious agents or lesions of any kind attributable to the bulk suspension.

If the presence of B virus is demonstrated, the measures concerning vaccine production described in Part E, section E.1.2.2, should be taken.

A test for the presence of Marburg virus may be carried out in guinea pigs.

E.1.4.2 Monovalent bulk (after filtration)- tests for retroviruses

Test samples from the filtered monovalent bulk should be examined for the presence of retroviruses by an assay for reverse transcriptase (RTase) acceptable to the NRA.

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Appendix 1

Overview of virus seeds used in OPV production

The history of the poliovirus strains used in the production of OPV is well documented (*1*, *2*, *3*). This appendix gives an overview of virus seeds used in OPV production.

The flow diagrams in Figures 1 and 2 show the history of seed virus and reference materials used in manufacture of OPV from Sabin 1 and Sabin 2 (Figure 1) and Sabin 3 (Figure 2) strains. Concentric circles indicate progressive virus passages made to prepare master seed stocks, working seed stocks and production lots of vaccine. Where relevant, sub-master seed stocks are identified in the footnotes. Different seed viruses are identified as SO (Sabin Original), SOM (Merck stock of SO), SOB (Behringwerke stock of SO), Pfizer (otherwise known as re-derived SO, or RSO), SOJ (Japanese stock of SO) and SOR (Russian stock of SO).

These figures provide only a historical overview of the use of different seeds derived from the Sabin vaccine strain in OPV production. They do not indicate any WHO "qualification" or "approval" of the strains or vaccines in the context of this document.

The origin of the nOPV given Emergency Use Listing by WHO has been published (4) but it is not yet supplied as a seed by WHO. The design, modifications and their purposes are described in Figure 3.

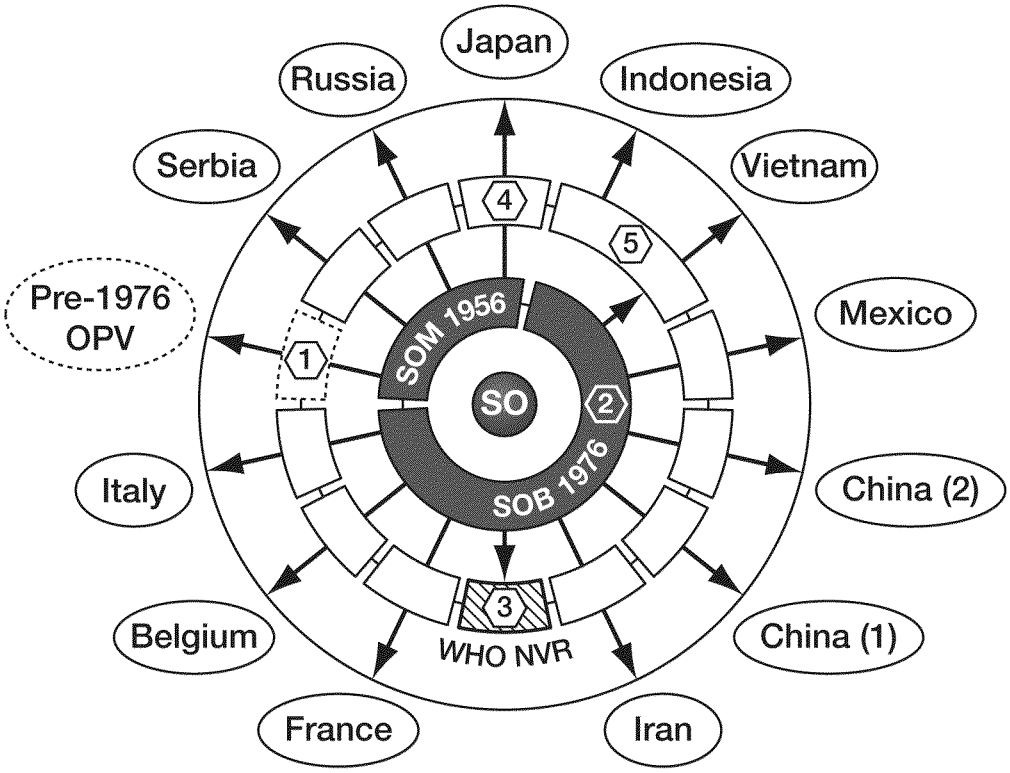

Figure 1. Types 1 and 2 OPV

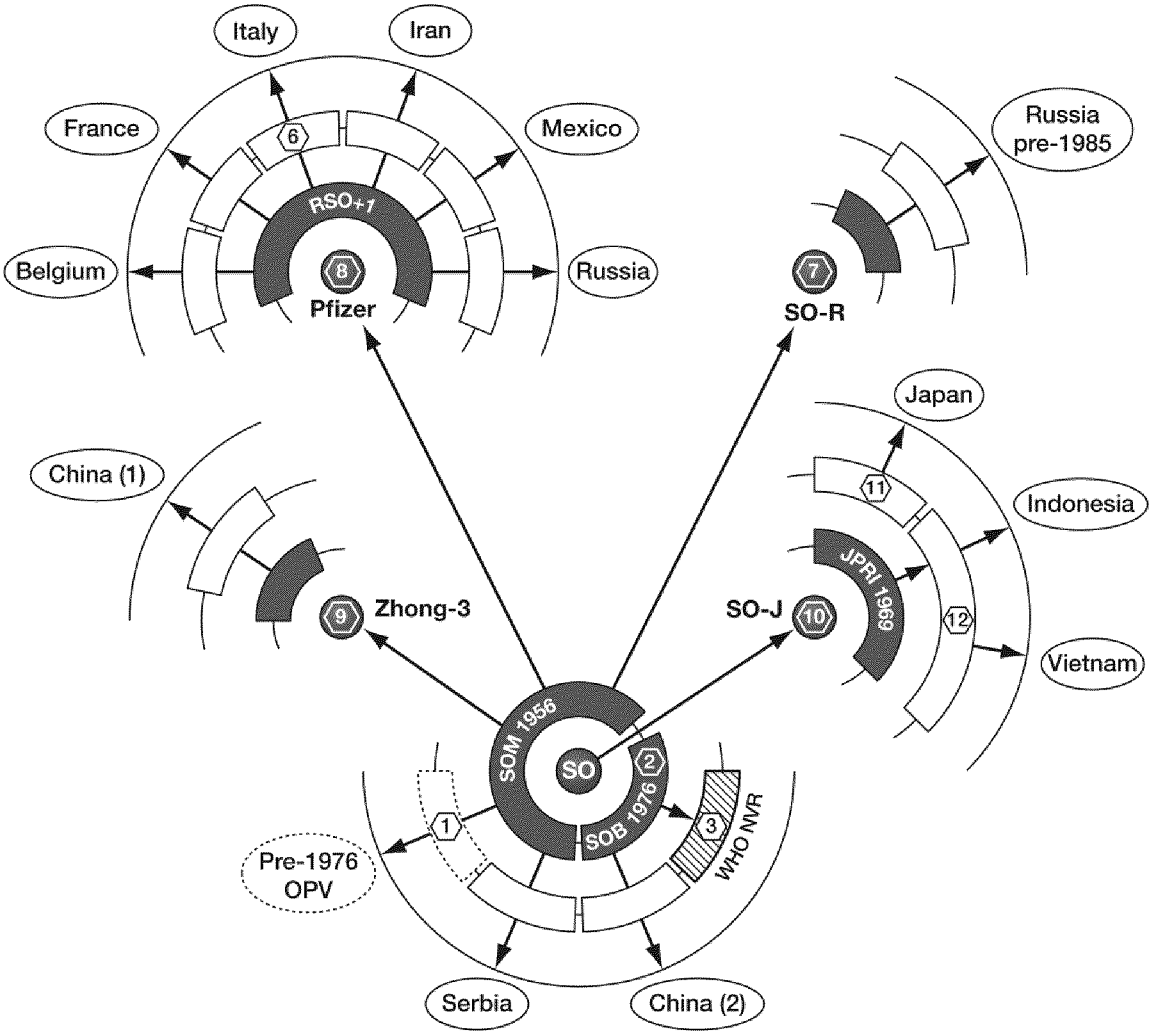

Figure 2. Type 3 OPV

Schematic of the nOPV2 genome showing modifications and their locations. The sequence of 5'UTR domain V (*S15 domV*) prevents an increase in *domV* thermostability by single point mutations; to prevent replacement of *domV* attenuation elements by recombination, the cre element, essential for poliovirus replication, was relocated from its original position in the 2C coding region to the 5'UTR (5' cre5). The original cre was inactivated by mutations (cre^{mut}); $3D^{pol}$ mutations *HiFi* (D53N) and *Rec1* (K38R) reduce overall virus adaptation capacity by reducing mutation and recombination rates, respectively.

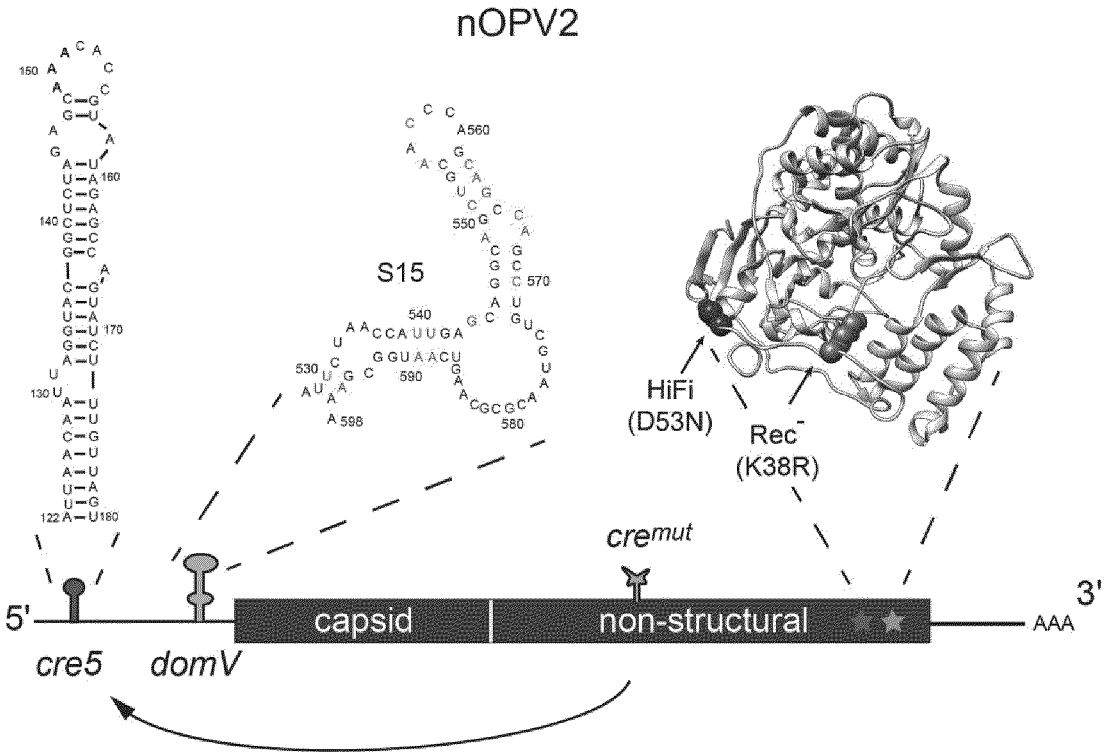

Figure 3. nOPV2 Vaccine Design

Names of manufacturers shown on Figures 1 and 2 are as follows:

Belgium	GlaxoSmithKline Biologicals
China (1)	Institute of Medical Biology, Kunming
China (2)	China National Biotec Group, Beijing Tiantan Biological Products Company
France	Sanofi Aventis
Indonesia	PT Bio Farma
Iran	Razi Vaccine and Serum Research Institute
Italy	Novartis Vaccines
Japan	Japan Poliomyelitis Research Institute
Mexico	Biologics and Reagents Laboratories of Mexico
Russia	Chumakov Federal Scientific Center for Research & Development of Immune-and-Biological Products of Russian Academy of Sciences
Serbia	Torlak Institute of Virology, Vaccines and Serum
Viet Nam	Center for Research and Production of Vaccines and Biologicals

Notes:

1. Working seeds produced by different manufacturers before 1976.
2. WHO master seed stock.
3. WHO neurovirulence reference preparation.
4. Type 1 seed stock prepared at JPRI by four passages of SOM, including three terminal dilution passages (passage level SO+5). Type 2 seed stock prepared at JPRI by one passage of SOM (SO+2).
5. Seed stock prepared at JPRI by one passage of SOB (SO+2).
6. Novartis performed an additional passage to prepare sub-master seed stock from which a working seed was produced.
7. Six plaques were selected, pooled together, and grown to produce seed stock in Russia.
8. Pfizer (RSO): re-derived Sabin Original, produced by RNA plaque purification, passage.
9. Zhong-3: plaque purification, passage.
10. Produced by JPRI in 1969 from SO stock by one passage (SO+1).
11. Prepared from SOJ by passages in AGMK cells (SOJ+9), including two plaque purifications and three terminal passages (SO+10).
12. Prepared from SOJ by passages in AGMK cells (SOJ+6), including two plaque purifications (SO+7).

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Appendix 2

In vivo tests for neurovirulence, and considerations in relation to assay choice

Live attenuated vaccines were developed by Sabin in large part by use of nonhuman primates, particularly old world monkeys for measuring the level of residual neurovirulence. In the 1980s, tests of vaccine bulks and seeds were standardized as a single dose of test material given by intraspinal inoculation tested concurrently with a homologous reference. Vaccines derived from the Sabin strains that pass the monkey neurovirulence test (MNVT) have been shown to have an acceptable safety profile. However, in its current form, the MNVT is regarded as a test of consistency and it is not known whether vaccines that fail the test are virulent in human recipients. Tests designed to replace the MNVT should be able to detect the same changes from batch to batch with similar sensitivity. A neurovirulence test in transgenic mice (TgmNVT) expressing the human poliovirus receptor (TgPVR21 mice) has been developed as an alternative to the MNVT for all three poliovirus serotypes.

Summaries of the MNVT and TgmNVT for Sabin OPV are given below, along with the implementation process for the TgmNVT. It is assumed that the in vivo neurovirulence test procedures and acceptance criteria applied to Sabin OPV are suitable for the evaluation of nOPV.

1. Summary of the MNVT

1.1 Key features

A detailed set of standard operating procedures for the "Neurovirulence test of types 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in monkey" is available from WHO.[1] Between 5.5 and 6.5 $\log_{10}$ $CCID_{50}$ of monovalent virus is delivered in a single dose by intraspinal inoculation into the lumbar cord. A back titration of the inoculum should be carried out after the inoculation step is completed. Residual paralysis, if any, is noted over the following 17–22 days. The animals are sacrificed at the end of the test or earlier on humane grounds and prepared for histological examination of the central nervous system. Regions are scored for damage on a scale from 1 to 4, and a mean lesion score is calculated for each monkey and then for all the monkeys in the test. The clinical signs do not form part of the assessment or of the pass/fail criteria. The homologous WHO/SO+2 reference is tested in parallel. For a new laboratory, the implementation process should be agreed with the NRA.

1.2 Number of animals

The number of monkeys has been chosen on statistical grounds, considering the variability of the test, such that a satisfactory vaccine will only twice give the lesion score of a reference preparation in 1% of tests and therefore be incorrectly scored as a fail. Valid animals must show some sign of histological damage as evidence of correct placement of active virus. The number of valid monkeys required per virus preparation is 11 for types 1 and 2 and 18 for type 3.

[1] Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/poliomyelitis.

Because a reference must be tested at the same time, the total number of monkeys is at least 22 for types 1 and 2 and 36 for type 3.

1.3 Sections examined

Sections are examined from defined regions of the spinal cord and brain and scored histologically for virus activity on a scale of 1 (cellular infiltration only) to 4 (massive neuronal damage). At least 29 sections are examined per monkey, as specified in the WHO standard operating procedures for the MNVT. The readings are used to generate the mean lesion score for the animal, and the mean lesions scores for all animals are then used to generate the mean lesion score for the test as a whole.

1.4 Pass/fail criteria

The pass/fail criteria are based on the variation in the test from run to run, established from the scores obtained with the reference preparation and specific to each laboratory and operator. The within-test variance is used to calculate the statistical constants C_1, C_2 and C_3. If the mean lesion score of the test vaccine is greater than that of the concurrently tested reference by more than C_1, the vaccine is not acceptable. If the test vaccine gives a higher score than the reference but the difference in scores lies between C_1 and C_2, the vaccine may be retested and the results pooled; if the difference for the pooled test results is greater than C_3, the vaccine fails.

The values for C_1, C_2 and C_3 are initially established on the basis of the data accumulated after four qualifying tests. These values should then be updated after every test until nine tests have been performed. After that, the C values are based on the last 10 tests performed. The C values must be established for each testing laboratory.

2. Summary of the TgmNVT

2.1 Key features

The detailed operating procedures for the TgmNVT, "WHO neurovirulence test of type 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus", are available from WHO.[1] The test for neurovirulence of polio vaccines in transgenic mice involves the intraspinal inoculation of a defined strain of transgenic mice carrying the human receptor for poliovirus with small volumes of the test vaccine. Two virus concentrations are used and the read-out of the test is based on the clinical dose response. A reference preparation is tested at the same time and a clearly defined process has been established for implementation of the test in a new laboratory.

2.2 Strain of transgenic mouse

Different transgenic mouse lines differ in their sensitivity to polio infection depending on the particular transgenic construct and the genetic background, and only strains from a source approved by WHO should be used. Currently the only approved transgenic mouse strain is

[1]Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/poliomyelitis.

TgPVR21, developed in Japan and sourced from the developers or from an approved subcontractor.

2.3 Titration of virus

Two doses of virus are inoculated in a volume of five microlitres: for type 1, 1.75 and 2.75 $CCID_{50}$; for type 2, 5.0 and 6.0 $CCID_{50}$; and for type 3, 3.5 and 4.5 $CCID_{50}$. The inocula must be prepared and titrated accurately to ensure that these doses are given; the precision of the determinations should be better than +/- 0.3 $\log_{10}$. A back titration of the inoculum should be performed after the inoculation step is completed.

2.4 Inoculation and observation of animals

Animals procured at age 5–6 weeks are randomized to cages and allowed to recover for at least seven days. They are then appropriately anaesthetized and inoculated with five microlitres of diluted test virus between the last thoracic and first lumbar vertebrae. Animals are observed for clinical signs once a day for the next 14 days and ultimately scored either as normal (slight weakness or no signs) throughout or paralysed (paresis on two consecutive days or paralysis on a single day). The lower and higher doses of the reference should give more than 5% and less than 95% of animals paralysed, respectively, for the test to be valid. A test requires 128 mice for one vaccine plus the reference tested concurrently, or 192 for two vaccines and the reference. The reference is the same as that used in the monkey test; the use of other references may be acceptable but should be validated.

The vaccine passes if it is not significantly more virulent than the reference defined in terms of the log odds ratio and statistical constants L1 and L2 which are based on the reproducibility of the test and define the pass/fail criteria and the grey zone in which a retest is required. The acceptance and rejection limits, L1 and L2, were selected so that a test vaccine which is equivalent to the reference will have a 0.95 probability of passing and a 0.01 probability of failing, respectively. The constants are regularly updated. Statistical evaluation of test validity includes linearity and dose and gender effects.

3. Implementation process of the TgmNVT

If a manufacturer wishes to use the transgenic mouse test, relevant validation data should be available for their specific product to demonstrate the test's applicability. This may include reference to the extensive collaborative studies by which the test was originally developed. A clear stepwise process for implementing the TgmNVT has been established; it involves training in the inoculation technique through injection of Indian ink, tests with vaccines, and testing of a blinded evaluation panel containing vaccines that pass, fail or marginally fail the test. Competence in clinical scoring is acquired through a standardized training procedure which involves parallel scoring with an experienced scorer and criteria for declaring a trainee competent.

Testing should be performed according to procedures specified in the WHO standard operating procedures for the TgmNVT, using appropriate WHO reference materials unless modified

procedures have been validated and shown to be suitable. The test chosen should be used to test virus seeds and bulks, as described in section A.4.4.7.2.

4. Considerations for the choice of assay for the evaluation of Sabin OPV

The following specific issues suggest that care should be taken in the selection of the in vivo tests to be performed for neurovirulence and that the selection should be justified. The report of the WHO Working Group Meeting to Discuss the Revision of the WHO Recommendations for OPV: TRS Nos. 904 and 910 provides more detailed discussions (1).

4.1 Types 1 and 2 Sabin vaccine viruses

The relative sensitivity of the transgenic mouse and monkey tests performed according to WHO procedures with respect to the presence of mutations in the 5'untranslated region (UTR) in types 1 and 2 appears to be comparable but significantly lower than that in type 3 (2, 3). It is unknown whether these two models are equally sensitive to other potential neurovirulent mutations. Most manufacturers use essentially identical seeds of types 1 and 2, in contrast to the situation with type 3.

4.2 Type 3 Sabin vaccine virus

4.2.1 Molecular biology

Studies of the molecular biology of the Sabin polio vaccine virus strains have suggested that few mutations are involved in attenuation and that, for the type 3 strain, there may be only two: one base change in the 5'noncoding region of the genome at base 472 and one coding change at base 2034 that introduces an amino acid change in the virus protein VP3. A third mutation at position 2493 has been described (*4*). Growth of Sabin 3 virus in cell culture or in vaccine recipients results in rapid accumulation of U instead of C at nucleotide 2493 (changing Thr to Ile at amino acid 6 of capsid protein VP1), and all Sabin 3 OPV batches contain variable amounts of these mutants. This mutation does not affect neurovirulence as determined in the monkey test but there is evidence that it influences the results obtained in the transgenic mouse test, as described in the WHO operating procedures (*5*). Variations in the virulence of vaccine batches measured in monkeys correlate well with variations in the base in the 5'-UTR as measured by MAPREC. Changes in the amino acid in VP3, or changes at other positions that suppress its effect, are not thought to be generated in the course of well controlled production runs, although this is possible in principle.

4.2.2 Current type 3 seed viruses

Seed viruses currently used for global vaccine production contain variable proportions of the bases found at position 2493 (C or U):

- The original WHO reference material (passage level SO+2) for neurovirulence testing contained about an equal mixture of both forms (2493 C or U).
- Batches prepared from RSO, the seeds most commonly used in production in Europe, typically contain about 5% or less of 2493-U (mutant).
- Seed viruses used in production by some manufacturers (a plaque purified from SO) contain 100% of mutant form (2493-U) (6).

All OPVs currently in use are believed to have an acceptable safety profile.

5. Experience using the MNVT and TgmNVT with type 3 Sabin seeds and vaccines

There is evidence that the transgenic mouse test, as described in the WHO operating procedures for the TgmNVT, is sensitive to the presence of 2493-U, whereas the monkey test is not sensitive to this mutation. Thus, batches produced from RSO seed will pass both the monkey and transgenic mouse tests, whereas batches produced from the alternative seeds that contain 100% 2493-U will pass the monkey test but may fail the transgenic mouse test, although still having an acceptable safety profile in clinical use.

WHO's current standard operating procedures for the TgmNVT specify the doses and the WHO reference material to be used and include the proportion of mice affected at the two doses of virus given for the test to be valid. The WHO reference material for TgmNVT is the same as that used in the monkey test and has approximately 50% 2493-C, and it was validated primarily against vaccines made from SO or RSO seeds. However, if used to test vaccines derived from 2493-U containing seed, it may fail them even if they contain little 472-C and would pass MNVT. The TgmNVT could be adapted for testing 2493-U containing bulks – e.g. by changing the reference material, the doses and/or the validity criteria. Manufacturers may wish to do this to make it applicable to their product. Any modified test should be validated and approved by the NRA.

References

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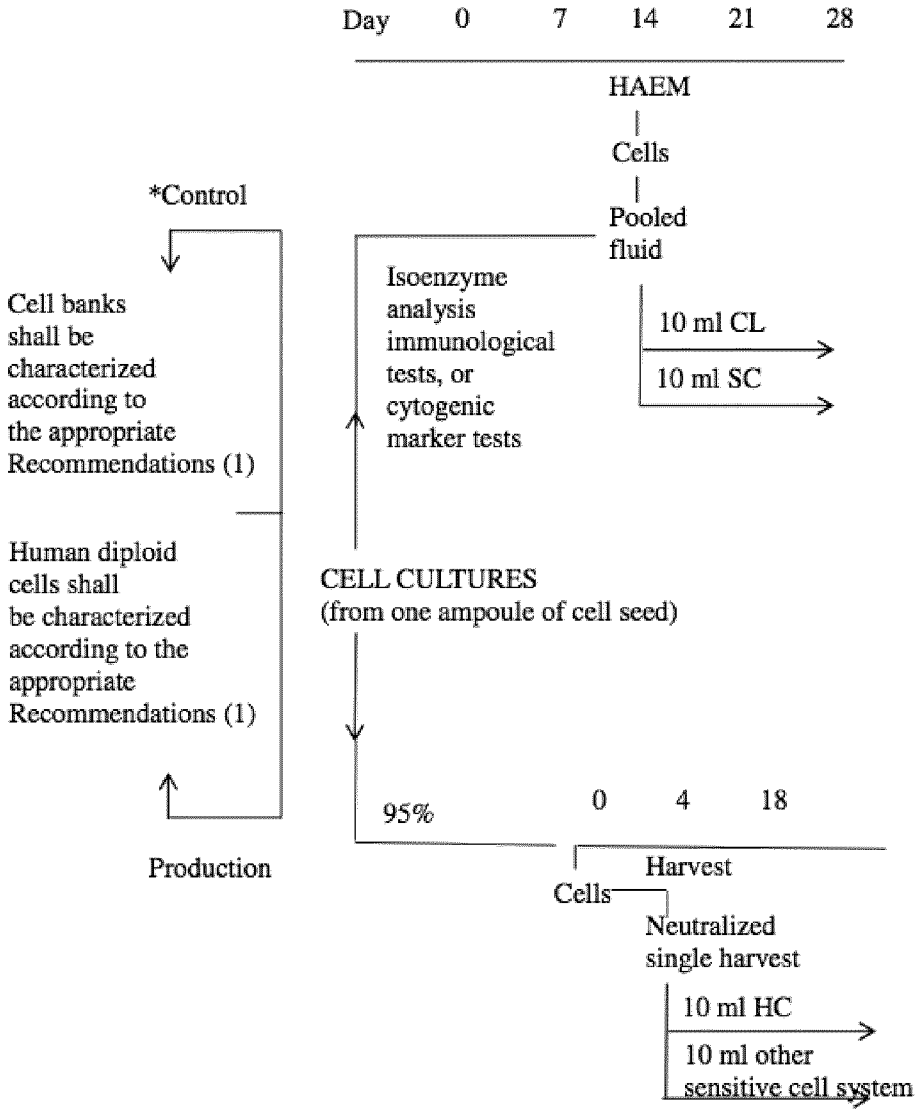
Appendix 3

Preparation of poliomyelitis vaccines (oral, live, attenuated) using cell banks – example of a flowsheet



* Control cells: 5% of the total or 500 ml of cell suspension, or 100 million cells.
HAEM = test for haemadsorbing viruses.
CL = cell line used for production, but not the same batch of cells used for production of the virus.
SC = when a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line. When a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line (*1*).
HC = human cells.

Note: This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the NRA, this flowsheet should not be considered as an integral part of the requirements and has been included solely for guidance. Manufacturing establishments should prepare their own flowsheet in order to clarify the procedures used.

Reference

1. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 3 (WHO Technical Report Series, No. 978; https://www.who.int/publications/m/item/animal-cell-culture-trs-no-978-annex3, accessed 9 May 2022)

Appendix 4

Cell-culture techniques for determining the virus content of poliomyelitis vaccines (oral, live, attenuated)

This appendix describes a method for the determination of the virus content of live attenuated OPV in cell cultures. It is an example that is provided for guidance only.

The preparation to be assayed and the reference preparation are diluted in an appropriate medium. It is convenient to make tenfold dilution steps of the virus suspensions initially, but for dilutions that are to be inoculated into Hep-2 (Cincinnati) cell cultures the dilutions should be prepared in 1.0 $\log_{10}$ or smaller steps. A preliminary assay may be required to ensure that, in the test, the dilution range selected encompasses at least three dilutions that will infect between 0% and 100% of the cultures inoculated.

Titrate the vaccine for infectious virus using no fewer than three separate containers of vaccine following the method described below. Titrate one container of an appropriate virus reference preparation in triplicate to validate each assay. The virus titre of the reference preparation is monitored using a control chart, and a titre is established on a historical basis by each laboratory.

If the vaccine contains more than one poliovirus type, titration of the individual serotypes is undertaken separately using mixtures of appropriate type-specific antiserum (or preferably a monoclonal antibody) to neutralize each of the other types present.

For titration of individual serotypes, inoculate a suitable number of wells (ideally 8–10) in a flat-bottomed microtitre plate with equal volumes of the selected dilutions of virus and the appropriate antisera mixture. Total virus content is determined, without any prior incubation, by directly diluting the vaccine in the assay medium. The assay is then incubated for 1–3 hours at 34–36 °C, followed by the addition of an appropriate volume of a suitable cell. The plates are further incubated at 34–36 °C and examined between day 5 and day 9 for the presence of viral cytopathic effect.

The cytopathic effect can be observed by direct reading or after an appropriate staining (vital or fixed staining). The individual virus concentration for each polio serotype and reference preparation is then calculated using an appropriate method.

The assay is considered valid if:

- the estimated virus concentration for the reference preparation is ± 0.5 $\log_{10}$ $CCID_{50}$ of the established value for this preparation;
- the confidence interval ($P = 0.95$) of the estimated virus concentration of the three replicates of the reference preparation is not greater than ± 0.3 $\log_{10}$ $CCID_{50}$.

The assay is repeated and results are averaged if:

- the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 $\log_{10}$ $CCID_{50}$.

The assay should be validated for nOPV.

Appendix 5

Model summary protocol for the manufacturing and control of poliomyelitis vaccines (oral, live, attenuated)

The following protocol is intended for guidance and indicates the minimum information that should be provided by the manufacturer to the NRA or NCL.

Information and tests may be added or omitted as necessary with the approval of the NRA or NCL. In case the testing method is different from the one listed in this model protocol, it should be approved by the NRA. For example, if molecular methods (such as NAT, HTS) are used for the testing of adventitious agents or mycoplasma, their key parameters and information should be identified and provided, with the minimum of testing method, date of testing, specification and result.

It is possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO recommendations on a particular product should be provided in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet (package insert) that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it should also be accompanied by a lot release certificate from the NRA or NCL of the country in which the vaccine was produced and/or released stating that the product meets the national requirements as well as Part A of these WHO Recommendations.

Summary information on finished product (final vaccine lot)

International name: _____

Trade name: _____

Product licence (marketing authorization) number: _____

Country: _____

Name and address of manufacturer: _____

Name and address of licence holder, if different: _____

Virus strain: _____

Origin and short history: _____

Finished product (final lot):

Batch number: _____

Final bulk: _____

Type of container: _____

Number of doses per container: _____

Number of filled containers in this final lot: _____

Bulk numbers of monovalent bulk Type 1 Type 2 Type 3

suspensions blended in monovalent/bivalent/trivalent vaccine: _____

Site of manufacture of each monovalent bulk: _____

Date of manufacture of each monovalent bulk: _____

Date of manufacture of final bulk (blending): _____

Date of manufacture (filling) of finished product: _____

Date on which last determination of virus titre was started, or date of start of period of validity: _____

Shelf-life approved (months): _____

Expiry date:

Storage conditions: _____

Volume of human dose (in drops and/or ml): _____

Virus titre per single human dose: _____

Type 1: _____

Type 2: _____

Type 3: _____

Nature and concentration of stabilizer: _____

Nature of any antibiotics present in vaccine and amount per human dose: _____

Release date: _____

Summary of source materials

The information requested below is to be presented on each submission. Full details on master and working seed lots should be provided upon first submission only and whenever a change has been introduced.

The following sections are intended for recording the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. If any test has to be repeated, this must be indicated. Any abnormal result must be recorded on a separate sheet.

If any cell lot or virus harvest intended for production is rejected during the control testing, this should also be recorded either in the following sections or on a separate sheet.

Control of source materials (section A.3)

Cell banks (every submission)

Information on cell banking system:

Name and identification of substrate:

Origin and short history: _____

Authority that approved the cell bank: _____

Master cell bank (MCB) and working cell bank (WCB) lot numbers and date of preparation: _____

Date the MCB and WCB were established: _____

Date of approval by national regulatory authority: _____

Total number of ampoules stored: _____

Passage level (or number of population doublings) of cell bank: _____

Maximum passage approved: _____

Storage conditions: _____

Method of preparation of cell bank in terms of number of freezes and efforts made to ensure that a homogeneous population is dispersed into the ampoules: _____

Tests on MCB and WCB, first submission only

Percentage of total cell-bank ampoules tested: _____

Identification test:

Method: _____

Specification: _____

Date of test: _____

Result: _____

Growth characteristics: _____

Morphological characteristics: _____

Immunological marker: _____

Cytogenetic data: _____

Biochemical data: _____

Results of other identity tests: _____

Tests for adventitious agents _____

Method used: _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Tests for bacteria, fungi and mycoplasma
Tests for bacteria and fungi

Method used: _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Volume of medium per vial: _____

Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control:	_____	_____	_____	_____	_____

Test for mycoplasma

Method used: _____

Volume tested: _____

Media used: _____

Temperature of incubation: _____

Observation period (specification): _____

Positive controls (list of species used and results):

	Date of start of test	Date of end of test	Results
Subcultures at day 3:	_____	_____	_____
Subcultures at day 7:	_____	_____	_____
Subcultures at day 14:	_____	_____	_____
Subcultures at day 21:	_____	_____	_____

Indicator cell-culture method (if applicable)

Cell substrate used: _____

Inoculum: _____

Date of test: _____

Passage number: _____

Negative control: _____

Positive controls: _____

Date of staining: _____

Results: _____

Results of tests for tumorigenicity (if applicable): _____

Virus seed A.3.2 (Every submission)

Vaccine virus strain(s) and serotype(s): _____

Substrates used for preparing seed lots: _____

Origin and short history: _____

Authority data approved virus strains: _____

Date of approval: _____

Information and seed lot preparation, every submission (section A.3.2.1)

Virus master seed (VMS), virus sub-master seed, and virus working seed (VWS)

Source of VMS: _____

VMS and VWS lot number: _____

Name and address of manufacturer: _____

VWS passage level from VMS: _____

Dates of inoculation: _____

Dates of harvest: _____

Numbers of containers: _____

Conditions of storage: _____

Dates of preparation: _____

Maximum passages levels authorized: _____

Tests on virus master seed (VMS), virus sub-master seed, and virus working seed (VWS), first submission only

Test for adventitious agents

Date(s) of satisfactory test(s) for freedom from adventitious agent: _____

Volume of virus seed samples for neutralization and testing: _____

Batch number of antisera used for neutralization virus seed: _____

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Identity test _____

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Absence of SV40

Method used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

In vitro tests for molecular characteristics

MAPREC (for Sabin OPV)

Date of test: _____

Type 1 _____

Ratio of % of the sum of both mutations 480-A, 525-C of bulk sample to the International Standard or level of mutations:

Result of test of consistency of production: _____

Result of test of comparison with the International Standard: _____

Type 2 _____

Ratio of % 481-G of bulk sample to the International Standard or level of mutations:

Result of test of consistency of production: _____

Result of test of comparison with the International Standard: _____

Type 3 _____

Ratio of %472C of bulk sample to the International Standard or level of mutations:

Result of test of consistency of production _____

Result of test of comparison with the International Standard _____

HTS (for virus seed, if applicable) Type1 Type2 Type3

Specification: _____

Date of test: _____

Result: _____

In vivo tests for neurovirulence

Neurovirulence test in monkeys:

Result of blood serum test in monkeys prior to inoculation: _____

Number and species of monkeys inoculated: _____

Quantity (CCID50) inoculated in each test monkey: _____

Number of “valid” monkeys inoculated with test sample: _____

Number of positive monkeys observed inoculated with test sample or with reference: _____

Reference preparation: _____

Number of "valid" monkeys inoculated with reference: _____

Number of positive monkeys observed: _____

Mean Lesion Score of test sample: _____

Mean Lesion Score of reference: _____

(see also attached forms giving details of histological observations and assessment)

C1 constant value: _____

Neurovirulence test in transgenic mice

Strain of mice inoculated: _____

For each dose of the seed sample: _____

Number of mice inoculated: _____

Number of mice excluded from evaluation: _____

Number of mice paralysed: _____

Results of validity tests for each dose of the reference virus: _____

Number of mice inoculated: _____

Number of mice excluded from evaluation: _____

Number of mice paralysed: _____

Virus assay results for each dose inoculated (residual inoculums): _____

Paralysis rates for test vaccine at each dose: _____

Paralysis rates for reference virus at each dose: _____

Results: _____

Log odds ratio: _____

L1 and L2 values: _____

Pass/fail decision: _____

Freedom from bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Method used: _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Volume of medium per vial: _____

Observation period (specification) _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Test for mycoplasma

Method used: _____

Volume tested: _____

Media used: _____

Temperature of incubation: _____

Observation period (specification): _____

Positive controls (list of species used and results):

	Date of start of test	Date of end of test	Results
Subcultures at day 3	_____	_____	_____
Subcultures at day 7	_____	_____	_____
Subcultures at day 14	_____	_____	_____
Subcultures at day 21	_____	_____	_____

Indicator cell-culture method (if applicable)

Cell substrate used: _____

Inoculum: _____

Date of test: _____

Passage number: _____

Negative control: _____

Positive controls: _____

Date of staining: _____

Results: _____

Virus titration

Date of test: _____

Reference batch number: _____

Date of test: _____

Result: _____

Genotype characterization

Method used: _____

Date of test: _____

Result _____

Test for mycobacteria

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Control of vaccine production (section A.4)

Control of production cell cultures
Lot number of MCB: _____

Lot number of WCB: _____

Date of thawing of ampoule of WCB: _____

Passage number of production cells: _____

Date of preparation of control cell cultures: _____

Results of microscopic observation: _____

Tests on control cell cultures

Ratio of control to production cell cultures: _____

Incubation conditions: _____

Period of observation of cultures: _____

Dates observation started/ended:

Ratio or proportion of cultures discarded for nonspecific reasons: _____

Results of observation: _____

Date of supernatant fluid collected: _____

Tests for haemadsorbing viruses

Quantity of cell tested: _____

Method used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

Tests for adventitious agents in cell supernatant fluids _____
Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Control of single harvests (section A.4.3)

Lot number(s) _____

Date of inoculation: _____

Temperature of incubation: _____

Date of harvest: _____

Volume harvested: _____

Storage time and approved storage period: _____

Date of sampling: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Virus titration

Method used: _____

Reference batch number: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Result of reference: _____

Tests of neutralized single harvests for adventitious agents _____

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Freedom from bacteria, fungi and mycoplasmas
Tests for bacteria and fungi
Method used _____

Number of vials tested: _____

Volume of inoculum per vial:

Volume of medium per vial: _____

Observation period (specification) _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Test for mycoplasma
Method used: _____

Volume tested: _____

Media used: _____

Temperature of incubation: _____

Observation period (specification): _____

Positive controls (list of species used and results): _____

	Date of start of test	Date of end of test	Results
Sub cultures at 3rd day	_____	_____	_____
Sub cultures at 7th day	_____	_____	_____
Sub cultures at 14th day	_____	_____	_____
Sub cultures at 21th day	_____	_____	_____

Indicator cell-culture method (if applicable)

Cell substrate used: _____

Inoculum: _____

Date of test: _____

Passage number: _____

Negative control: _____

Positive controls: _____

Date of staining: _____

Results: _____

Test for mycobacteria

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Tests for molecular consistency

MAPREC (for Sabin OPV, if applicable)

Date of test: _____

Type 1

Ratio of % of the sum of both mutations 480-A, 525-C of bulk sample to the International Standard or level of mutations: _____

Result of test of consistency of production: _____

Result of test of comparison with the International Standard: _____

Type 2

Ratio of % 481-G of bulk sample to the International Standard or level of mutations: _____

Result of test of consistency of production: _____

Result of test of comparison with the International Standard: _____

Type 3

Ratio of % 472C of bulk sample to the International Standard or level of mutations: _____

Result of test of consistency of production: _____

Result of test of comparison with the International Standard: Type1 Type2 Type3

HTS (if applicable) _____

Specification: _____

Date of test: _____

Result:

Control of monovalent bulk (section A.4.4)

Lot number: _____

Date of filtration of bulk: _____

Porosity of filters used: _____

Date of sampling: _____

Identity test
Method used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

Lot number of reference reagents: _____

Virus titration

Date of test: _____

Reference batch number: _____

Result: _____

Tests for bacteria and fungi
Method used _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Volume of medium per vial: _____

Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control:	_____	_____	_____	_____	_____

Test for mycobacteria
Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Tests for consistency of virus characteristics

MAPREC (for Sabin OPV)
Date of test: _____

Type 1
Ratio of % of the sum of both mutations 480-A, 525-C of bulk sample to the International Standard or level of mutations: _____
Result of test of consistency of production: _____
Result of test of comparison with the International Standard: _____
Type 2
Ratio of % 481-G of bulk sample to the International Standard or level of mutations: _____
Result of test of consistency of production _____

Result of test of comparison with the International Standard: _____
Type 3
Ratio of % 472C of bulk sample to the International Standard or level of mutations _____
Result of test of consistency of production: _____
Result of test of comparison with the International Standard: _____

HTS (if applicable)	Type1	Type2	Type3
Specification:			
Date of test:			
Result:			

Neurovirulence tests for Sabin OPV

Neurovirulence test in monkeys:

Result of blood serum test in monkeys prior to inoculation: _____

Date of inoculation of monovalent bulk: _____

Number and species of monkeys inoculated: _____

Quantity (CCID50) inoculated in each test monkey: _____

Number of "valid" monkeys inoculated with test sample: _____

Number of positive monkeys observed inoculated with test sample or with reference: _____

Reference preparation: _____

Number of "valid" monkeys inoculated with reference: _____

Number of positive monkeys observed: _____

Mean Lesion Score of test sample: _____

Mean Lesion Score of reference: _____

(see also attached forms giving details of histological observations and assessment)

C1 constant value: _____

Neurovirulence test in transgenic mice

Strain of mice inoculated: _____

For each dose of the bulk sample: _____

Number of mice inoculated: _____

Number of mice excluded from evaluation: _____

Number of mice paralysed: _____

Results of validity tests for each dose of the reference virus: _____

Number of mice inoculated: _____

Number of mice excluded from evaluation: _____

Number of mice paralysed: _____

Virus assay results for each dose inoculated (residual inoculums): _____

Paralysis rates for test vaccine at each dose: _____

Paralysis rates for reference virus at each dose: _____

Results: _____

Log odds ratio: _____

L1 and L2 values: _____

Pass/fail decision: _____

Final bulk A.4.5

Preparation of bulk (types as appropriate):	Type 1	Type 2	Type 3
Monovalent bulks in blend:	_____	_____	_____
Volume in blend:	_____	_____	_____
Nature and volume of stabilizer:	_____	_____	_____
Nature and volume of diluent:	_____	_____	_____

Total volume of blend: _____

Storage time and approved storage period: _____

Tests for bacteria and fungi

Method used: _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Volume of medium per vial: _____

Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Filling and containers (section A.5)

Total volume for final filling: _____

Date of filling: _____

Number of vials after inspection: _____

Number of vials filled: _____

Control tests on final lot A.6
Inspection of final containers
Appearance: _____

Date of test: _____

Results: _____

Extractable volume
Extractable volume (ml): _____

The number of drops, using the approved dropper, in a minimum of five individual final containers: _____

pH

Date of test: _____

Result: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

Lot number of reference reagents _____

Tests for bacteria and fungi
Method used: _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Volume of medium per vial: _____

Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results

20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Virus titration

Date of test: _____

Reference batch number: _____

Titre of individual virus types: _____

Batch numbers of antiserum used in test: _____

Date of test: _____

Results	Vaccine	Reference
Type 1:	_____	_____
Type 2:	_____	_____
Type 3:	_____	_____

Thermal stability

Date of test: _____

Batch numbers of antiserum used in test _____

Results:	Vaccine at 37 ºC	Vaccine	Difference
Total virus:	_____	_____	_____

Residual antibiotics (if applicable)

Date of start of test: _____

Date of end of test: _____

Results _____

Level of stabilizer (if applicable)

Date of start of test: _____

Date of end of test: _____

Results: _____

Additional information for production in monkey kidney-cell cultures

Production in monkey kidney-cell cultures

Control of vaccine production _____

Control of monkeys

Monkey species used for production: _____

Quarantine batch number: _____

Percentage of monkeys surviving quarantine period: _____

Nature and concentration of antibiotics used in the production cell culture maintenance medium: _____

Tests for antibodies to simian immunodeficiency virus, SV40, foamy viruses and B virus

Methods used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

Production details

Production monkey number: _____

Date of trypsinizing: _____

Number of cultures prepared: _____

Cell cultures for vaccine production

Virus seed lot number: _____

Virus titre/cell ratio: _____

Number of cultures inoculated: _____

Date of inoculation: _____

Date of harvest: _____

Temperature of incubation: _____

Period of incubation: _____

Number of cultures harvested: _____

Tests on pooled supernatant fluids

Date of sampling from production cell cultures: _____

Tests for adventitious agents: _____

Volume tested/cell culture type: _____

Observation period: _____

Date of completion of tests: _____

Results: _____

Date of sampling from cell cultures inoculated with the pooled fluid _____

Tests for adventitious agents: _____

Volume tested/cell culture type: _____

Date of completion of tests: _____

Results: _____

Tests in rabbit kidney-cell cultures

Volume tested: _____

Date of completion of tests: _____

Results: _____

Control of cell cultures

Ratio of control to production cell cultures or control cell cultures as a proportion of production cell cultures: _____

Period of observation of cultures: _____

Ratio or proportion of cultures discarded for nonspecific reasons: _____

Results: _____

Tests for haemadsorbing viruses

Methods: _____

Results: _____

Tests for other adventitious agents _____

Methods: _____

Results: _____

Control of single harvests

Volume harvested: _____

Date of sampling: _____

Tests for bacteria, fungi, and mycoplasmas: _____

Results: _____

Tests on neutralized single harvests in monkey kidney-cell and human cell cultures

Batch number of antiserum used: _____

Volume tested: _____

Date of starting primary cell culture tests: _____

Period of observation: _____

Date of sampling cell culture fluids: _____

Period of observation: _____

Date of completion of tests: _____

Results: _____

Control of monovalent bulk

Tests in rabbits

Number and weight of animals: _____

Date of inoculation: _____

Results of injection: _____

Quantity injected: _____

Results (survival numbers, etc.): _____

Date of filtration of bulk: _____

Porosity of filters used: _____

Date of sampling: _____

Tests for retroviruses

Methods: _____

Date: _____

Results : _____

1
2

Certification by the manufacturer

Name of head of production and/or quality control (typed) _____

Certification by the person from the control laboratory of the manufacturing company taking overall responsibility for the production and quality control of the vaccine:

I certify that lot no. _____ of poliomyelitis vaccine (oral), whose number appears on the label of the final container, meets all national requirements and/or satisfies Part A of the WHO *Recommendations to assure the quality, safety and efficacy of live attenuated poliomyelitis vaccine (oral)*.

Signature: _____

Name (typed): _____
Date: ____

Certification by the NRA/NCL

If the vaccine is to be exported, attach the model NRA/NCL Lot Release Certificate for poliomyelitis vaccine (oral) (as shown in Appendix 6), a label from a final container and an instruction leaflet for users.

Appendix 6

Model certificate for the release of poliomyelitis vaccines (oral, live, attenuated) by NRAs

This certificate is to be provided by the NRA or NCL of the country in which the vaccine has been manufactured, and/or released on request by the manufacturer.

Certificate no. _____

The following lot(s) of live attenuated poliomyelitis vaccine (oral) produced by _____[(1)] in _____[(2)] whose numbers appear on the labels of the final containers, comply with the relevant specification in the marketing authorization[(3)] and the provisions for the release of biological products and Part A[(4)] of WHO's Recommendations to assure the quality, safety and efficacy of live attenuated poliomyelitis vaccines (oral) (_____)[(5)], and comply with WHO good manufacturing practices: main principles for pharmaceutical products[(6)], WHO Good manufacturing practices for biological products[(7)] and WHO Guidelines for independent lot release of vaccines by regulatory authorities[(8)].

The release decision is based on _____[(9)].

The release decision is based on _____
Final lot number _____
Number of human doses released in this final lot _____
Expiry date _____

The certificate may also include the following information:
- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and/or common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and/or expiry date
- storage conditions;
- signature and function of the authorized person and authorized agent to issue the certificate;
- date of issue of certificate;
- certificate number.

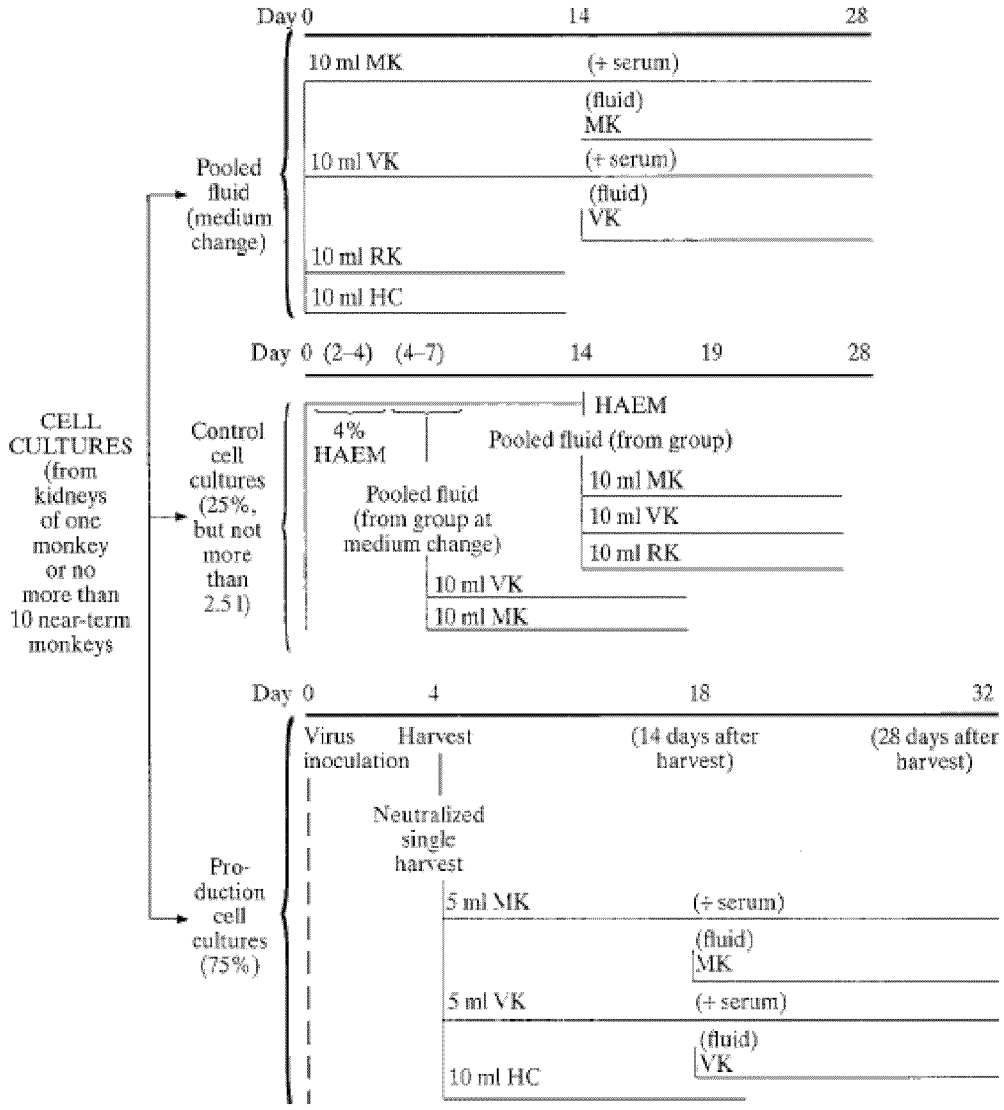
The Director of the NRA/NCL (or other appropriate authority):
Name (typed) _____
Signature _____
Date _____

Footnotes:

1 Name of manufacturer.
2 Country of origin.
3 If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the NRA or NCL.
4 With the exception of provisions on distribution and transport, which the NRA or NCL may not be in a position to assess.
5 WHO Technical Report Series, No. (xx, xxxx).
6 WHO Technical Report Series, No. 986, Annex 2.
7 WHO Technical Report Series, No. 999, Annex 2.
8 WHO Technical Report Series, No. 978, Annex 2.
9 Evaluation of the product-specific summary protocol, independent laboratory testing, and/or specific procedures laid down in defined document, and so on as appropriate.

Appendix 7

Preparation of poliomyelitis vaccines (oral, live, attenuated) using primary monkey kidney cells – example of a flowsheet



HAEM = test for haemadsorbing viruses.
MK = monkey kidney cells from species (but not the same animal) used for production.
VK = kidney cells from vervet monkey or one sensitive to SV40 virus.
RK = rabbit kidney cells.
HC = human cells sensitive to measles.

Note: This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the NRA, this flowsheet should not be considered as an integral part of the requirements and has been included solely for guidance. Manufacturing establishments should prepare their own flowsheet in order to clarify the procedures used.

Appendix 8

International reference materials for poliomyelitis vaccines (oral, live, attenuated)

This appendix describes available international reference materials for OPV, which were developed for Sabin OPV and can be accessed through NIBSC[1] and WHO[2] catalogues. International reference materials for nOPV will be needed, particularly WHO International Standards for the three serotype versions of nOPV should be developed which will likely require monovalent and trivalent formulations. Current neurovirulence references used for MNVT and TgmNVT for Sabin OPV are suitable for nOPV products. Similarly, international standards for anti-poliovirus antibodies, S19 hyper-attenuated poliovirus strains and anti-polio monoclonal antibody sera are suitable for nOPV quality control assays. Finally, specific international reference materials for molecular quality control assays based on HTS will be required for Sabin OPV and nOPV products, respectively.

WHO International Standards and International Reference Preparations are available to ensure manufacture and quality control testing of the different versions of Sabin OPV meet appropriate regulatory requirements.

International Standards for the potency testing of tOPV have been available since 1995. More recently, new International Standards have been established for bOPV, mOPV1, mOPV2 and mOPV3, with compositions and potencies similar to vaccines needed for the final phase of the GPEI.

> The 1st International Standard for the potency estimation of trivalent OPV (NIBSC Catalogue number: 85/659) was established by the WHO ECBS in 1995 as a mixture of three commercially produced monovalent bulks - one of each poliovirus (Sabin) types 1, 2 and 3 (1). Following depletion of stocks of this standard, the 2nd International Standard was established by the WHO ECBS in 2004 (2), calibrated against International Standard 85/659. The composition of the 2nd International Standard was kept as close as possible to the 1st International Standard to allow direct comparison of the two preparations e.g. in stability studies. The 2nd International Standard (02/306) was also prepared by mixing three commercially produced and released monovalent bulks - one of each poliovirus (Sabin) types 1, 2 and 3. The passage level of the virus in the bulks was: Sabin original (SO) + 3 for type 1, SO+3 for type 2 and a re-derived SO (RSO)+3 for type 3. All three bulks used in the production of this standard were produced on primary monkey cells (PMKC). The standard was prepared by blending the three polio serotypes monovalent bulks in MEM with 1% w/v bovine albumin and sodium bicarbonate buffer. The assigned potency for 02/306 was set at: 7.51, 6.51, 6.87 and 7.66 $\log_{10}$ TCID50/ml for type 1, 2, 3 and total virus content, respectively. The same bulk materials used to produce the 2nd International Standard 02/306 were used to prepare candidate preparations for bOPV, mOPV1, mOPV2 and mOPV3 in a similar manner, which were established as International Standards by WHO ECBS in 2017 (3). The 1st International Standard for

[1] https://www.nibsc.org/products/brm_product_catalogue.aspx
[2] https://www.who.int/activities/providing-international-biological-reference-preparations

bOPV 1+3 (16/164) was assigned potencies 7.19, 6.36 and 7.32 log10 TCID50/ml for type 1, 3 and total poliovirus content, respectively. The 1st International Standards for mOPV1 (16/196), mOPV2 (15/296) and mOPV3 (16/202) were assigned potencies 7.19, 6.36 and 7.32 log10 TCID50/ml for type 1, 2 and 3 poliovirus, respectively. Monoclonal antibody sera against type 1, 2 and 3 poliovirus, 02/256 (NIBSC batch number 425), 02/258 (NIBSC batch number 267) and 02/260 (NIBSC batch number 495), respectively, are available and routinely used globally by a number of manufacturers and NRAs for potency assays of bivalent and trivalent OPV.

Additionally, low titer monovalent type 1, 2 and 3 poliovirus WHO reference reagents are available for use in reference laboratories to measure the sensitivity of cell cultures for poliovirus infection.

Low titer monovalent Sabin type 1, 2 and 3 poliovirus reference reagents were prepared using the same bulk materials used to produce the current International Standards for monovalent, bivalent and trivalent OPV with assigned potencies as follows (4):

– Type 1 (01/528): 5.1 log 10 CCID50/0.1ml in RD cells and 4.9 log 10 CCID50/0.1 ml in L20B cells;
– Type 2 (01/530): 5.1 log 10 CCID50/0.1ml in RD cells and 4.8 log 10 CCID50/0.1ml in L20B cells;
– Type 3 (01/532): 5.3 log 10 CCID50/0.1ml in RD cells and 4.9 log 10 CCID50/0.1ml in L20B cells.

Following depletion of stocks of these reagents, new virus reference stocks were prepared from the same original material. The new monovalent reference reagents were established with assigned potencies as follows:

- Type 1 (10/164): 5.5 log 10 CCID50/0.1ml in RD cells and 5.3 log 10 CCID50/0.1 ml in L20B cells;
- Type 2 (10/166): 5.1 log 10 CCID50/0.1ml in RD cells and 4.8 log 10 CCID50/0.1ml in L20B cells;
- Type 3 (10/168): 5.3 log 10 CCID50/0.1ml in RD cells and 4.8 log 10 CCID50/0.1ml in L20B cells.

An International Standard for anti-poliovirus types 1, 2 and 3 antibodies (human) is also available for the standardization of neutralizing antibody tests for poliovirus.

The 1st International Standards for anti-poliovirus sera types 1, 2 and 3 were established by the WHO ECBS in 1963 from serotype-specific polyclonal antisera produced by hyper-immunisation of rhesus monkeys with live virus suspensions (5). Each of the standards was specific to one serotype only. They were established through a collaborative study (WHO, 1963) and assigned a unitage of 10 IU/vial, for each of the polio serotypes (5). The 2nd International Standard (66/202) was established by the WHO ECBS in 1991 to replace the 1st International Standards. Stocks of the 1st International Standards ran very low around 1989 and a replacement for the 1st International Standards was selected through a collaborative study (6). In contrast to the 1st International Standard, the 2nd International Standard was a single serum that contained activity against each of the three poliovirus serotypes (7). The following unitage was assigned to the 2nd International Standard: 25 IU of anti-poliovirus serum (type 1) human; 50 IU of anti-poliovirus serum (type 2) human;

and 5 IU of anti-poliovirus serum (type 3) human. Following exhaustion of 66/202, the 3rd International Standard for anti-poliovirus sera (Human) types 1, 2 and 3 (82/585) was established by the WHO ECBS in 2006 with assigned unitage of 11, 32 and 3 IU per vial of neutralising antibody to type 1, 2 and 3 poliovirus respectively (8).

International Standards for MAPREC analysis of poliovirus types 1, 2 and 3 (Sabin) and International Reference Reagents for control of MAPREC assays of poliovirus type 1, 2 and 3 (Sabin) are available (9). Some of these references might be useful for HTS assays (10) or new reference might be needed for this purpose.

International Standards and Reference Reagents were prepared from commercial vaccines and viruses generated by cell culture infection. The list of MAPREC references currently available is as follows (11-13):

- 00/410: MAPREC assay of poliovirus type 1 (Sabin).100% 480-A, 525-C DNA (1st International Reference Preparation).
- 00/416: MAPREC assay of poliovirus type 1 (Sabin) Low Mutant Virus Reference (1st International Reference Preparation).
- 00/418: MAPREC assay of poliovirus type 1 (Sabin) (1st International Standard)
- 00/422: MAPREC assay of poliovirus type 1 (Sabin) High Mutant Virus Reference(1st International Reference Preparation).
- 97/758: MAPREC analysis of Poliovirus type 2 (Sabin) Synthetic DNA 0.67%481-G (1st International Standard)
- 98/524: MAPREC analysis of Poliovirus type 2 (Sabin) Synthetic DNA, 100%481-G (1st International Standard)
- 98/596: MAPREC analysis of Poliovirus type 2 (Sabin), high virus reference 1.21% 481-G (1st International Reference Preparation)
- 94/790: MAPREC analysis of Poliovirus type 3 (Sabin). Synthetic DNA 100% 472-C. WHO (1st International Standard)
- 95/542: MAPREC analysis of Poliovirus type 3 (Sabin) Synthetic DNA 0.9% 472-C. WHO (1st International Standard)
- 96/572: MAPREC analysis of Poliovirus type 3 (Sabin) Low virus reference 0.7%472-C (1st International Reference Preparation)
- 96/578: MAPREC analysis of Poliovirus type 3 (Sabin) High virus reference 1.1%472-C (1st International Reference Preparation)
- 97/756: MAPREC analysis of Poliovirus type 2 (Sabin), low virus reference 0.65%481-G (1st International Reference Preparation)

Reference preparations at the SO+2 passage level, designated WHO/I for type 1 virus, WHO/II for type 2 virus and WHO/III for type 3 virus, are available upon request through WHO. These reference preparations are for use in in vivo neurovirulence tests with vaccines. The relevant reference materials should be included in each test of vaccine (see section A.4.4.7.2). Virus panels for validation and implementation of the transgenic mouse neurovirulence test, as specified in the WHO SOP (14), are also available.

New non-pathogenic, hyper-attenuated poliovirus strains, S19, are available for quality control assays of OPV (15). S19 strains are polioviruses that replicate in tissue culture but are unlikely to

replicate at all in humans should they be exposed even to large amounts and for this reason they can be used outside GAPIII containment requirements.

> The strains are genetically stable and include a portfolio of strains containing the capsid proteins (and thus having the antigenic properties) of the Sabin OPV strains or the wild type strains used most commonly in the production of inactivated polio vaccine. In December 2018, the WHO Containment Advisory Group concluded that the S19 strain can be used outside of the containment requirements of GAPIII for neutralization assays (16). Organizations wishing to use S19 poliovirus strains should follow a detailed validation process to ensure the genetic properties of S19 strains are maintained and can be used to replace current original poliovirus strains. There is a seed-lot system to produce banks of highly characterized S19 strains resembling vaccine production. NIBSC suggests that S19 strains should be tested on a seed lot basis to minimize the risks of reversion and will work with any suitable facility to help generate and validate further banks.

The reference materials listed above are available from the National Institute for Biological Standards and Control, Potters Bar, United Kingdom[1].

References

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[1] National Institute for Biological Standards and Control, Potters Bar, United Kingdom: https://www.nibsc.org/

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9. WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage (MAPREC) for Oral Poliovirus (Sabin) Vaccine Types 1, 2 or 3. Version 5 (2012). https://www.who.int/publications/m/item/maprec-sop-for-opv-types-1-2-or-3, accessed 9 May 2022.
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Comments and suggestions from reviewer

Title: **Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccine (oral, live, attenuated)**
Proposed replacement of Annex 2 of WHO Technical Report Series, No. 980

(WHO/BS/2022.2423)

Reviewer's Name (first name/last name): **Affiliation:** **Country:** **Email:**

(Table is expandable)

Sections/page and line No.	Original Text	Comment	Suggested Amendment	Internal Use Only [blank]
General comments (if you have)				
Introduction				
Purpose and scope				
Terminology				

Sections/page and line No.	Original Text	Comment	Suggested Amendment	Internal Use Only [blank]
General considerations				
International reference materials				
Part A. Manufacturing recommendations				
Part B. Nonclinical evaluation of poliomyelitis vaccines (oral, live, attenuated)				
Part C. Clinical evaluation of poliomyelitis vaccines (oral, live, attenuated)				
Part D. Recommendations for NRAs				
Part E. Recommendations for poliomyelitis vaccines (oral, live, attenuated) prepared in primary monkey kidney cells				
Authors and acknowledgements				
References				

Sections/page and line No.	Original Text	Comment	Suggested Amendment	Internal Use Only [blank]
Appendix 1. Overview of virus seeds used in OPV production				
Appendix 2. In vivo tests for neurovirulence, and considerations in relation to assay choice				
Appendix 3. Preparation of poliomyelitis vaccines (oral, live, attenuated) using cell banks – example of a flowsheet				
Appendix 4. Cell-culture techniques for determining the virus content of poliomyelitis vaccines (oral, live, attenuated)				
Appendix 5. Model summary protocol for the manufacturing and control of poliomyelitis vaccines (oral, live, attenuated)				
Appendix 6. Model certificate for the release of poliomyelitis vaccines (oral, live, attenuated) by NRAs				
Appendix 7. Preparation of poliomyelitis vaccines (oral, live, attenuated) using primary monkey kidney cells – example of a flowsheet				
Appendix 8. International reference materials for poliomyelitis vaccines (oral, live, attenuated)				
Other comments (if you have)				

Wu, Tong (HC/SC)

From: Wu, Tong (HC/SC)
Sent: 2023-07-19 6:55 PM
To: Wall, Michael (HC/SC)
Subject: IAS for SV40 promoter

Hi Mike,

Co agreed to have an IAS for the SV40 promoter sequence as we discussed today. We can talk about it tomorrow.

Thanks,
tong

Wu, Tong (HC/SC)

From: Wu, Tong (HC/SC)
Sent: 2023-08-03 11:34 AM
To: Davis, Elisabeth (HC/SC)
Subject: FW: Draft clarifax questions RE: SV40

From: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>
Sent: Thursday, July 27, 2023 9:17 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Subject: Draft clarifax questions RE: SV40

Hi Tong, here are a couple of ideas for clarifax questions to Pfizer.

1. Sequence analysis of plasmids [redacted] and [redacted] reveal the presence of the SV40 promoter, including [redacted] and the purpose of the SV40 promoter in these plasmids.
2. The WHO guidance for residual DNA in biologic products stipulates that fragments should not be greater than 200bp. As such, provide the following information:
    a. Is there, or has there ever been, a size analysis performed for residual DNA template in COMIRNATY DS?
    b. Is there, or has there ever been, an analysis performed for residual intact circular DNA template in the linearized DNA template starting material?

Mike

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-08-04 9:23 AM
To: Wu, Tong (HC/SC)
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Excellent Tong and thanks for the copy on that!
Dean

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Friday, August 4, 2023 9:08 AM
To: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Cc: Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Kenneth,

Can you send out the following Quality Clarifax for the above mentioned NDS-CV:

1. **Sequence analysis of plasmid [redacted] (XBB 1.5) indicates the presence of the regulatory region sequences of Simian Virus (SV40), located in the plasmid region of [redacted] Provide the following:**
   a. **[redacted]**
   b. **Justification for the SV40 regulatory elements in the plasmid.**

2. **Update Table 3.2.S.2.3-1 (Functional Elements of [redacted] Omicron XBB.1.5) to include the following plasmid [redacted] XBB 1.5) sequences:**
   a **[redacted]**
   b
   c

3. **Concerning the residual plasmid DNA in the drug substance, provide data/information characterizing the following:**
   a. **the size distribution of the residual DNA fragments.**
   b. **residual intact circular plasmid.**

We will accommodate the timeline requested by Pfizer for the response.

Thanks,
tong

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Monday, July 31, 2023 3:22 PM
To: Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>; Blahoianu, Maria (HC/SC) <maria.blahoianu@hc-sc.gc.ca>; Tabbasum, Valentina (HC/SC) <valentina.tabbasum@hc-sc.gc.ca>; Willcott, Marsha (HC/SC)

<marsha.willcott@hc-sc.gc.ca>; Lacroix, Jeremie (HC/SC) <jeremie.lacroix@hc-sc.gc.ca>; Clark-Knowles, Katherine (HC/SC) <Katherine.Clark-Knowles@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; NG, Queenie (HC/SC) <queenie.ng@hc-sc.gc.ca>; Adoni, Harsha Vardhan (HC/SC) <harsha.vardhan.adoni@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; Eng, David (HC/SC) <david.eng@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Hanna, Jessy (HC/SC) <jessy.hanna@hc-sc.gc.ca>
Cc: HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Fandja, Patrick (HC/SC) <patrick.fandja@hc-sc.gc.ca>; Fung, Winnie (HC/SC) <winnie.fung@hc-sc.gc.ca>; BRDD ORIRM / BRRGR DMBR (HC/SC) <brdd.orirm-brrgr.dmbr@hc-sc.gc.ca>
Subject: RE: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The Rolling Sequence #2 is now available for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302: HC6-024-e276302 (0002) Biologic Dossier

Notes:

- Due to delays, the sponsor has combined the planned files for Roll #2 with Roll #3. The application plan has been updated.
- The following documents have been provided in this roll:
    - Annotated and non-annotated English PM. The French PM will be provided during the review.
    - Non-Canadian Labelling
    - Revised Canadian Addendum RMP
    - Company Core Data Sheet (the CDS has not yet been updated to include the simplified posology proposed in the Canadian Product Monograph and SmPC)
    - Module 2.3 Introduction, QOS and 2.3.S, 2.3.P and 2.3.A documents sections
    - Module 3 documents to supported the updated antigen composition for Omicron XBB.1.5; clone documents in Module 3 from previous Comirnaty, Comirnaty Original & Omicron BA.4/5 submissions

Thank you,
Kenneth

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Friday, July 21, 2023 6:52 PM
To: Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>; Blahoianu, Maria (HC/SC) <maria.blahoianu@hc-sc.gc.ca>; Tabbasum, Valentina (HC/SC) <valentina.tabbasum@hc-sc.gc.ca>; Willcott, Marsha (HC/SC) <marsha.willcott@hc-sc.gc.ca>; Lacroix, Jeremie (HC/SC) <jeremie.lacroix@hc-sc.gc.ca>; Clark-Knowles, Katherine (HC/SC) <Katherine.Clark-Knowles@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; NG, Queenie (HC/SC) <queenie.ng@hc-sc.gc.ca>; Adoni, Harsha Vardhan (HC/SC) <harsha.vardhan.adoni@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; Eng, David (HC/SC) <david.eng@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Hanna, Jessy (HC/SC) <jessy.hanna@hc-sc.gc.ca>
Cc: HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>
Subject: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Good afternoon all,

The following submission is ready for review:

Submission Type: NDS-CV **Control No.:** 276302

Product Name: Comirnaty Omicron XBB.1.5 (raxtozinameran)

Sponsor Name: Pfizer Canada ULC on behalf of BioNTech

Link to sequence on docubridge rolling sequence #1: HC6-024-e276302 (0000) Biologic Dossier
Link to screening clarifax response sequence: HC6-024-e276302 (0001) Biologic Dossier

Link to screening report: HC6-024-e276302 (1.0) Reg Info - Health Product ***New***

Submission Notes:

- This submission provides for a 30 µg, 10 µg and 3 µg formulation of the BNT162b2 Omicron (XBB.1.5) Variant drug product.
- The sponsor notes that the NDS-CV is supported largely by data previously filed under the COMIRNATY and COMIRNATY ORIGINAL & OMICRON BA.4/BA.5.
- The sponsor states that all Module 2, 4 and 5 components previously filed to Health Canada have been cloned as part of this first roll.
- According to the Application Plan, the following Rolling Sequences will be provided with the following information:
  - Roll #1 (June 29, 2023) – Module 1 Administrative and Product Information documents, Cloned Module 2, 4, 5 documents
  - Roll #2 (July 17, 2023) – English Product Monograph (Annotated and Non-annotated), Foreign Labels, Cloned Module 3, EU RMP (TBD)
  - Roll #3 (August 2023) – French Product Monograph (Non-annotated), LASA Assessment, Canadian Addendum RMP (TBD), How to Administer & Quick References Guides

Update* Roll #2 has been delayed and will be combined with Roll 3 (Week of July 24). Due to the expedited timelines of review, the courtesy copy of the PM has been requested by ORA.

LABEL:

- According to the Application Plan, the sponsor will be submitting the MAA vial and carton labels; EUA, USPI and/or SmPC in Roll #2. A Health Risk Product Communication (HPRC) is required for this submission due to the use of global labels.

- Updated *How to Administer* and *Quick Reference* Guides will be provided in Roll #3 (August 2023).

MHPD:

- The EU RMP is scheduled to be submitted (TBD) in Roll #3 (August 2023) in accordance with the Application Plan.
- In a correspondence, the sponsor will be requesting the submit the Canadian Addendum RMP Post-Approval of this submission under Terms and Conditions. Further discussion will be carried out between MHPD and the sponsor for the submission of the Canadian Addendum RMP.

Thanks,

Kenneth Garay
Senior Regulatory Affairs Officer
Office of Regulatory Affairs
Biologic and Radiopharmaceutical Drugs Directorate

Health Products and Food Branch/Health Canada
kenneth.garay@hc-sc.gc.ca / Tel: 437-324-8892

Agent principal des affaires réglementaires
Bureau des affaires réglementaires
Direction des médicaments biologiques et radiopharmaceutiques
Direction générale des produits de santé et des aliments / Santé Canada
kenneth.garay@hc-sc.gc.ca / Tel: 437-324-8892

Smith, Dean (HC/SC)

Subject: Brief chat on SV40 and residual plasmid DNA in DP
Location: Microsoft Teams Meeting

Start: Thu 2023-08-17 7:00 AM
End: Thu 2023-08-17 7:15 AM
Show Time As: Tentative

Recurrence: (none)

Meeting Status: Not yet responded

Organizer: Smith, Dean (HC/SC)
Required Attendees: [redacted]@ema.europa.eu)

Hi [redacted]
If this works for you and your holiday commitments, I'll open the Teams meeting when the RAG meeting finishes, regardless of the time.
However, as mentioned in my last email, if this doesn't work for you, we can postpone until next week or whenever you're back from your holiday.
All the best!
Dean

Microsoft Teams meeting

Join on your computer, mobile app or room device
Click here to join the meeting

Meeting ID: 281 600 994 924
Passcode: [redacted]
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Video Conference ID: 118 578 656 4
Alternate VTC instructions

Or call in (audio only)
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Téléchargez Teams | Participez sur le web

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teams@hc-sc.phac-aspc.video.canada.ca
No de vidéoconférence : 118 578 656 4
Autres instructions VTC

Ou composez le numéro de téléphone (audio seulement)
+1 343-644-9946,,918451399# Canada, Ottawa
No de conférence téléphonique : 918 451 399#
Recherchez un numéro local | Réinitialisez le NIP

Pour en savoir plus | Options de réunion

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-08-21 9:45 AM
To: [redacted]
Subject: RE: [redacted] RE: Quick follow up on the Ad-Hoc Cluster meeting this week

Hi [redacted]
Thanks for the feed back. I haven't seen the agenda as yet.
If the SV40 topic were added, it would just be an FYI as to what we plan to do, as you and I discussed, to see if we are all on the same page. This is a pubic topic now and we are approaching the next vaccination season for these vaccines.
If it seems to need more than that, then that we would have to be scheduled for a follow up.
Thanks again,
Dean

From: [redacted]@ema.europa.eu>
Sent: Monday, August 21, 2023 9:39 AM
To: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: [redacted] RE: Quick follow up on the Ad-Hoc Cluster meeting this week

Hi Dean,

I would be fine, but consider FDA dropped a number of other very relevant topics from the agenda…

[redacted]

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Monday, 21 August 2023 15:31
To: [redacted]@ema.europa.eu>
Subject: [redacted] RE: Quick follow up on the Ad-Hoc Cluster meeting this week

Hi [redacted]
I'm considering writing to [redacted] to see if we can add a short discussion on the SV40 topic at the end of the Cluster meeting this Thursday, just to begin to coordinate that.
Any concerns or thoughts on that?
Dean

Dean Smith, Ph.D.
Senior Scientific Evaluator
Vaccine Quality Division 1
Advisor
Centre for Vaccines, Clinical Trials & Biostatistics
Health Canada, 100 Eglantine Driveway, Tunney's Pasture
Ottawa, Ontario
Canada K1A 0K9
Mob: +1 613-799-5689

Wu, Tong (HC/SC)

From: Wu, Tong (HC/SC)
Sent: 2023-08-23 11:57 AM
To: Smith, Dean (HC/SC)
Subject: RE: FYI Tong RE: [redacted] and SV40 enhance/promoter sequence and other non-essential sequences in Pfizer's plasmid for their COVID-19 vaccines

Thanks!

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Wednesday, August 23, 2023 11:55 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Subject: FYI Tong RE: [redacted] and SV40 enhance/promoter sequence and other non-essential sequences in Pfizer's plasmid for their COVID-19 vaccines

FYI

From: [redacted]@fda.hhs.gov>
Sent: Wednesday, August 23, 2023 11:13 AM
To: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: [EXTERNAL] [redacted] RE: SV40 enhance/promoter sequence and other non-essential sequences in Pfizer's plasmid for their COVID-19 vaccines

Dear Dean,

Nice job, as always, facilitating a global discussion among NRAs.

Thank you for raising the topic. Yes, the topic has been discussed internally and with [redacted]

"No objection" from CBER/OVRR to adding a brief mention of this topic at tomorrow's Cluster meeting.

Lookig forward to seeing tomorrow.

Be well,
[redacted]

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Wednesday, August 23, 2023 10:42 AM
To: [redacted]@fda.hhs.gov>
Subject: [EXTERNAL] [redacted] RE: SV40 enhance/promoter sequence and other non-essential sequences in Pfizer's plasmid for their COVID-19 vaccines
Importance: High

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Dear [redacted]

It was good to see you at the CEPI-WHO RAG meeting last Thursday morning, in spite of the hour. 😊

I understand that there have been internal discussions at CBER regarding the presents of an SV40 enhancer/promoter sequence, noting that its presence is unrelated to the purpose of the Pfizer's plasmid as a transcription template for their mRNA COVID-19 vaccine.

Pfizer has communicated to us recently, that they apparently chose not to mention this information to EMA, FDA or HC at the time of their initial or subsequent submissions. However, as of April of this year this information was independently made public, which has resulted to questions coming to agencies.

At HC we do not view this an urgent risk topic. However, given the fall COVID-19 vaccination campaigns, it may be useful to be on the same page on this topic between agencies. Time permitting, a brief exchange at the end of tomorrow's Cluster Meeting could also help inform potential exchanges between Agencies and Pfizer on the SV40 topic, as well as future actions we may collectively consider taking subsequently to encourage Pfizer to remedy the situation prior to a potential fall 2024 COVID-19 vaccine campaign. It would be unfortunate if the information circulating had a negatively impact on public acceptance of the vaccine this year or in the future.

Following the last CEPI-WHO RAG meeting, [redacted] and I spoke about the SV40 sequence in the plasmid. [redacted] is open to a brief mention of this issue at the Cluster Meeting if time permits and CBER agrees.

Attached is the pre-print posted in April 2023 (also available at this link) which was also presented at the June 15 VRBPAC by Dr. Kevin McKernan during the Public Comments section of the meeting (i.e., it is under 5 minutes).

If you have any questions or concerns, I'm happy to follow up with you.

Thank you in advance for considering this request.

Best regards,
Dean

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-08-23 2:54 PM
To: [redacted]
Subject: FW: [EXTERNAL] [redacted] RE: SV40 enhance/promoter sequence and other non-essential sequences in Pfizer's plasmid for their COVID-19 vaccines
Attachments: Sequencing of bivalent_4-11-23.docx

From: [redacted]@fda.hhs.gov>
Sent: Wednesday, August 23, 2023 11:13 AM
To: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: [EXTERNAL] [redacted] RE: SV40 enhance/promoter sequence and other non-essential sequences in Pfizer's plasmid for their COVID-19 vaccines

Dear Dean,

Nice job, as always, facilitating a global discussion among NRAs.

Thank you for raising the topic. Yes, the topic has been discussed internally and with [redacted]

"No objection" from CBER/OVRR to adding a brief mention of this topic at tomorrow's Cluster meeting.

Lookig forward to seeing tomorrow.

Be well,
[redacted]

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Wednesday, August 23, 2023 10:42 AM
To: [redacted]@fda.hhs.gov>
Subject: [EXTERNAL] [redacted] RE: SV40 enhance/promoter sequence and other non-essential sequences in Pfizer's plasmid for their COVID-19 vaccines
Importance: High

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Dear [redacted]

It was good to see you at the CEPI-WHO RAG meeting last Thursday morning, in spite of the hour. 😊

I understand that there have been internal discussions at CBER regarding the presents of an SV40 enhancer/promoter sequence, noting that its presence is unrelated to the purpose of the Pfizer's plasmid as a transcription template for their mRNA COVID-19 vaccine.

Pfizer has communicated to us recently, that they apparently chose not to mention this information to EMA, FDA or HC at the time of their initial or subsequent submissions. However, as of April of this year this information was independently made public, which has resulted to questions coming to agencies.

At HC we do not view this an urgent risk topic. However, given the fall COVID-19 vaccination campaigns, it may be useful to be on the same page on this topic between agencies. Time permitting, a brief exchange at the end of tomorrow's Cluster Meeting could also help inform potential exchanges between Agencies and Pfizer on the SV40 topic, as well as future actions we may collectively consider taking subsequently to encourage Pfizer to remedy the situation prior to a potential fall 2024 COVID-19 vaccine campaign. It would be unfortunate if the information circulating had a negatively impact on public acceptance of the vaccine this year or in the future.

Following the last CEPI-WHO RAG meeting, [redacted] and I spoke about the SV40 sequence in the plasmid. [redacted] is open to a brief mention of this issue at the Cluster Meeting if time permits and CBER agrees.

Attached is the pre-print posted in April 2023 (also available at this link) which was also presented at the June 15 VRBPAC by Dr. Kevin McKernan during the Public Comments section of the meeting (i.e., it is under 5 minutes).

If you have any questions or concerns, I'm happy to follow up with you.

Thank you in advance for considering this request.

Best regards,
Dean

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-08-22 4:44 PM
To: Baca-Estrada, Maria (HC/SC)
Cc: Ekewenu, Edith (HC/SC); Willcott, Marsha (HC/SC); Blahoianu, Maria (HC/SC); Tabbasum, Valentina (HC/SC)
Subject: Re: EMA/FDA/HC Cluster meeting agenda

Hi Maria,

I assumed you and your clinical colleagues would be addressing all the questions given the explicit clinical focus of the meeting . :)

If there is time I will raise the SV40 topic.

All the best!
Dean

Sent from my iPhone

> On Aug 22, 2023, at 3:35 PM, Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca> wrote:
>
> Hi Edith,
> Here are the names of HC speakers for the Cluster meeting.
>
> **Dean,** if you don't mind, we would like to address the last point on question 5 regarding the periodic updated COVID-19 vaccines (Valentina will address the first part regarding intervals).
>
> *Subject: Interval between last dose of COVID-19 vaccine [i.e., bivalent (Original plus Omicron BA.4/BA.5) or monovalent prototype (Original)] and **periodic updated COVID-19 vaccine**.*
>
> We think this is related to how HC will support address periodic updates, as we have discussed many times it would be in collaboration with international partners etc… we feel you are in a better position to address this given your international collaborations etc…
>
> Let me know if you have any questions
> Thank you all
> Maria
> <(EMA-FDA-HC)_Vaccine_Cluster_TC_FINAL_Agenda_2023_Aug_24_.(002).docx>

REVISED MEETING AGENDA
VACCINE CLUSTER
EMA/FDA/HC
THURSDAY, AUGUST 24, 2023

Washington, DC/Ottawa TIME **09:00am - 11:00Am (EDT)//**

Amsterdam/Geneva TIME **15:00 – 17:00 (CET)**

EMA Co-Chair: [redacted]
FDA/CBER Co-Chair: [redacted]
HC Co-Chair: Co Pham

European Medicines Agency (EMA): [redacted] **(Lead)**
EMA Liaison Officer to the FDA: [redacted]
EMA, Health Threats and Vaccines Strategy: [redacted]
EMA Anti-infectives and Vaccines [redacted]
EMA, Pediatrics: [redacted]
EMA, Scientific Advice: [redacted]
EMA, Quality of Medicines: [redacted]
EMA ETF and Vaccine Working Party: [redacted]

Additional CHMP experts: [redacted]
Additional experts: [redacted]

US Food & Drug Administration (FDA):
CBER Office of Vaccines Research and Review: [redacted]

CBER Office of the Center Director: [redacted]
CBER Office of Biostatistics and Epidemiology: [redacted]
CBER Office of Compliance and Biologics Quality: [redacted]
CBER International Affairs: [redacted]
FDA Office of International Program: [redacted]

Health Canada (HC)

BRDD - Centre for Vaccines, Clinical Trials and Biostatistics: Co Pham, Catherine Njue, Gina Coleman, Fabian Schwarz, Stubbert Lawton, Tong Wu, Dean Smith, Jason Fernandes, Stella Yuen, Naomi De Silva, Liang Ming, Maria Baca-Estrada, Richard Siggers, Julie Joseph, Judy Zhang; Cao Ming YU, Rudy Zimmer, Mark Akins, Gayle Pulle, Guanhua Wang, Ningli Zhang, Cynthia Allen, Nathalie Fortin, Kim Pronovost, Sanath Rao, Charlene Young, Richard Isbrucker

BRDD – Centre of Policy, Pediatrics and International Collaboration: Edith Ekewenu,

Item	Topics	Speakers	Duration
	Co-chairs: [redacted] Co Pham		
1.	**Introductions** **Confidentiality Statement**	EMA Co-Chair: [redacted] FDA/CBER Co-Chair: [redacted] HC Co-Chair: Co Pham	5 min
2.	**Adoption of the agenda**	All	5 min
3.	**Subject:** Pediatric age cut-off for single dose versus two or more dose series ➢ Exchange of viewpoints and perspectives.	EMA ( ) HC (Maria Blahoianu) FDA ()	min
	Actions Items:		

4.	**Subject:** Harmonization of immunization schedule in 4–5-year-olds ➢ Exchange of viewpoints and perspectives.	EMA ( ) HC (Maria Blahoianu) FDA ( )	min
	Actions Items:		
5.	**Subject:** Dosing interval for additional doses in certain immunocompromised individuals 6 months of age and older. ➢ Exchange of viewpoints and perspectives.	EMA ( ) FDA ( ) HC (Marsha Willcott)	min
	Actions Items:		
6.	**Subject:** Additional doses in higher risk individuals from 50 – 64 years of age ➢ Exchange on viewpoints and perspectives.	EMA ( ) FDA ( ) HC (Marsha Willcott)	min
	Actions Items:		
7.	**Subject:** Interval between last dose of COVID-19 vaccine [i.e., bivalent (Original plus Omicron BA.4/BA.5) or monovalent prototype (Original)] and periodic updated COVID-19 vaccine. ➢ Exchange on viewpoints and perspectives.	EMA ( ) FDA ( ) HC (Valentina Tabbasum and Dean Smith)	min

	Actions Items:		
8.	**Review Action Items and Closing Remarks**	EMA Co-Chair: [redacted] FDA/CBER Co-Chair: [redacted] HC Co-Chair: Co Pham	5 min

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FDA ZOOM

[redacted]fda.hhs.gov is inviting you to a scheduled ZoomGov meeting.

Join ZoomGov Meeting
https://fda.zoomgov.com/j[redacted]

Meeting ID: 161 759 3709
Passcode: [redacted]
One tap mobile
+16692545252,,1617593709#,,,,*030823# US (San Jose)
+16469641167,,1617593709#,,,,*030823# US (US Spanish Line)

Dial by your location
+1 669 254 5252 US (San Jose)
+1 646 964 1167 US (US Spanish Line)
+1 646 828 7666 US (New York)
+1 415 449 4000 US (US Spanish Line)
+1 551 285 1373 US
+1 669 216 1590 US (San Jose)
833 568 8864 US Toll-free
833 435 1820 US Toll-free
Meeting ID: 161 759 3709
Passcode: [redacted]
Find your local number: https://fda.zoomgov.com/[redacted]

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-08-25 12:50 PM
To: Wu, Tong (HC/SC); Baca-Estrada, Maria (HC/SC)
Cc: Wall, Michael (HC/SC); Davis, Elisabeth (HC/SC); Siggers, Richard (HC/SC); Joseph, Julie (HC/SC); Irwin, Chad (HC/SC)
Subject: RE: SV40 sequence - Pfizer's response

Thanks Tong.

Glad this was raised this yesterday and not all is lost.

There is a small window still to work on this, when the current authorizations are behind everyone.

Additionally, at least Pfizer's reply acknowledges "minimal risk". [redacted]

It will still require FDA and EMA support, but that may still be achievable.

And, as you pointed out before your clarifax when out, the negative reply supports our need to coordinate with our international partners.

In the current climate, that is a win for us and thank to you and your team for that!!

Dean

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Friday, August 25, 2023 12:21 PM
To: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>; Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>
Cc: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Joseph, Julie (HC/SC) <julie.joseph@hc-sc.gc.ca>; Irwin, Chad (HC/SC) <chad.irwin@hc-sc.gc.ca>
Subject: SV40 sequence - Pfizer's response

As expected, Pfizer didn't commit.

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-08-29 3:33 PM
To: Wu, Tong (HC/SC); Wall, Michael (HC/SC)
Cc: Joseph, Julie (HC/SC); Davis, Elisabeth (HC/SC); Siggers, Richard (HC/SC)
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Tong,

How is this hybrid?

1. In the initial clarifax response, it was suggested that the regulatory sequence elements in question were non-functional with respect to the manufacturing process. [redacted]

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Tuesday, August 29, 2023 11:54 AM
To: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Smith, Dean (HC/S C) <dean.smith@hc-sc.gc.ca>
Cc: Joseph, Julie (HC/SC) <julie.joseph@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear both,

I agree with Dean that we should not tell Pfizer our interaction with EMA and FDA on this (especially they do not seem to care much at this moment). However, we can not say nothing! Please see the following text that Julie and I worked out. Let me know your thoughts:

The following comment relates to Pfizer's response to the Quality Clarifax dated August 22, 2023. It is for information only and a formal response is not required at this time:

1. [redacted]

Thanks,
tong

From: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>
Sent: Tuesday, August 29, 2023 9:52 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Tong,

Here's a draft of the clarifax questions:

1. In the previous clarifax responses, it was indicated that the provided sequence elements were non-functional with regard to the manufacturing process. [redacted]

2. Health Canada will continue to work with international regulatory partners to achieve harmonisation regarding removal of these sequence elements from the plasmid for future strain changes.

Mike

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Monday, August 28, 2023 2:21 PM
To: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Thanks!
None.

From: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>
Sent: Monday, August 28, 2023 2:15 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>
Subject: FW: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Tong, Lis,

The latest draft for the XBB.1.5 review report is attached.

Mike

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Tuesday, August 22, 2023 4:20 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>
Cc: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Joseph, Julie (HC/SC) <julie.joseph@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>

Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The quality clarifax #2 has been issued for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302 : HC6-024-e276302 (1.0) Reg Info - Health Product

The response is due Thursday, August 24, 2023.

Kind Regards,
Kenneth

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Tuesday, August 22, 2023 2:56 PM
To: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>
Cc: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Joseph, Julie (HC/SC) <julie.joseph@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Gary,

Could you please send out the following quality clarifax?

1. **Health Canada is not aware of any peer-reviewed scientific evidence that would raise safety concerns over** [redacted] **Please comment.**

2. **Health Canada notes the commitment to provide additional data and/or information characterizing the size distribution of residual DNA fragments and residual intact circular plasmid. We ask that the data package also address whether the residual DNA plasmid is capable of replication in bacteria.**

Thanks,
tong

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Friday, August 11, 2023 1:57 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Cc: Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines

Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>; Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The response sequence for the Quality Clarifax for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302 is now available on docubridge.

Link to sequence in dB: HC6-024-e276302 (0004) Biologic Dossier

Notes:

- Further to the response to the quality clarifax, the sponsor has included the Canadian Addendum RMP and the second language PM (pre-approval).

Thank you,
Kenneth

From: Garay, Kenneth (HC/SC)
Sent: Friday, August 4, 2023 1:37 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Cc: Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The quality clarifax was issued Comirnaty Omicron XBB.1.5, NDS-CV Control # 276302: HC6-024-e276302 (1.0) Reg Info - Health Product

The response is due August 9, 2023.

Kind Regards,
Kenneth

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Friday, August 4, 2023 9:08 AM
To: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Cc: Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Kenneth,

Can you send out the following Quality Clarifax for the above mentioned NDS-CV:

ATIA - 20(1)(b) ATIA - 20(1)(c)

1. **Sequence analysis of plasmid [redacted] XBB 1.5) indicates the presence of the regulatory region sequences of Simian Virus (SV40), located in the plasmid region of [redacted] Provide the following:**
   a. **[redacted]**
   b. **Justification for the SV40 regulatory elements in the plasmid.**

2. **Update Table 3.2.S.2.3-1 (Functional Elements of pVV-01505 Omicron XBB.1.5) to include the following plasmid [redacted] XBB 1.5) sequences:**
   a. **[redacted]**
   b. **[redacted]**
   c. **[redacted]**

3. **Concerning the residual plasmid DNA in the drug substance, provide data/information characterizing the following:**
   a. **the size distribution of the residual DNA fragments.**
   b. **residual intact circular plasmid.**

We will accommodate the timeline requested by Pfizer for the response.

Thanks,
tong

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Monday, July 31, 2023 3:22 PM
To: Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>; Blahoianu, Maria (HC/SC) <maria.blahoianu@hc-sc.gc.ca>; Tabbasum, Valentina (HC/SC) <valentina.tabbasum@hc-sc.gc.ca>; Willcott, Marsha (HC/SC) <marsha.willcott@hc-sc.gc.ca>; Lacroix, Jeremie (HC/SC) <jeremie.lacroix@hc-sc.gc.ca>; Clark-Knowles, Katherine (HC/SC) <Katherine.Clark-Knowles@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; NG, Queenie (HC/SC) <queenie.ng@hc-sc.gc.ca>; Adoni, Harsha Vardhan (HC/SC) <harsha.vardhan.adoni@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; Eng, David (HC/SC) <david.eng@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Hanna, Jessy (HC/SC) <jessy.hanna@hc-sc.gc.ca>
Cc: HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Fandja, Patrick (HC/SC) <patrick.fandja@hc-sc.gc.ca>; Fung, Winnie (HC/SC) <winnie.fung@hc-sc.gc.ca>; BRDD ORIRM / BRRGR DMBR (HC/SC) <brdd.orirm-brrgr.dmbr@hc-sc.gc.ca>
Subject: RE: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The Rolling Sequence #2 is now available for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302: HC6-024-e276302 (0002) Biologic Dossier

Notes:

- Due to delays, the sponsor has combined the planned files for Roll #2 with Roll #3. The application plan has been updated.
- The following documents have been provided in this roll:
  - Annotated and non-annotated English PM. The French PM will be provided during the review.
  - Non-Canadian Labelling
  - Revised Canadian Addendum RMP

- Company Core Data Sheet (the CDS has not yet been updated to include the simplified posology proposed in the Canadian Product Monograph and SmPC)
- Module 2.3 Introduction, QOS and 2.3.S, 2.3.P and 2.3.A documents sections
- Module 3 documents to supported the updated antigen composition for Omicron XBB.1.5; clone documents in Module 3 from previous Comirnaty, Comirnaty Original & Omicron BA.4/5 submissions

Thank you,
Kenneth

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Friday, July 21, 2023 6:52 PM
To: Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>; Blahoianu, Maria (HC/SC) <maria.blahoianu@hc-sc.gc.ca>; Tabbasum, Valentina (HC/SC) <valentina.tabbasum@hc-sc.gc.ca>; Willcott, Marsha (HC/SC) <marsha.willcott@hc-sc.gc.ca>; Lacroix, Jeremie (HC/SC) <jeremie.lacroix@hc-sc.gc.ca>; Clark-Knowles, Katherine (HC/SC) <Katherine.Clark-Knowles@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; NG, Queenie (HC/SC) <queenie.ng@hc-sc.gc.ca>; Adoni, Harsha Vardhan (HC/SC) <harsha.vardhan.adoni@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; Eng, David (HC/SC) <david.eng@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Hanna, Jessy (HC/SC) <jessy.hanna@hc-sc.gc.ca>
Cc: HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>
Subject: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Good afternoon all,

The following submission is ready for review:

Submission Type: NDS-CV **Control No.:** 276302

Product Name: Comirnaty Omicron XBB.1.5 (raxtozinameran)

Sponsor Name: Pfizer Canada ULC on behalf of BioNTech

Link to sequence on docubridge rolling sequence #1: HC6-024-e276302 (0000) Biologic Dossier
Link to screening clarifax response sequence: HC6-024-e276302 (0001) Biologic Dossier

Link to screening report: HC6-024-e276302 (1.0) Reg Info - Health Product ***New***

Submission Notes:

- This submission provides for a 30 µg, 10 µg and 3 µg formulation of the BNT162b2 Omicron (XBB.1.5) Variant drug product.
- The sponsor notes that the NDS-CV is supported largely by data previously filed under the COMIRNATY and COMIRNATY ORIGINAL & OMICRON BA.4/BA.5.
- The sponsor states that all Module 2, 4 and 5 components previously filed to Health Canada have been cloned as part of this first roll.
- According to the Application Plan, the following Rolling Sequences will be provided with the following information:
  - Roll #1 (June 29, 2023) – Module 1 Administrative and Product Information documents, Cloned Module 2, 4, 5 documents

- Roll #2 (July 17, 2023) – English Product Monograph (Annotated and Non-annotated), Foreign Labels, Cloned Module 3, EU RMP (TBD)
- Roll #3 (August 2023) – French Product Monograph (Non-annotated), LASA Assessment, Canadian Addendum RMP (TBD), How to Administer & Quick References Guides

Update* Roll #2 has been delayed and will be combined with Roll 3 (Week of July 24). Due to the expedited timelines of review, the courtesy copy of the PM has been requested by ORA.

LABEL:

- According to the Application Plan, the sponsor will be submitting the MAA vial and carton labels; EUA, USPI and/or SmPC in Roll #2. A Health Risk Product Communication (HPRC) is required for this submission due to the use of global labels.

- Updated *How to Administer* and *Quick Reference* Guides will be provided in Roll #3 (August 2023).

MHPD:

- The EU RMP is scheduled to be submitted (TBD) in Roll #3 (August 2023) in accordance with the Application Plan.
- In a correspondence, the sponsor will be requesting the submit the Canadian Addendum RMP Post-Approval of this submission under Terms and Conditions. Further discussion will be carried out between MHPD and the sponsor for the submission of the Canadian Addendum RMP.

Thanks,

Kenneth Garay
Senior Regulatory Affairs Officer
Office of Regulatory Affairs
Biologic and Radiopharmaceutical Drugs Directorate
Health Products and Food Branch/Health Canada
kenneth.garay@hc-sc.gc.ca / Tel: 437-324-8892

Agent principal des affaires réglementaires
Bureau des affaires réglementaires
Direction des médicaments biologiques et radiopharmaceutiques
Direction générale des produits de santé et des aliments / Santé Canada
kenneth.garay@hc-sc.gc.ca / Tel: 437-324-8892

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-08-30 2:35 PM
To: Wu, Tong (HC/SC)
Subject: RE: 3rd Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Looks good Tong!
Thanks for this effort!!
Dean

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Wednesday, August 30, 2023 1:25 PM
To: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>
Cc: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Joseph, Julie (HC/SC) <julie.joseph@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: 3rd Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Kenneth,

Please send the following clarifax/comments to Pfizer, which doesn't require a response.

The following comment relates to Pfizer's response to the Quality Clarifax dated August 22, 2023. It is for information only and a formal response is not required at this time:

1. [redacted]

Thanks,
tong

From: Pal, Yasmeen (HC/SC) <yasmeen.pal@hc-sc.gc.ca>
Sent: Friday, August 25, 2023 11:44 AM
To: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>
Cc: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Joseph, Julie (HC/SC) <julie.joseph@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: Response: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The response to the quality clarifax issued for NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302 is now available on dB and has been pushed to your workload: HC6-024-e276302 (0008) Biologic Dossier

Thanks,
Yasmeen (on behalf of Ken)

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Tuesday, August 22, 2023 4:20 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>
Cc: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Joseph, Julie (HC/SC) <julie.joseph@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The quality clarifax #2 has been issued for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302 : HC6-024-e276302 (1.0) Reg Info - Health Product

The response is due Thursday, August 24, 2023.

Kind Regards,
Kenneth

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Tuesday, August 22, 2023 2:56 PM
To: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>
Cc: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Joseph, Julie (HC/SC) <julie.joseph@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Gary,

Could you please send out the following quality clarifax?

1. **Health Canada is not aware of any peer-reviewed scientific evidence that would raise safety concerns over [redacted]. Please comment.**

2. **Health Canada notes the commitment to provide additional data and/or information characterizing the size distribution of residual DNA fragments and residual intact circular plasmid. We ask that the data package also address whether the residual DNA plasmid is capable of replication in bacteria.**

Thanks,
tong

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Friday, August 11, 2023 1:57 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Cc: Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>; HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>; Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The response sequence for the Quality Clarifax for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302 is now available on docubridge.

Link to sequence in dB: HC6-024-e276302 (0004) Biologic Dossier

Notes:

- Further to the response to the quality clarifax, the sponsor has included the Canadian Addendum RMP and the second language PM (pre-approval).

Thank you,
Kenneth

From: Garay, Kenneth (HC/SC)
Sent: Friday, August 4, 2023 1:37 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Cc: Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The quality clarifax was issued Comirnaty Omicron XBB.1.5, NDS-CV Control # 276302: HC6-024-e276302 (1.0) Reg Info - Health Product

The response is due August 9, 2023.

Kind Regards,

Kenneth

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Friday, August 4, 2023 9:08 AM
To: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Cc: Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: Quality Clarifax: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Kenneth,

Can you send out the following Quality Clarifax for the above mentioned NDS-CV:

1. **Sequence analysis of plasmid [redacted] XBB 1.5) indicates the presence of the regulatory region sequences of Simian Virus (SV40), located in the plasmid region of [redacted] Provide the following:**
   a. [redacted]
   b. **Justification for the SV40 regulatory elements in the plasmid.**

2. **Update Table 3.2.S.2.3-1 (Functional Elements of [redacted] Omicron XBB.1.5) to include the following plasmid [redacted] (XBB 1.5) sequences:**
   a. [redacted]
   b. [redacted]
   c. [redacted]

3. **Concerning the residual plasmid DNA in the drug substance, provide data/information characterizing the following:**
   a. **the size distribution of the residual DNA fragments.**
   b. **residual intact circular plasmid.**

We will accommodate the timeline requested by Pfizer for the response.

Thanks,
tong

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Monday, July 31, 2023 3:22 PM
To: Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>; Blahoianu, Maria (HC/SC) <maria.blahoianu@hc-sc.gc.ca>; Tabbasum, Valentina (HC/SC) <valentina.tabbasum@hc-sc.gc.ca>; Willcott, Marsha (HC/SC) <marsha.willcott@hc-sc.gc.ca>; Lacroix, Jeremie (HC/SC) <jeremie.lacroix@hc-sc.gc.ca>; Clark-Knowles, Katherine (HC/SC) <Katherine.Clark-Knowles@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; NG, Queenie (HC/SC) <queenie.ng@hc-sc.gc.ca>; Adoni, Harsha Vardhan (HC/SC) <harsha.vardhan.adoni@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; Eng, David (HC/SC) <david.eng@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Hanna, Jessy (HC/SC) <jessy.hanna@hc-sc.gc.ca>
Cc: HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Fandja, Patrick (HC/SC) <patrick.fandja@hc-sc.gc.ca>; Fung, Winnie (HC/SC) <winnie.fung@hc-sc.gc.ca>; BRDD ORIRM / BRRGR DMBR (HC/SC) <brdd.orirm-brrgr.dmbr@hc-sc.gc.ca>
Subject: RE: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The Rolling Sequence #2 is now available for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302: HC6-024-e276302 (0002) Biologic Dossier

Notes:

- Due to delays, the sponsor has combined the planned files for Roll #2 with Roll #3. The application plan has been updated.
- The following documents have been provided in this roll:
  - Annotated and non-annotated English PM. The French PM will be provided during the review.
  - Non-Canadian Labelling
  - Revised Canadian Addendum RMP
  - Company Core Data Sheet (the CDS has not yet been updated to include the simplified posology proposed in the Canadian Product Monograph and SmPC)
  - Module 2.3 Introduction, QOS and 2.3.S, 2.3.P and 2.3.A documents sections
  - Module 3 documents to supported the updated antigen composition for Omicron XBB.1.5; clone documents in Module 3 from previous Comirnaty, Comirnaty Original & Omicron BA.4/5 submissions

Thank you,
Kenneth

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Friday, July 21, 2023 6:52 PM
To: Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>; Blahoianu, Maria (HC/SC) <maria.blahoianu@hc-sc.gc.ca>; Tabbasum, Valentina (HC/SC) <valentina.tabbasum@hc-sc.gc.ca>; Willcott, Marsha (HC/SC) <marsha.willcott@hc-sc.gc.ca>; Lacroix, Jeremie (HC/SC) <jeremie.lacroix@hc-sc.gc.ca>; Clark-Knowles, Katherine (HC/SC) <Katherine.Clark-Knowles@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; NG, Queenie (HC/SC) <queenie.ng@hc-sc.gc.ca>; Adoni, Harsha Vardhan (HC/SC) <harsha.vardhan.adoni@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; Eng, David (HC/SC) <david.eng@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Hanna, Jessy (HC/SC) <jessy.hanna@hc-sc.gc.ca>
Cc: HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>
Subject: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Good afternoon all,

The following submission is ready for review:

Submission Type: NDS-CV **Control No.:** 276302

Product Name: Comirnaty Omicron XBB.1.5 (raxtozinameran)

Sponsor Name: Pfizer Canada ULC on behalf of BioNTech

Link to sequence on docubridge rolling sequence #1: HC6-024-e276302 (0000) Biologic Dossier
Link to screening clarifax response sequence: HC6-024-e276302 (0001) Biologic Dossier

Link to screening report: HC6-024-e276302 (1.0) Reg Info - Health Product *New*

Submission Notes:

- This submission provides for a 30 µg, 10 µg and 3 µg formulation of the BNT162b2 Omicron (XBB.1.5) Variant drug product.
- The sponsor notes that the NDS-CV is supported largely by data previously filed under the COMIRNATY and COMIRNATY ORIGINAL & OMICRON BA.4/BA.5.
- The sponsor states that all Module 2, 4 and 5 components previously filed to Health Canada have been cloned as part of this first roll.
- According to the Application Plan, the following Rolling Sequences will be provided with the following information:
  - Roll #1 (June 29, 2023) – Module 1 Administrative and Product Information documents, Cloned Module 2, 4, 5 documents
  - Roll #2 (July 17, 2023) – English Product Monograph (Annotated and Non-annotated), Foreign Labels, Cloned Module 3, EU RMP (TBD)
  - Roll #3 (August 2023) – French Product Monograph (Non-annotated), LASA Assessment, Canadian Addendum RMP (TBD), How to Administer & Quick References Guides

Update* Roll #2 has been delayed and will be combined with Roll 3 (Week of July 24). Due to the expedited timelines of review, the courtesy copy of the PM has been requested by ORA.

LABEL:

- According to the Application Plan, the sponsor will be submitting the MAA vial and carton labels; EUA, USPI and/or SmPC in Roll #2. A Health Risk Product Communication (HPRC) is required for this submission due to the use of global labels.

- Updated *How to Administer* and *Quick Reference* Guides will be provided in Roll #3 (August 2023).

MHPD:

- The EU RMP is scheduled to be submitted (TBD) in Roll #3 (August 2023) in accordance with the Application Plan.
- In a correspondence, the sponsor will be requesting the submit the Canadian Addendum RMP Post-Approval of this submission under Terms and Conditions. Further discussion will be carried out between MHPD and the sponsor for the submission of the Canadian Addendum RMP.

Thanks,

Kenneth Garay
Senior Regulatory Affairs Officer
Office of Regulatory Affairs
Biologic and Radiopharmaceutical Drugs Directorate
Health Products and Food Branch/Health Canada
kenneth.garay@hc-sc.gc.ca / Tel: 437-324-8892

Agent principal des affaires réglementaires
Bureau des affaires réglementaires
Direction des médicaments biologiques et radiopharmaceutiques
Direction générale des produits de santé et des aliments / Santé Canada
kenneth.garay@hc-sc.gc.ca / Tel: 437-324-8892

Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

Kevin McKernan, Yvonne Helbert, Liam T. Kane, Stephen McLaughlin
Medicinal Genomics, 100 Cummings Center, Suite 406-L, Beverly Mass, 01915

Several methods were deployed to assess the nucleic acid composition of four expired vials of the Moderna and Pfizer bivalent mRNA vaccines. Two vials from each vendor were evaluated with Illumina sequencing, qPCR, RT-qPCR, Qubit™ 3 fluorometry and Agilent Tape Station™ electrophoresis. Multiple assays support DNA contamination that exceeds the European Medicines Agency (EMA) 330ng/mg requirement and the FDAs 10ng/dose requirements. These data may impact the surveillance of vaccine mRNA in breast milk or plasma as RT-qPCR assays targeting the vaccine mRNA cannot discern DNA from RNA without RNase or DNase nuclease treatments. Likewise, studies evaluating the reverse transcriptase activity of LINE-1 and vaccine mRNA will need to account for the high levels of DNA contamination in the vaccines. The exact ratio of linear fragmented DNA versus intact circular plasmid DNA is still being investigated. Quantitative PCR assays used to track the DNA contamination are described.

Introduction

Several studies have made note of prolonged presence of vaccine mRNA in breast milk and plasma (Bansal et al. 2021; Hanna et al. 2022; Castruita et al. 2023). This could be the result of the stability of N1-methylpseudouridine (m1Ψ) in the mRNA of the vaccine. Nance *et al*. depict a vaccine mRNA synthesis method that utilizes a dsDNA plasmid that is first amplified in *E.coli* prior to an *in-vitro* T7 polymerase synthesis of vaccine mRNA (Nance and Meier 2021). Failure to remove this DNA could result in the injection of spike encoded nucleic acids more stable than the modified RNA. The EMA has stated limits at 330ng/mg of DNA to RNA (Josephson 2020-11-19). The FDA has issued guidance for under 10ng/dose in vaccines (Sheng-Fowler et al. 2009). Residual injected DNA can result in type I interferon responses and can increase the potential for DNA integration(Ulrich-Lewis et al. 2022).

Results

To assess the nucleic acid composition of the vaccines, vaccine DNA was deeply sequenced using two different methods. The first method used a commercially available New England Biolabs RNA-seq method that favored the sequencing of the RNA but still presented over 500X coverage for the unanticipated DNA vectors (Figure 1 and 2). The RNA-seq assemblies had truncated poly A tracts compared to the constructs described by Nance *et al*. The second method eliminated the RNA with RNase A treatment and sequenced only the DNA using a Watchmaker Genomics fragment library kit. The DNA focused assemblies delivered vector assemblies with more intact poly A tracts (Figure 3).

These assemblies were utilized to design multiplex qPCR and RT-qPCR assays that target the spike sequence present in both the vaccine mRNA and the DNA vector while also targeting the origin of replication sequence present only in the DNA vector (Figure 3). The assembly of Pfizer vial 1 contains a 72bp insertion not present in the assembly of Pfizer vial 2. This indel is known

for its enhancement to the SV40 promoter and its nuclear localization signal (Dean et al. 1999) (Moreau et al. 1981).

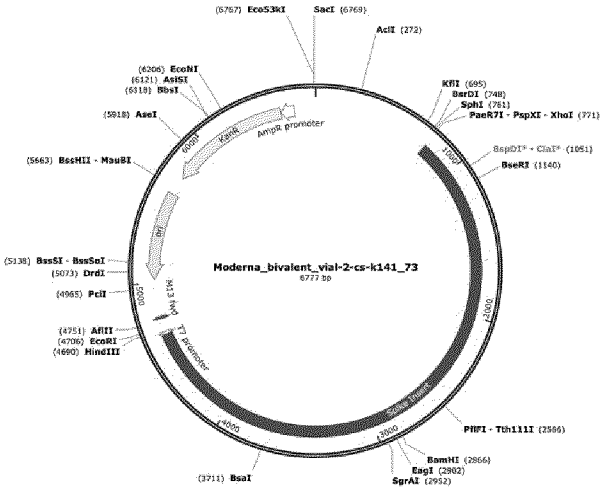


Figure 1. A Moderna vector assembly of an RNA-seq library with a spike insert (red), Kanamycin resistance gene (green) driven by an AmpR promoter and a high copy bacterial origin of replication (yellow).

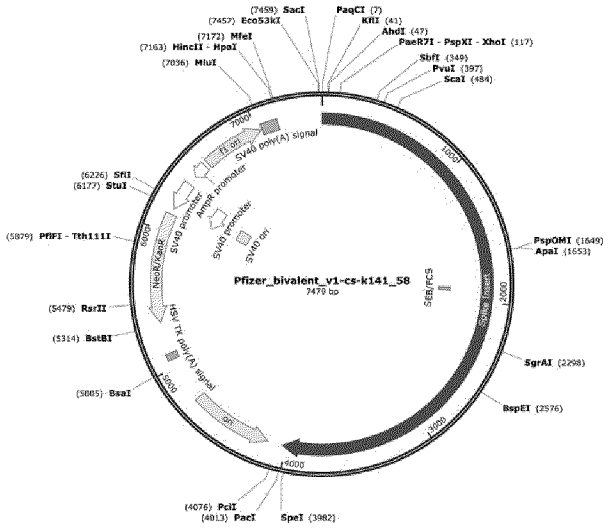


Figure 2. Pfizer bivalent vaccine assembly of the RNA-seq library. Annotated with SEB/FCS, spike insert (red), bacterial origin of replication (yellow), Neo/Kan resistance gene(green), F1 origin (yellow) and an SV40 promoter (yellow and white).

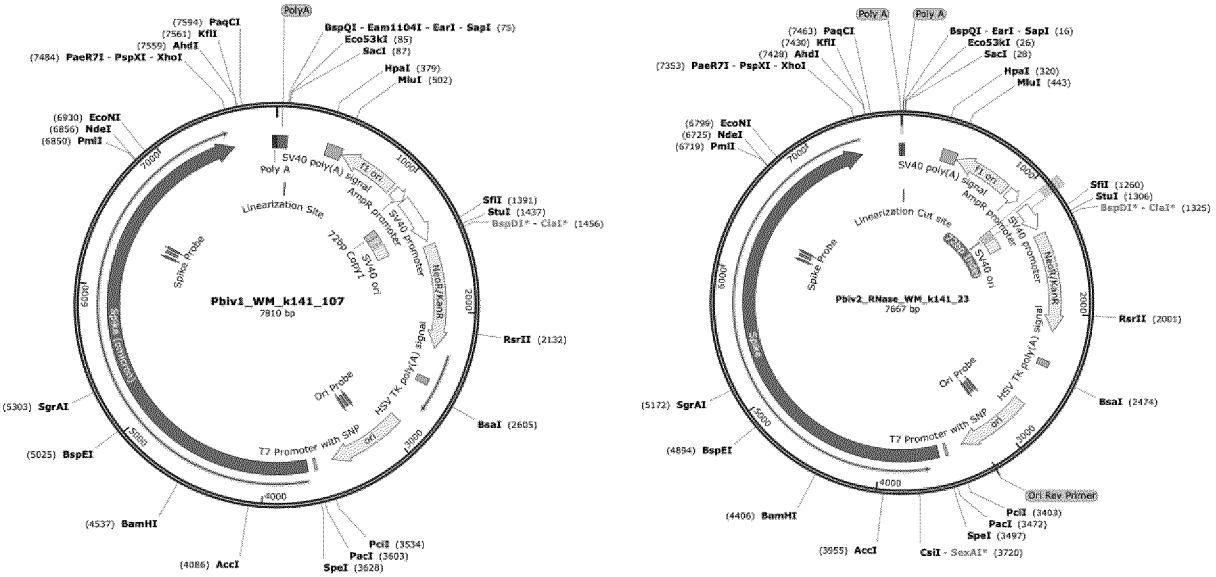


Figure 3. RNase treated vaccines were shotgun sequenced with Illumina (RNase-Seq not RNA-seq). Pfizer vectors from vial 1 (left) and vial 2 (right) contain a 72bp difference in the SV40 promoter (green and light blue annotation). qPCR assays are depicted in pink as Spike probe and Ori probe. The RNase sequencing provided better resolution over the Eam1104i linearization site and the Poly adenylation sequence. The vectors differ in the length of the polyA tail (likely sequencing artifact) and the 72bp indel.

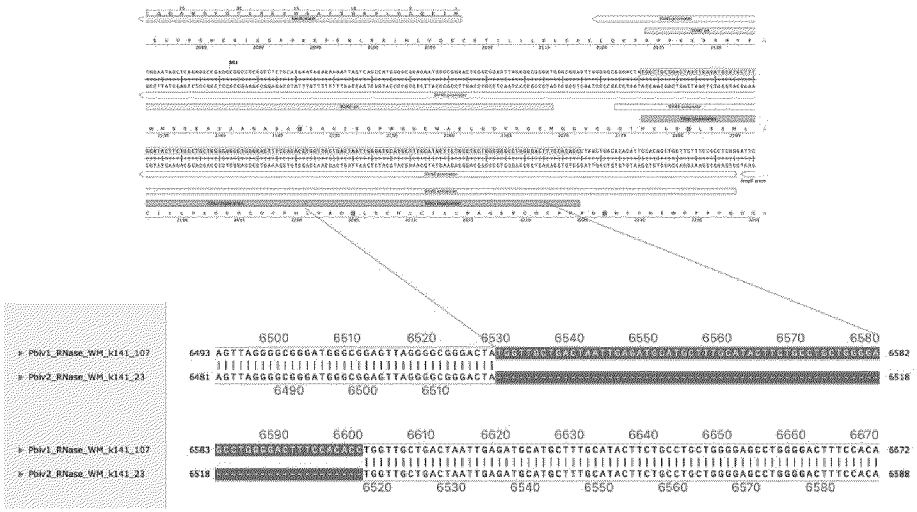


Figure 4. Local alignment of Pfizer vial 1 to Pfizer vial 2 vectors highlights the 72bp tandem duplication in blue.

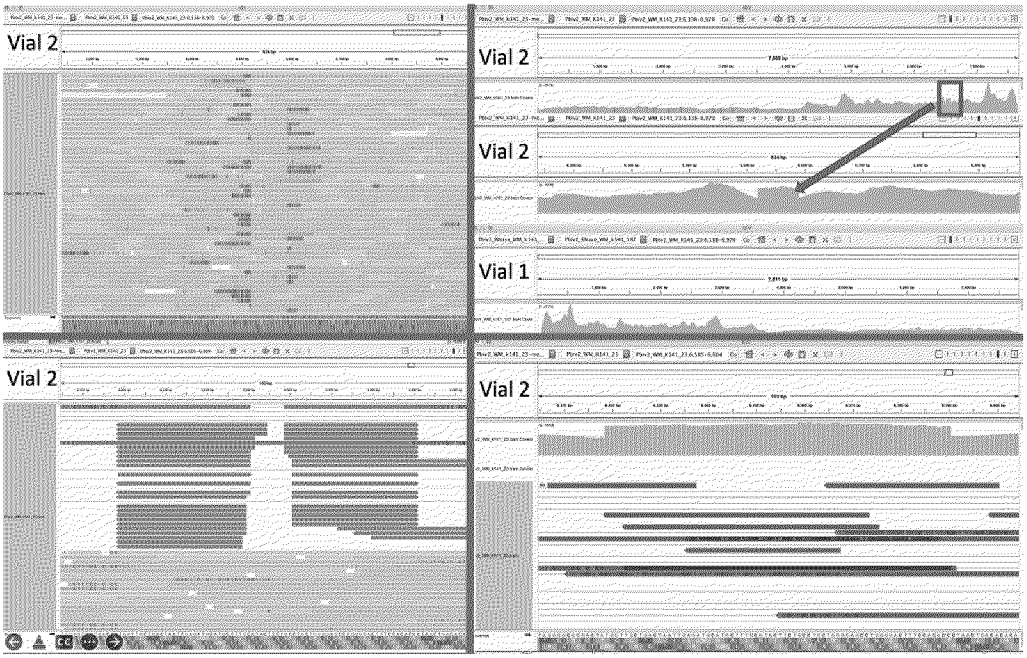


Figure 5A. Close inspection of the Integrative Genome Viewer (IGV) demonstrates the appearance of a 72bp insertion that is heteroplasmic in Pfizer vial 2. The upper left IGV view is a zoomed-out view where the colored marks depict the indel. The lower Left IGV view shows inverted paired reads as the 72bp insertion is a tandem repeat and paired reads shorter than 72bp can be mapped two different ways. Upper Right IGV view demonstrates a read coverage pile up or 'Plateau'. This occurs when the reference has one copy of the 72bp repeat and the sample has 2 copies. Note- In the upper right IGV depiction, the sequence in Vial 1 is in the opposite orientation in IGV as Vial 2. Lower right IGV view is a zoomed view of the upper right IGV screen.

Since the two Pfizer vials share the same lot number, finding a heterozygous copy number change between the two vials is unexpected. It was hypothesized that the appearance of a heteroplasmic copy number change is instead the result of the Megahit assembler collapsing what is actually two copies of the 72bp sequence into a single copy due to the insert sizes in the sequencing libraries being too short (105bp). It is noteworthy that the longer paired-end reads in the library resolve the 72bp tandem repeat.

When references have a single copy of the 72bp repeat and the sample has two copies of the repeat, reads should pile up to twice the coverage over the single copy 72bp loci as seen in Figure 5A. To test this hypothesis, we added a second 72bp sequence to the shorter plasmid assembly and observed that the reads map without artifact and no evidence of heteroplasmy (Figure 5B).

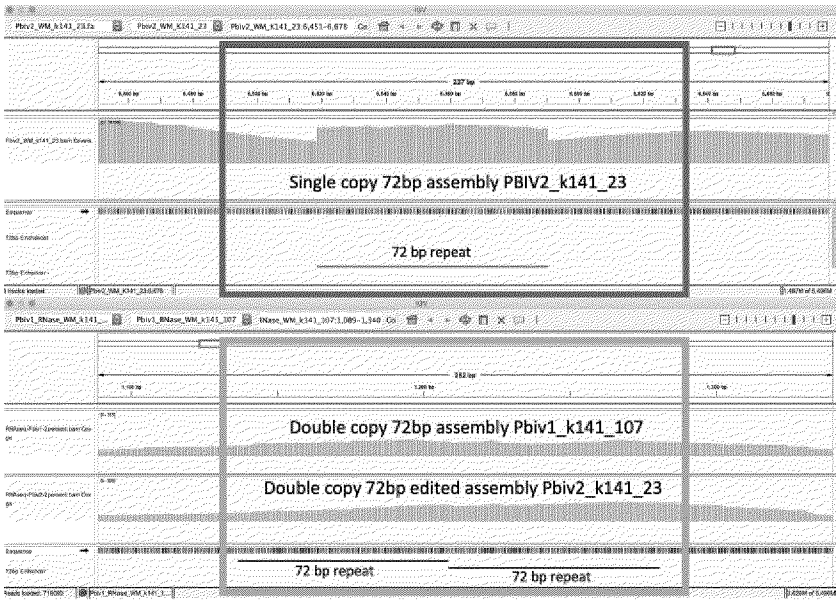


Figure 5B. IGV view of the read coverage over Pbiv2_k141_23 shows a discrete 72bp plateau in coverage (red rectangle). Editing the Pbiv2_k141_23 reference to include 2 copies of the 72bp sequence, and remapping the sequence data to this corrected sequence shows that the coverage over both vectors is more normal with no coverage plateau in Pfizer vial 2.

These data conclude that all Pfizer vectors contain a homoplastic 2 copy 72bp SV40 Enhancer associated with more robust expression and nuclear localization. The initial heteroplastic indel was an artifact of the Megahit assembler and short insert libraries.

To estimate the size of the DNA, the purified vaccines were evaluated on an Agilent Tape Station™ using DNA (genomic DNA screen tapes) and RNA based (high sensitivity RNA tapes) electrophoresis tapes.

Agilent Tape Station™ electrophoresis reveal 7.5 - 11.3 ng/µl of dsDNA compared to the 23.7 - 55.9ng/µl of mRNA detected in each 300µl sample. Qubit™ 3 fluorometry estimated 1-2.8ng/µl of DNA and 21.8ng - 52.8ng/µl of RNA. There is higher fragmentation seen in the DNA electrophoresis. The total RNA levels are less than the anticipated 30ug (100ng/µl) and 100ug (200ng/µl) doses suggesting a loss of yield in DNA and RNA isolation, manufacturing variance or RNA decay with expired lots.

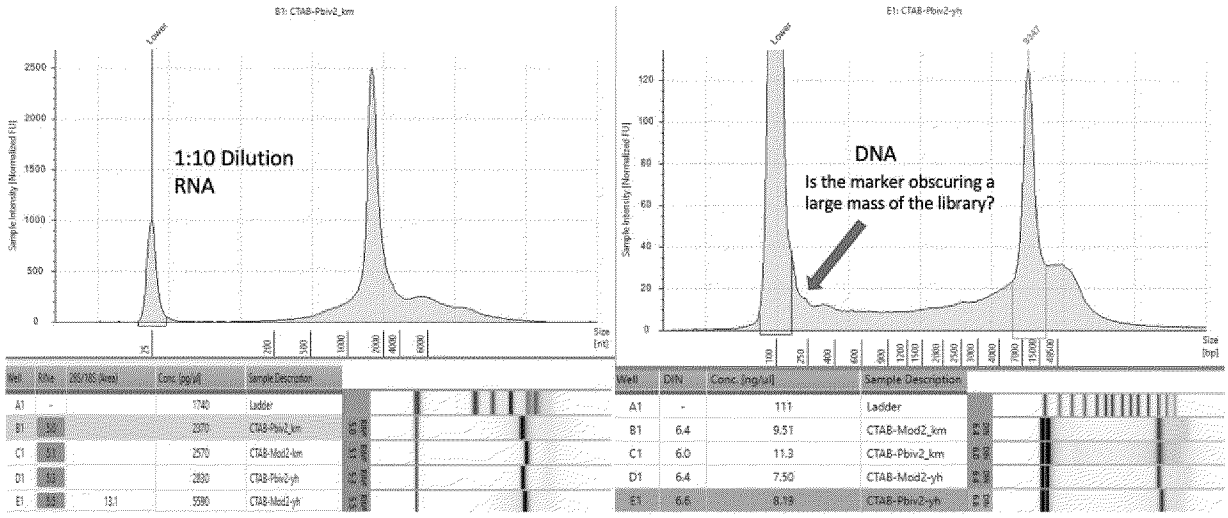


Figure 6. Agilent Tape Station™ electrophoresis demonstrates 23.7ng/µl – 55.9ng/µl of RNA (left). 7.5ng-11.3ng/µl are observed on DNA based Tape Station™. While the DNA electropherogram shows a peak suggestive of a full-length plasmid, this sample is known to have high amounts of N1-methylpseudouridine RNA present. DNA hybrids with N1-methylpseudouridine mRNA may provide enough intercalating dye cross talk to produce a peak. The sizing of the peak on the RNA tape on the left is shorter than expected. This may be the results of N1 methylpseudouridine changing the secondary structure or the mass to charge ratio of the DNA.

Quantitative PCR assays were designed using IDTs Primer Quest software targeting a region in the spike protein that was identical between Moderna and Pfizer spike sequences and a shared sequence in the vectors' origin of replication. This allowed the qPCR and RT-qPCR assessment of the vaccines. qPCR only amplifies DNA while RT-qPCR amplifies both DNA and RNA. Gradient qPCR was utilized to explore conditions where both targets would perform under the same cycling conditions for both RT-qPCR and PCR (gradient PCR data not shown).

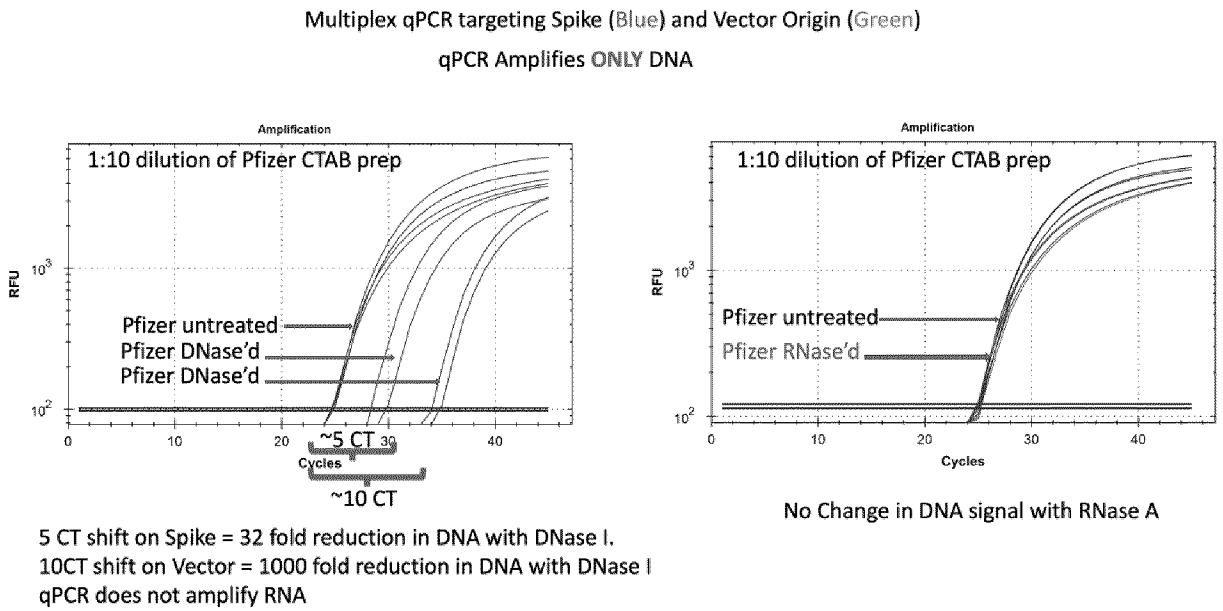


Figure 7. qPCR of Pfizer's bivalent vaccine with and without DNase I (left) and RNase A (right). Untreated mRNA demonstrates equal CTs for Spike and Vector assays as expected. Vector is more DNase I sensitive than the Spike suggesting the modRNA may inhibit nuclease activity of DNase I against complementary DNA targets. RNase A treatment doesn't alter the qPCR signal.

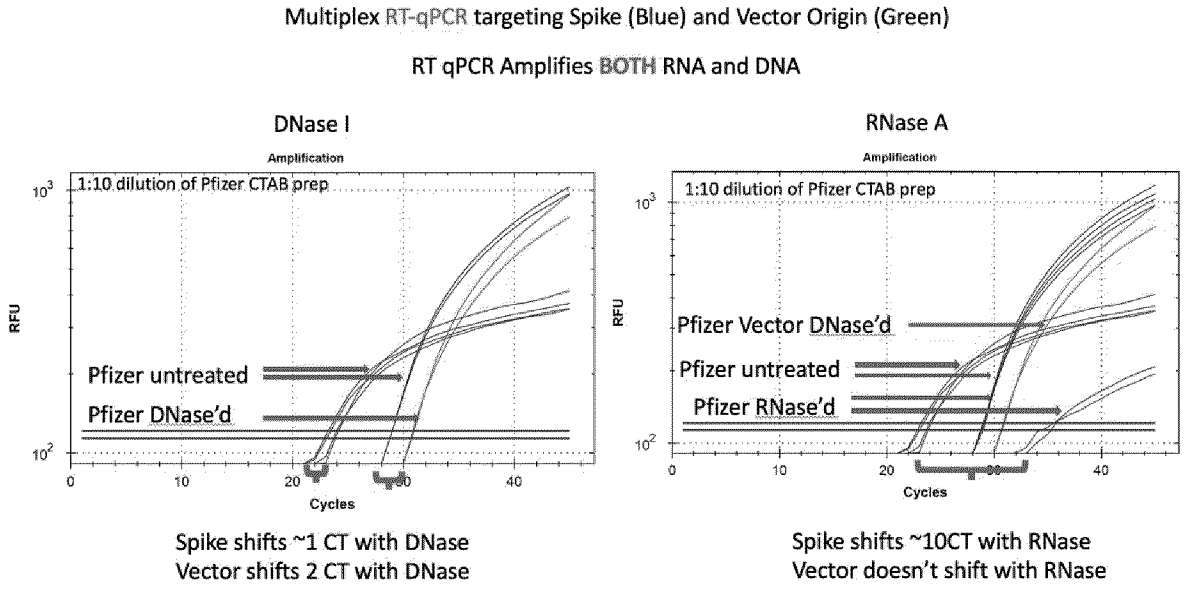


Figure 8. RT-qPCR amplifies both DNA and RNA. The untreated samples show a large CT offset with Pfizer Spike and Vector assays (Left Blue versus Green). This is anticipated as the T7 polymerization should create more mRNA over spike than over the vector. Small 1-2 CT shifts are seen with DNase I treatment. This is expected if the DNA is less than equal concentration of

nucleic acid in RT-PCR. RNase treatment (Right) shows a 10 CT offset but doesn't alter the DNA vector CT.

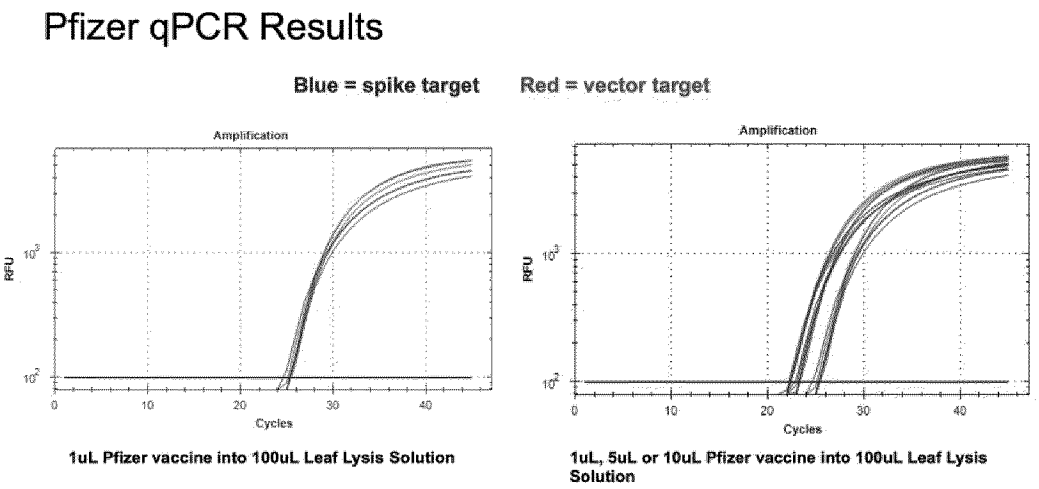


Figure 9. 1µl of the Pfizer bivalent vaccine placed in 100µl Leaf Lysis buffer for an 8 minute boil step delivers a CT of 24 for both Vector and Spike targets in qPCR (Left). Assay is responsive to 1,5,10µl of input (Right).

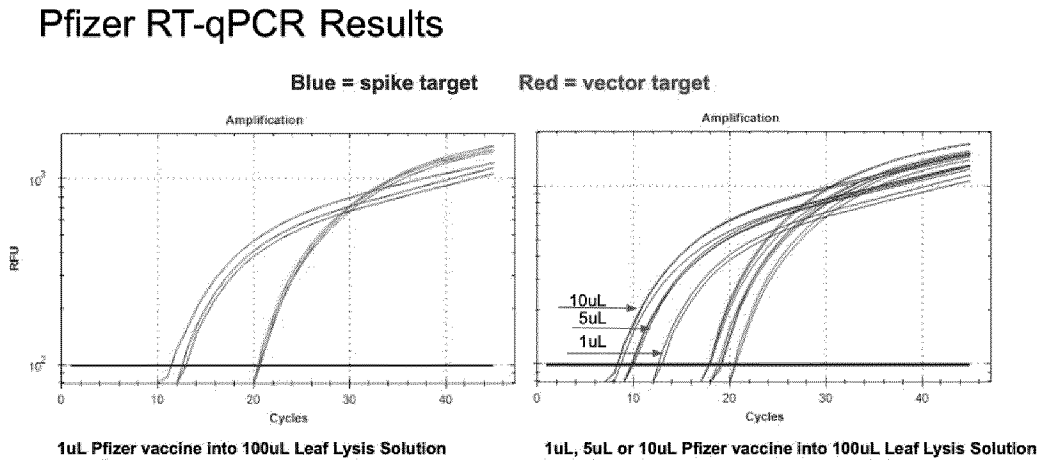


Figure 10. 1µl of the Pfizer bivalent vaccine placed in 100µl Leaf Lysis buffer for an 8 minute boil step delivers a CT of 20 and 12 for both Vector and Spike targets in RT-qPCR (Left). Assay is responsive to 1,5,10µl of input (Right).

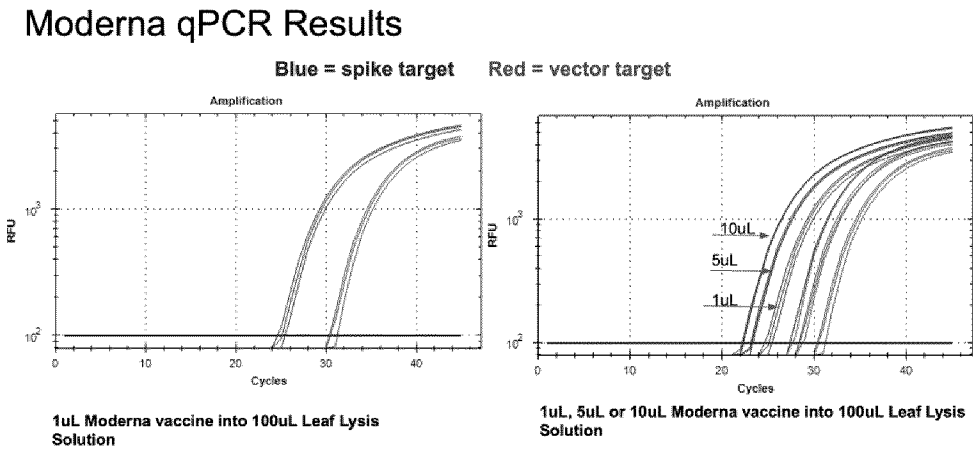


Figure 11. 1µl of the Moderna bivalent vaccine exhibits different CTs values for the spike and the vector targets (Left) with qPCR. This needs to be explored further as the assays provide equal CT scores on Pfizers' vaccines and the sequence of the amplicon is identical between the two vector origins. There are 2 mismatches in the spike amplicons between Moderna and Pfizer but none of the mismatches are under a primer or probe. The assay is responsive to 1,5,10µl of direct boil mRNA (Right).

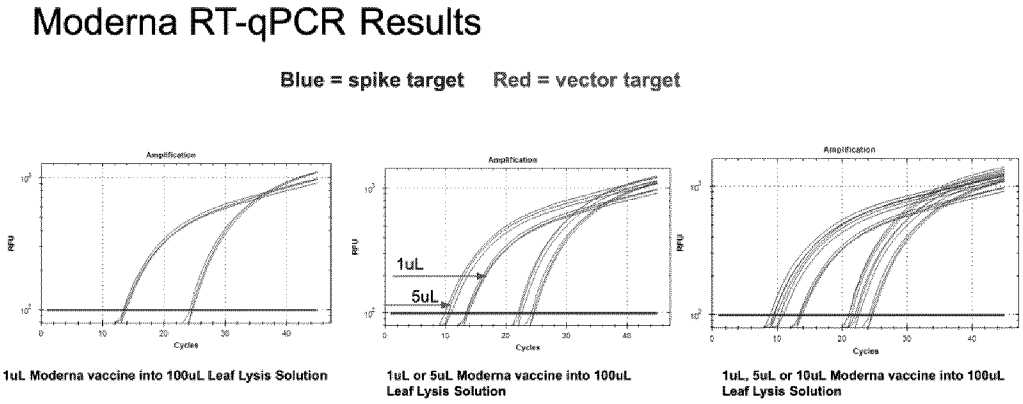


Figure 12. 1µl of the Moderna bivalent vaccine exhibits different CTs values for the spike and the vector targets (Left) with RT-qPCR. The large 10 CT shift between Spike and Vector needs to take into consideration that qPCR control shows a 5 CT offset. The boil preps can tolerate 1-10µl of vaccine (Middle and Right).

	Qubit DNA ng/µl	Qubit RNA ng/µl
Pbiv1	2.81	30.0
Pbiv2	1.47	52.8
Mod1	2.67	21.8
Mod2	1.04	49.0

Table 1. Qubit™ 3 Fluorometry estimates 1.04-2.8 ng/µl of dsDNA in the vaccines and 21.8ng-52.8ng/µl of RNA.

Synthetic templates were synthesized with IDT to build RT-qPCR standard curves to benchmark CTs to the mass of DNA in the reaction. This method uses ideal templates and fails to quantitate DNA molecules smaller than the amplicon size. As expected, this method delivers lower DNA concentration estimates than Qubit™ 3 fluorometry or Agilent Tape Station™. It also represents an ideal environment which doesn't capture the inhibition or primer depletion that can occur when large quantities of mRNA with identical sequence to your DNA target are co-present in a qPCR assay.

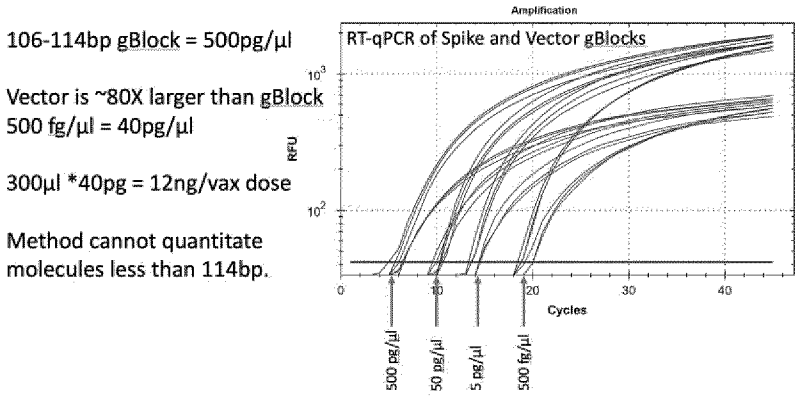


Figure 13. Two gBlocks were synthesized at IDT for Spike and Ori positive control templates used in an RT-qPCR assays. 10-fold serial dilutions were run in triplicate to correlate CT scores with picograms of DNA. The threshold is lowered from 10^2 for review of the background. CT of ~20 = 500fg/RT-qPCR reaction. Since 100bp targets only represent 1/80th of the vector DNA present as a potential contaminant, 500 fg/µl manifests in 40pg/µl of vector DNA. Any DNA that is DNase I treated and is smaller than the amplicon size cannot amplify or be quantitated with this method. This method will under quantitate DNase I treated samples compared to Qubit™ 3 or Agilent Tape Station™.

This work was further validated by testing 8 unopened Pfizer monovalent vaccines with both qPCR and RT-qPCR.

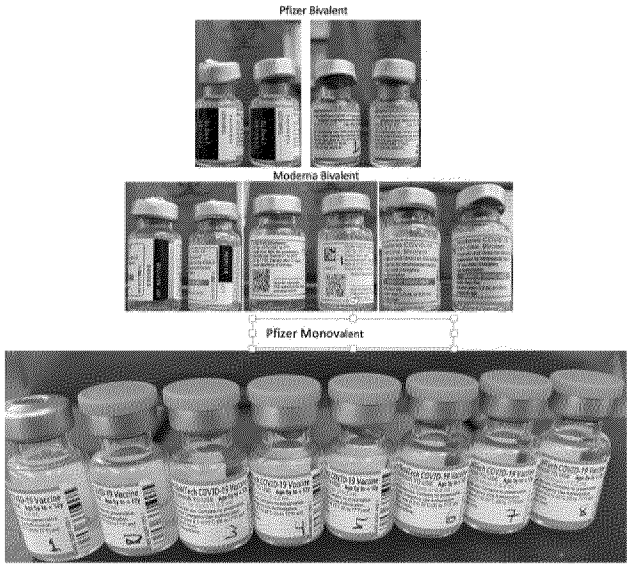


Figure 14. Moderna and Pfizer Bivalent vaccines (Top). 8 Monovalent Pfizer mRNA vaccines. These were unopened but past expiration (Bottom).

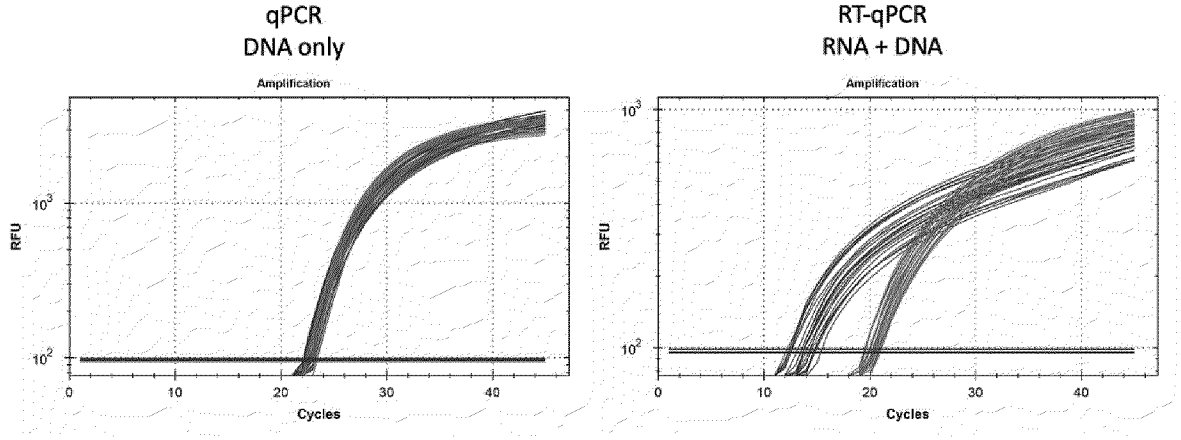


Figure 15. 1µl of vaccine boiled in 100µl of Leaf Lysis buffer was subjected to qPCR (left) and RT-qPCR (right) for Vector (red) and Spike (blue). 8 samples were tested in triplicate.

qPCR-Spike	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	23.12	22.98	22.58	22.33	22.36	22.08	22.20	22.06	0.401
Replicate 2	23.16	22.90	22.70	22.36	22.20	22.16	22.29	22.22	0.373
Replicate 3	23.22	22.84	22.59	22.29	22.44	22.26	22.29	22.11	0.366
STDEV	0.05	0.07	0.07	0.03	0.12	0.09	0.05	0.08	

qPCR: (Vector-Spike)	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	0.20	0.08	0.27	(0.00)	0.18	0.18	0.10	0.24	0.090
Replicate 2	0.16	0.22	0.29	0.11	0.18	0.12	0.03	0.13	0.079
Replicate 3	0.14	0.31	0.20	0.17	0.31	0.19	0.20	0.13	0.069
STDEV	0.03	0.11	0.05	0.09	0.08	0.04	0.08	0.06	

qPCR-Vector	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	23.33	23.06	22.85	22.32	22.54	22.26	22.30	22.30	0.411
Replicate 2	23.32	23.12	23.00	22.47	22.38	22.28	22.32	22.35	0.419
Replicate 3	23.36	23.15	22.79	22.46	22.75	22.46	22.49	22.23	0.383
STDEV	0.02	0.04	0.11	0.08	0.19	0.11	0.11	0.06	

RATIO RNA/DNA	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	1	1	1	1	1	1	1	1	0.068
Replicate 2	1	1	1	1	1	1	1	1	0.062
Replicate 3	1	1	1	1	1	1	1	1	0.056
STDEV	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1	

Table 2. CT values for Spike and Vector during qPCR (DNA only). Standard deviation for the triplicate measurements run horizontally in black font. Standard deviation for vial to vial run vertically in Red. Delta CT or (Vector CT minus Spike CT) represents the ratio of Spike to Vector DNA and should = 1.

RT-Spike	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	14.05	14.77	13.18	13.77	13.79	12.52	12.62	13.53	0.749
Replicate 2	14.29	14.74	14.38	14.82	13.78	13.82	12.57	12.38	0.925
Replicate 3	14.49	14.91	15.43	13.84	13.74	13.55	12.36	12.19	1.141
STDEV	0.22	0.09	1.12	0.59	0.02	0.69	0.14	0.72	

RT: (Vector-Spike)	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	6.74	5.93	7.20	6.40	6.51	7.31	7.33	5.97	0.570
Replicate 2	6.33	6.06	5.92	5.67	6.34	6.13	6.92	7.06	0.478
Replicate 3	6.33	6.07	5.43	6.39	6.13	6.38	7.09	7.18	0.562
STDEV	0.24	0.07	0.91	0.42	0.19	0.62	0.21	0.67	

RT-Vector	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	20.80	20.71	20.39	20.16	20.30	19.83	19.95	19.50	0.439
Replicate 2	20.62	20.80	20.30	20.49	20.12	19.96	19.49	19.45	0.499
Replicate 3	20.81	20.98	20.86	20.23	19.88	19.93	19.45	19.37	0.638
STDEV	0.11	0.14	0.30	0.17	0.21	0.07	0.28	0.07	

RATIO RNA/DNA	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	107	61	147	84	91	159	161	63	41.54
Replicate 2	80	67	61	51	81	70	121	134	29.25
Replicate 3	80	67	43	84	70	83	136	145	34.79
STDEV	15.5	3.3	55.8	19.2	10.4	47.9	20.3	44.6	

Table 3. CT values for Spike and Vector during RT-qPCR (RNA+DNA). Ratio of RNA:DNA ranges from 43:1 To 161:1. EMA allowable limit is 3030:1. This is 18-70 fold over the EMA limit.

Discussion

Multiple methods highlight high levels of DNA contamination in the both the monovalent and bivalent vaccines. While the Qubit™ 3 and Agilent Tape Station™ differ on their absolute quantification, both methods demonstrate it is orders of magnitude higher than the EMAs limit of 330ng DNA/ 1mg RNA. qPCR and RT-qPCR confirms the relative RNA to DNA ratio. An 11-12 CT offset should be seen between Spike and Vector RT-qPCR signals to represent a 1:3030

contamination limit (2^11.6 = 3100). Instead, we observe much smaller CT offsets (5-7 CTs) when looking at qPCR and RT-qPCR data with these vaccines. It should be noted that Qubit™ 3 and Agilent methods stain all DNA in solution while qPCR measures only amplifiable molecules without DNase I cut sites between the primers. The further apart you space the qPCR primers, the fewer Qubit™ 3 and Agilent detectable molecules will amplify. The primers used in this study are 106bp and 114bp apart, thus any molecules that are DNase I cut below this length will be undercounted with the qPCR methods relative to more general dsDNA measurements from Qubit™ 3 or Agilent Tape Station™.

This also implies that qPCR standard curves using 100% intact synthetic DNA standards will amplify more efficiently and thus undercount the total digested DNA contamination. For example, standard curves with 106-114bp synthetic templates provide CTs under 20 in the picogram range (not low nanogram range) suggesting large portions of the library are smaller than the minimum amplifiable size. Pure standards also do not contain high concentrations of modified mRNA with identical sequence which could serve as a competitive primer sink or inhibitor to qPCR methods.

Alternatively, the Qubit™ 3 and the Agilent Tape Station™ could be inflating the DNA quantification due to intercalating dye cross talk with N1-methylpseudouridine RNA. For this reason, we believe the ratio we observed when these molecules are more scrupulously interrogated with polymerases specific for each template type in qPCR and RT-qPCR is a more relevant metric. The EMA metric is also stated as such a ratio.

This also brings into focus if these EMA limits took into consideration the nature of the DNA contaminants. Replication competent DNA should arguably have a more stringent limit. DNA with mammalian promoters or antibiotic resistance genes may also be of more concern than just random background *E.coli* genomic DNA from a plasmid preparation (Sheng-Fowler et al. 2009). Background *E.coli* DNA was measured with qPCR and had CT over 35.

There has been a healthy debate about the capacity for SARs-CoV-2 to integrate into the human genome(Zhang et al. 2021). This work has inspired questions regarding the capacity for the mRNA vaccines to also genome integrate. Such an event would require LINE-1 driven reverse transcription of the mRNA into DNA as described by Alden *et al*. (Alden et al. 2022). dsDNA contamination of sequence encoding the spike protein wouldn't require LINE-1 for Reverse Transcription and the presence of an SV40 nuclear localization signal in Pfizer's vaccine vector would further increase the odds of integration. This work does not present evidence of genome integration but does underscore that LINE-1 activity is not required given the dsDNA levels in these vaccines. The nuclear localization of these vectors should also be verified.

Prior sequencing of the monovalent vaccines from Jeong *et al.* only published the consensus sequence (Dae-Eun Jeong 2021). The raw reads for this project are not available and should be scrutinized for the presence of vector sequence.

Given these vaccines exceed the EMA limits (330ng/mg DNA/RNA) with the Qubit™ 3 and Agilent data and these data also exceed the FDA limit (10ng/dose) with the more conservative qPCR standard curves, we should revisit the lipopolysaccharide (LPS) levels. Plasmid contamination from *E.coli* preps are often co-contaminated with LPS. Endotoxins contamination can lead to anaphylaxis upon injection (Zheng et al. 2021).

A limitation of this study is the unknown provenance of the vaccine vials under study. These vials were sent to us anonymously in the mail without cold packs. RNA is known to degrade faster than DNA and it is possible poor storage could result in faster degradation of RNA than DNA. RNA as a molecule is very stable but in the presence of metals and heat or background ubiquitous RNases, it can degrade very quickly. All of the vaccines in this study are past the expiration date listed on the vial suggesting more work is required to understand the DNA to RNA ratios in fresh lots. The publication of these qPCR primers may assist in surveying additional lots with more controlled supply chains. Studies evaluating vaccine longevity in breast milk or plasma may benefit from vector DNA surveillance as this sequence is unique to the vaccine and may persist longer than mRNA.

While the sequencing delivered full coverage of the plasmid backbones, it is customary to assemble plasmids from DNase I fragmented libraries. These methods have not discerned the ratio of linear versus circular DNA in the vials. While plasmid DNA is more competent and stable, linear DNA may have higher genome integration risks.

The intercalating dyes used in the Qubit™ 3 and Agilent systems are known to have low fluorescent cross talk with DNA and RNA but it is unknown to what degree N1-methylpseudouridine alters the specificity of these intercalating dyes. As a result, we have relied on the CT offsets between RT-qPCR and qPCR with the vector and spike sequence as the best relative assessment of the EMA ratio-metric regulation. These qPCR and RT-qPCR reagents may be useful in tracking these contaminants in vaccines, blood banks or patient tissues in the future.

Methods

Purifying the mRNA from the LNPs

LiDs/SPRI purification

100µl of each vial was sampled (1/3rd to 1/5th of a dose)

- 5µl of 2% LiDs was added to 100µl of Vaccine to dissolve LNPs
- 100µl of 100% Isopropanol
- 233µl of Ampure (Beckman Genomics)
- 25µl of 25mM MgCl2 (New England Biolabs)

Samples were tip mixed 10X and incubated for 5 minutes for magnetic bead binding. Magnetic Beads were separated on a 96-well magnet plate for 10 minutes and washed twice with 200µl of 80% EtOH. The beads were left to air dry for 3 minutes and eluted in 100µl of ddH20. 2µl of eluted sample was run on an Agilent Tape Station™.

CTAB/Chloroform/SPRI purification of Vaccines

Some variability in qPCR performance was noted with our LiDs/SPRI purification method of the vaccines. This left some samples opaque and may represent residual LNPs in the purification. A CTAB/Chloroform/SPRI isolation was optimized to address this and used for further qPCR and Agilent electrophoresis. Briefly, 300µl of Vaccine was added to 500µl of CTAB (MGC solution A in SenSATIVAx MIP purification kit. #420004). The sample was then vortexed and heated for 5 minutes at 37°C. 800µl of chloroform was added, vortexed and spun at 19,000 rpms for 3 minutes. The top 250µl of aqueous phase was collected and added to 250µl of solution B and 1ml of magnetic binding buffer. Samples were vortexed and incubated for 5 minutes and magnetically separated. The supernatant was removed and the beads washed with 70% Ethanol two times. Samples were finally eluted in 300µl of MGC elution buffer.

Simple boil preparation for evaluating vaccine qPCR.

This boil prep process simply takes 1-10µl of the vaccine and dilutes it into a PCR compatible leaf lysis buffer and heats it (Medicinal Genomics part number 420208).

- 65°C for 6 minutes
- 95°C for 2 minutes

Library Construction for Sequencing

50µl of each 100µl sample was converted into RNA-Seq libraries for Illumina sequencing using the NEB NEBNext UltraII Directional RNA library Kit for Illumina (NEB#E7760S).

To enrich for longer insert libraries the fragmentation time was reduced from 15 minutes to 10 minutes and the First strand synthesis time was extended at 42°C to 50 minutes per the long insert recommendations in the protocol.

No Ribo depletion or PolyA enrichment was performed as to provide the most unbiased assessment of all fragments in the library. The library was amplified for 16 cycles according to the manufacturers protocol. A directional library construction method was used to evaluate the single stranded nature of the mRNA. This is an important quality metric in the EMA and TGA disclosure documents as dsRNA (>0.5%) can induce an innate immune response. dsRNA content is often estimated using an ELISA. Directional DNA sequencing offers a more comprehensive method for its estimation and was previously measured and 99.99% in Jeong et al. It is unclear how this may vary lot to lot or within the new manufacturing process for the newer bivalent vaccines.

RNase A treatment of the Vaccines

RNase A cleaves both uracils and cytosines. N1-methylpseudouridine is known to be RNAse-*L* resistant but RNase A will cleave cytosines which still exist in the mRNAs. This leaves predominantly DNA for sequencing. Vaccine mRNA that was previously sequenced and discussed here, was treated at 37°C for 30 minutes with 10µl of 20 Units/µl Monarch RNase A from NEB. The RNase reaction was purified using 1.5X of SenSATIVAx (Medicinal Genomics #420001). Sample were eluted in 20µl ddH20 after DNA purification. 15µl was used for DNA sequencing.

DNase treatment of the vaccines

50µl of CTAB purified vaccine was treated at 37°C for 30 minutes with 2µl DNase I and 6µl of DNase I buffer (Grim reefer MGC#420143). 2.5µl of LiDs Lysis buffer was added to stop the DNase reaction. Reactions were purified using 60µl 100% Isopropanol, 140µl Ampure, 15µl MgCl2. Magnetic beads were tip mixed 10 times, left for 5 minutes to incubate, magnetically separated and then washed twice with 80% EtOH.

Whole genome shotgun of RNase'd Vaccines.

15µl of the DNA was converted into sequence ready libraries using Watchmakers Genomics WGS library construction kit. This kit further fragments the DNA to smaller sizes making fragment length in the vaccines difficult to predict.

Qubit™ 3 Fluorometry

Qubit™ 3 fluorometry was performed using Biotum AccuBlue RNA Broad Range kit (#31073) and Biotum AccuGreen High Sensitivity dsDNA Quantitation Kit (#31066) according to the manufacturers instructions.

E.coli qPCR

Medicinal Genomics PathoSEEK™ E.coli Detection assay (#420102) was utilized according to the manufacturers instructions.

qPCR and RT-qPCR Spike Assay

- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Forward
- >AGATGGCCTACCGGTTCA
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Reverse
- >TCAGGCTGTCCTGGATCTT
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Probe
- >/56-FAM/CGAGAACCA/ZEN/GAAGCTGATCGCCAA/3IABkFQ/

qPCR and RT-qPCR Vector Origin Assay

- MedGen_Vax-vector_Ori_Forward
- >CTACATACCTCGCTCTGCTAATC
- MedGen_Vax-vector_Ori_Reverse
- GCGCCTTATCCGGTAACTATC
- MedGen_Vax-vector_Ori_Probe
- /5HEX/AAGACACGA/ZEN/CTTATCGCCACTGGC/3IABkFQ/

Elute primer to 100uM according to IDT instructions.

Make 50X primer-probe mix.

1. 25µl 100uM Forward Primer
2. 25µl 100uM Reverse Primer
3. 12.5µl 100uM Probe
4. 37.5µl nuclease free ddH20.

Use 15µl of this mixture in the **qPCR master mix** setup seen below. (0.5µl primer/probe per reaction)

Use 10µl of this mixture in the **RT-qPCR master mix** setup seen below.

Medicinal Genomics Master Mix kits used

1. https://store.medicinalgenomics.com/qPCR-Master-Kit-v3-200-rxns
2. https://store.medicinalgenomics.com/pathoseek-rt-qpcr-master-kit

Reaction setup for 30 reactions of qPCR

- 114µl Enzyme Mix (green tube)
- 24µl Reaction Buffer (blue tube)
- 246µl nuclease free ddH20
- 15µl of Primer-Probe set Spike
- 15µl of Primer-Probe set Ori

Use 13.8µl of above MasterMix and 5µl of purified sample (1µl Vax DNA/RNA + 4µl ddH20 if CT <15)

Reaction setup for 34 reactions of RT-qPCR

- 200µl Enzyme mix
- 96µl nuclease free ddH20
- 20µl RNase Inhibitor (purple tube)
- 4µl DTT (green tube)
- 10µl Primer-Probe set Spike
- 10µl Primer-Probe set Ori

10µl of MasterMix and 1µl of Vax DNA/RNA

Medicinal Genomics MIP DNA Purification Kit used

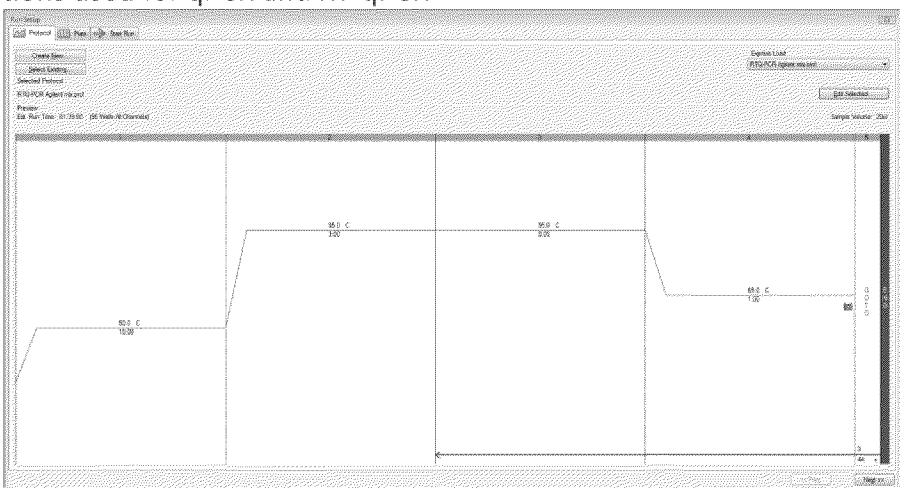
1. https://store.medicinalgenomics.com/SenSATIVAx-DNA-Extraction-Kit-200-reactions_2

he CTAB/Chloroform/SPRI based DNA/RNA isolation methods are described above.

Cycling conditions

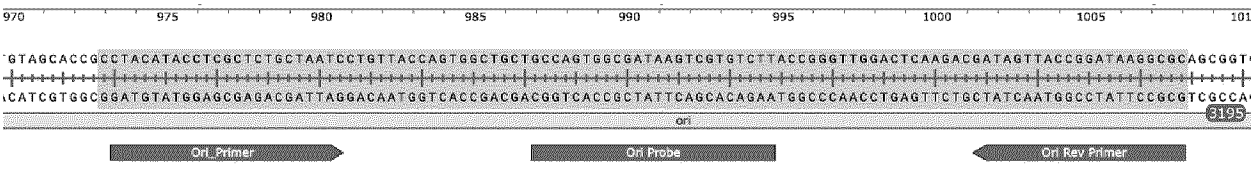
These conditions work for both qPCR and RT-qPCR. Note: The 50°C RT step can be skipped with qPCR. The MGC qPCR MasterMix kits used have a hot start enzyme which are unaffected by this 50°C step. For the sake of controlling RNA to DNA comparisons, we have put qPCR and RT-qPCR assays on the same plate and run the below program with the RT step included for all samples.

Cycling Conditions used for qPCR and RT-qPCR

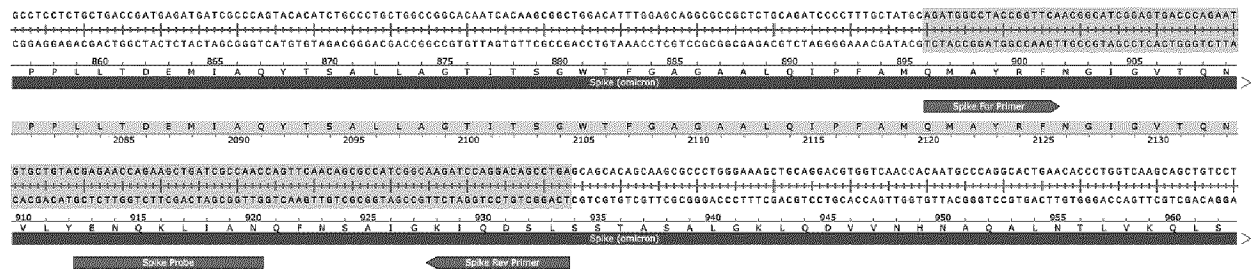


Sequences of amplicons for gBlock Positive Controls. Ori = 106bp, Spike = 114bp.

Ori target



Spike target



Sequencing Data

Raw Illumina Reads RNA-seq

- Pfizer Bivalent Vial 1 Forward reads
- Pfizer Bivalent Vial 1 Reverse reads
- Pfizer Bivalent Vial 2 Forward reads
- Pfizer Bivalent Vial 2 Reverse reads
- Moderna Vial 1 Forward reads
- Moderna Vial 1 Reverse reads
- Moderna Vial 2 Forward reads
- Moderna Vial 2 Reverse reads

Read files are run through sha256 (Hash and stash) and etched onto the DASH blockchain. The sha256 hash of the read file is spent into the OP_RETURN of an immutable ledger. If the hash of the file doesn't match the hash in these transactions, the file has been tampered with.

- Pfizer Vial 1 Forward hash
- Pfizer Vial 1 Reverse hash
- Pfizer Vial 2 Forward hash
- Pfizer Vial 2 Reverse hash
- Moderna Vial 1 Forward hash
- Moderna Vial 1 Reverse hash
- Moderna Vial 2 Forward hash
- Moderna Vial 2 Reverse hash

Megahit Assemblies

- Pfizer Vial 1
- Pfizer Vial 2
- Moderna Vial 1
- Moderna Vial 2

Illumina Reads mapped back to Megahit Assemblies

- Pfizer Vial 1 BAM File. Index File
- Pfizer Vial 2 BAM File. Index File
- Moderna Vial 1 BAM File. Index File
- Moderna Vial 2 BAM File. Index File

Q30 Filtered Illumina Reads (use these for transcriptional error rate estimates)

FastQ-Filter download: usage> fastq-filter -e 0.001 -o output.fastq input.fastq

- Pfizer bivalent Vial 1 Forward Reads
- Pfizer bivalent Vial 1 Reverse Reads
- Pfizer bivalent Vial 2 Forward Reads
- Pfizer bivalent Vial 2 Reverse Reads
- Moderna bivalent Vial 1 Forward Reads
- Moderna bivalent Vial 1 Reverse Reads
- Moderna bivalent Vial 2 Forward Reads
- Moderna bivalent Vial 2 Reverse Reads

Q30 BAM files. Q30 Reads mapped against Megahit assemblies

- Pfizer Vial 1 q30-BAM file. Index File
- Pfizer Vial 2 q30-BAM file. Index File
- Moderna Vial 1 q30-BAM file. Index File
- Moderna Vial 2 q30-BAM file. Index File

IGVtools error by base on q30 reads

Fields = Position in contig, Positive stand (+)A, +C, +G, +T, +N, +Deletion, +Insertion, Negative strand -A, -C, -G, -T, -N, -Deletion, -Insertion

- Moderna Vial 1

- Moderna Vial 2
- Pfizer Vial 1
- Pfizer Vial 2

Analysis pipeline

Reads were demultiplexed and processed with

- Trimgalore - Removes Illumina Sequencing adaptors.
- Megahit- assembles reads into contigs.
- Megahit for SARs-CoV-2
- Samtools- generates BAM files for viewing in IGV.
- Samtools stats used to calculate outie reads.
- BWA-mem- Short read mapper used to align reads back to the assembled references.
- SnapGene software- (www.snapgene.com)- Used to visualize and annotate expression vectors
- IGV- Integrated Genome Viewer used to visualize Illumina sequencing reads.

RNase Treated Libraries-BAM files

contig specific BAM files were created using samtools

samtools view -h input.bam contig_name -O BAM > contig.bam; samtools index contig.bam;

Samtools stats run on a each contig in each assembly.

for out_prefix in `ls *.sort.bam | perl -pe "s/.sort.bam//"`; do mkdir -p ${out_prefix}-samtools-stats; for contig in `samtools view -H ${out_prefix}.sort.bam | grep "^@SQ" | cut -f 2 | perl -pe "s/SN\://"`; do echo "Now calculating stats for ${contig}/$out_prefix..."; samtools stats ${out_prefix}.sort.bam $contig > ${out_prefix}-samtools-stats/${contig}-samtools-stats.txt; done; done

- Pbiv1_RNase_WM_k141_107.fa
- Pbiv1_RNase_WM_k141_107.bam
- Pbiv1_RNase_WM_k141_107.bam.bai
- Pbiv2_RNase_WM_k141_23.fa
- Pbiv2_RNase_WM_k141_23.bam

- Pbiv2_RNase_WM_k141_23.bam.bai

Author contributions

KJM- constructed the sequencing libraries, designed the qPCR assays, ran Qubit™ 3s and Agilent Tape Station™ and performed the analysis, drafted the manuscript.
YH-Optimized DNA isolations, Tape Station™ and qPCR results.
SM, LTK- assisted in demultiplexing and trimming the reads and assembly troubleshooting

Conflicts of interest- Authors of this paper are employees of Medicinal Genomics which manufacturers some of the qPCR and DNA isolation kits used in this study.

Acknowledgements.
We'd like to thank Lei Zhang for organizing the sequencing outsourcing. We'd like to thank Jessica Rose, Sabine Hazan, Jikkyleaks, Pathogenetics, Steve Massey, Valentine Bruttel, Lynn Fynn, Sasha Letypova, David Wiseman and Pharmacoconuts for helpful comments on this topic.

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De Rose, Carol (HC/SC)

From: Fortin, Nathalie (HC/SC)
Sent: 2023-09-12 3:09 PM
To: Pham, Co (HC/SC)
Subject: FW: QEES: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302
Attachments: 276302_QEES_CVCTB.docx

Hi Co
Please find comments for your consideration.
Merci
Nathalie

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Tuesday, September 5, 2023 11:01 AM
To: Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>
Cc: Wall, Michael (HC/SC) <michael.wall@hc-sc.gc.ca>; Davis, Elisabeth (HC/SC) <elisabeth.davis@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>
Subject: QEES: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Hi Co,

Please review the QEES and let me know if you have questions/comments. There is no T&Cs for this NDS-CV, as the commitments/comments are not considered critical to product quality.

Thanks,
tong

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Monday, July 31, 2023 3:22 PM
To: Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>; Blahoianu, Maria (HC/SC) <maria.blahoianu@hc-sc.gc.ca>; Tabbasum, Valentina (HC/SC) <valentina.tabbasum@hc-sc.gc.ca>; Willcott, Marsha (HC/SC) <marsha.willcott@hc-sc.gc.ca>; Lacroix, Jeremie (HC/SC) <jeremie.lacroix@hc-sc.gc.ca>; Clark-Knowles, Katherine (HC/SC) <Katherine.Clark-Knowles@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; NG, Queenie (HC/SC) <queenie.ng@hc-sc.gc.ca>; Adoni, Harsha Vardhan (HC/SC) <harsha.vardhan.adoni@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; Eng, David (HC/SC) <david.eng@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Hanna, Jessy (HC/SC) <jessy.hanna@hc-sc.gc.ca>
Cc: HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>; Fortin, Nathalie (HC/SC) <nathalie.fortin@hc-sc.gc.ca>; Fandja, Patrick (HC/SC) <patrick.fandja@hc-sc.gc.ca>; Fung, Winnie (HC/SC) <winnie.fung@hc-sc.gc.ca>; BRDD ORIRM / BRRGR DMBR (HC/SC) <brdd.orirm-brrgr.dmbr@hc-sc.gc.ca>
Subject: RE: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Dear all,

The Rolling Sequence #2 is now available for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302: HC6-024-e276302 (0002) Biologic Dossier

Notes:

- Due to delays, the sponsor has combined the planned files for Roll #2 with Roll #3. The application plan has been updated.
- The following documents have been provided in this roll:
  - Annotated and non-annotated English PM. The French PM will be provided during the review.
  - Non-Canadian Labelling
  - Revised Canadian Addendum RMP
  - Company Core Data Sheet (the CDS has not yet been updated to include the simplified posology proposed in the Canadian Product Monograph and SmPC)
  - Module 2.3 Introduction, QOS and 2.3.S, 2.3.P and 2.3.A documents sections
  - Module 3 documents to supported the updated antigen composition for Omicron XBB.1.5; clone documents in Module 3 from previous Comirnaty, Comirnaty Original & Omicron BA.4/5 submissions

Thank you,
Kenneth

From: Garay, Kenneth (HC/SC) <kenneth.garay@hc-sc.gc.ca>
Sent: Friday, July 21, 2023 6:52 PM
To: Baca-Estrada, Maria (HC/SC) <maria.baca-estrada@hc-sc.gc.ca>; Blahoianu, Maria (HC/SC) <maria.blahoianu@hc-sc.gc.ca>; Tabbasum, Valentina (HC/SC) <valentina.tabbasum@hc-sc.gc.ca>; Willcott, Marsha (HC/SC) <marsha.willcott@hc-sc.gc.ca>; Lacroix, Jeremie (HC/SC) <jeremie.lacroix@hc-sc.gc.ca>; Clark-Knowles, Katherine (HC/SC) <Katherine.Clark-Knowles@hc-sc.gc.ca>; Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>; NG, Queenie (HC/SC) <queenie.ng@hc-sc.gc.ca>; Adoni, Harsha Vardhan (HC/SC) <harsha.vardhan.adoni@hc-sc.gc.ca>; Rose, Jhona (HC/SC) <jhona.rose@hc-sc.gc.ca>; Eng, David (HC/SC) <david.eng@hc-sc.gc.ca>; Deng, Anvella (HC/SC) <anvella.deng@hc-sc.gc.ca>; Hanna, Jessy (HC/SC) <jessy.hanna@hc-sc.gc.ca>
Cc: HC.F ORA_COVID / BAR_COVID F.SC <hc.ora_covid-bar_covid.sc@hc-sc.gc.ca>; HC.F BBRS COVID Vaccines Management / BBRA COVID Vaccins Gestionnaires F.SC <bbrs.covid.vaccines.management-bbra.covid.vaccins.gestionnaires@hc-sc.gc.ca>
Subject: COVID-19: New NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302

Good afternoon all,

The following submission is ready for review:

Submission Type: NDS-CV **Control No.:** 276302

Product Name: Comirnaty Omicron XBB.1.5 (raxtozinameran)

Sponsor Name: Pfizer Canada ULC on behalf of BioNTech

Link to sequence on docubridge rolling sequence #1: HC6-024-e276302 (0000) Biologic Dossier
Link to screening clarifax response sequence: HC6-024-e276302 (0001) Biologic Dossier

Link to screening report: HC6-024-e276302 (1.0) Reg Info - Health Product ***New***

Submission Notes:

- This submission provides for a 30 µg, 10 µg and 3 µg formulation of the BNT162b2 Omicron (XBB.1.5) Variant drug product.
- The sponsor notes that the NDS-CV is supported largely by data previously filed under the COMIRNATY and COMIRNATY ORIGINAL & OMICRON BA.4/BA.5.

- The sponsor states that all Module 2, 4 and 5 components previously filed to Health Canada have been cloned as part of this first roll.
- According to the Application Plan, the following Rolling Sequences will be provided with the following information:
  - Roll #1 (June 29, 2023) – Module 1 Administrative and Product Information documents, Cloned Module 2, 4, 5 documents
  - Roll #2 (July 17, 2023) – English Product Monograph (Annotated and Non-annotated), Foreign Labels, Cloned Module 3, EU RMP (TBD)
  - Roll #3 (August 2023) – French Product Monograph (Non-annotated), LASA Assessment, Canadian Addendum RMP (TBD), How to Administer & Quick References Guides

Update* Roll #2 has been delayed and will be combined with Roll 3 (Week of July 24). Due to the expedited timelines of review, the courtesy copy of the PM has been requested by ORA.

LABEL:

- According to the Application Plan, the sponsor will be submitting the MAA vial and carton labels; EUA, USPI and/or SmPC in Roll #2. A Health Risk Product Communication (HPRC) is required for this submission due to the use of global labels.

- Updated *How to Administer* and *Quick Reference* Guides will be provided in Roll #3 (August 2023).

MHPD:

- The EU RMP is scheduled to be submitted (TBD) in Roll #3 (August 2023) in accordance with the Application Plan.
- In a correspondence, the sponsor will be requesting the submit the Canadian Addendum RMP Post-Approval of this submission under Terms and Conditions. Further discussion will be carried out between MHPD and the sponsor for the submission of the Canadian Addendum RMP.

Thanks,

Kenneth Garay
Senior Regulatory Affairs Officer
Office of Regulatory Affairs
Biologic and Radiopharmaceutical Drugs Directorate
Health Products and Food Branch/Health Canada
kenneth.garay@hc-sc.gc.ca / Tel: 437-324-8892

Agent principal des affaires réglementaires
Bureau des affaires réglementaires
Direction des médicaments biologiques et radiopharmaceutiques
Direction générale des produits de santé et des aliments / Santé Canada
kenneth.garay@hc-sc.gc.ca / Tel: 437-324-8892

To/À:	Security – Classification – de sécurité:
Dr. Co Pham Director Center for Vaccines, Clinical Trials, and Biostatistics	HC Protected B (when completed)
From/De: Dr. Tong Wu, Division Manager VQD3	**Date:** 2023-09-05

QUALITY EVALUATION EXECUTIVE SUMMARY (QEES)

Manufacturer / Sponsor	Pfizer Canada ULC., on behalf of BioNTech Manufacturing GmbH
Type of Submission	NDS-CV
Submission Review Target Date	Expedited (Sponsor has requested approval by Mid-September 2023)

Brand Name of Drug Product (Proper Name)	Control Number	Dossier ID	Sequences
COMIRNATY XBB.1.5	276302	HC6-024-e276203	0000, 0002, 0004, 0008

RECOMMENDATION
A Notice of Compliance is recommended.

Comments to be forwarded to the submission sponsor are provided below.

NAME	POSITION TITLE	SIGNATURE
Dr. Tong Wu	Division Manager	*This document has been signed electronically using the Health Canada docubridge system.*
Dr. Co Pham	Director	*This document has been signed electronically using the Health Canada docubridge system.*

THIS DOCUMENT CONTAINS CONFIDENTIAL THIRD PARTY INFORMATION: ☐ YES ☒ NO

COMMENTS TO BE FORWARDED TO THE SUBMISSION SPONSOR

Commitments

The sponsor is reminded of the following commitments:

1. Provide additional data and/or information characterizing the size distribution of residual DNA fragments and residual intact circular plasmid by Dec 1, 2023.
2. Provide a Certified Product Information Document when it becomes available.

Comments

The following comments are for information and a formal response is not required at this time:

1. The Sponsor is reminded of the obligation to report OOS results for on-going stability monitoring of COMIRNATY vaccines.

NOTES

None.

SUBMISSION BACKGROUND

COMIRNATY Omicron XBB.1.5 is a monovalent prophylactic vaccine developed to prevent COVID-19 caused by infection with SARS-CoV-2 virus. This vaccine contains a nucleoside-modified mRNA encoding the spike protein of the SARS-CoV-2 Omicron XBB.1.5 variant, and the mRNA is encapsulated in a lipid nanoparticle (LNP) stabilized in an aqueous cryoprotectant Tris-Sucrose buffer.

[redacted]

The Omicron XBB.1.5 drug product (DP) is produced by encapsulation of mRNA DS with four lipids into a LNP. The DP is supplied as a sterile, preservative-free, white to off-white frozen suspension for intramuscular injection, in a 2 mL glass vial sealed with a bromobutyl rubber stopper. Vials have an aluminum seal with a flip-off plastic cap and are to be stored at -90 to -60 °C. Once thawed the product can be stored at 2 to 5°C for up to 10 weeks. The table below summarizes the presentations in scope for the NDS-CV. Where a presentation is in scope, the cap colour is indicated:

Dose	MDV/DTU	SDV/RTU	MDV/RTU
30 µg		Light Grey	Dark Grey
10 µg	Orange	Light Blue	Dark Blue
3 µg	Maroon		

SDV: single-dose vial, MDV: multi-dose vial, DTU: dilute-to-use, RTU: ready-to-use

SUBMISSION SUMMARY

Manufacturing Process and Control Strategy

The manufacture and control of the Omicron XBB.1.5 is based on the BNT162b2 mRNA vaccine platform technology used for other COMIRNATY products, with minor changes to the plasmid sequence to match the Omicron XBB.1.5 variant and to the sequence of primers used for identity test of DS/DP.

<u>Drug Substance Manufacturing Process and Control</u>

The manufacturing process and control strategy for the COMIRNATY Omicron XBB.1.5 DS is considered equivalent to that approved for COMIRNATY Original & Omicron BA.4/BA.5, with the exception of minor sequence changes to the plasmid used for *in vitro* transcription and to the primers used for ID test, to match the Omicron XBB.1.5 variant. However, the structure and general properties of the Omicron XBB.1.5 variant DS remains highly similar to the Original

BNT162b2s DS and to the Omicron BA.1 and BA.4/BA.5 variant DS. The mRNAs contain the same structural elements with the exception of the S protein RNA coding sequence; the original RNA sequence has been replaced with the XBB.1.5-specific S protein RNA sequence. As such, the manufacturing process, process parameters and controls, analytical methods, and release specifications remain the same as those approved for COMIRNATY Original & Omicron BA.4/BA.5. Consistent manufacturing and control of Omicron XXB.1.5 DS is further supported by the following:

- Information concerning the source, history, and generation of plasmid [redacted] encoding the spike protein of the Omicron XBB.1.5 variant.
- Characterization and release testing results of the Omicron XXB.1.5 master cell bank used to prepare plasmid [redacted]
- Validation of the [redacted] and [redacted] PCR analytical procedures used for Omicron XBB.1.5-specific identity testing.
- Batch analysis for one batch of Omicron XBB.1.5 DS used to manufacture DP validation lots.

Drug Product Manufacturing Process and Control

Six presentations of the Omicron XBB.1.5 DP are proposed, each providing a dose of either 30, 10, or 3 µg of mRNA per dose. All doses are formulated in [redacted] Tris buffer, with [redacted] sucrose and a [redacted] and differ in their RNA concentration, fill volume, and requirements for dilution prior to administration (**Table 3.2.P-1**). The manufacturing process and control strategy for the COMIRNATY Omicron XBB.1.5 DP is considered equivalent to that approved for the Original (Tris/Sucrose formulation) and Bivalent (Original and Omicron BA.4/BA.5) DP at the various presentations.

Table 3.2.P-1. Drug Product Presentations

DP Presentation[a]	DP RNA Concentration (mg/mL)	Fill volume (mL)	Dilution with 0.9% sodium chloride (mL)	Injection Volume	Doses per vial
30 µg MDV	0.1	2.25	N/A	0.3	6
30 µg SDV	0.1	0.48	N/A	0.3	1
10 µg MDV	0.033	2.25	N/A	0.3	6
10 µg SDV	0.033	0.48	N/A	0.3	1
10 µg MDV (dilution required)	0.1	1.3	1.3	0.2	10
3 µg MDV (dilution required, 10 dose vial)	0.1	0.4	2.2	0.2	10

a. Dilution with 0.9% sodium chloride is only required for presentations described as "dilution required"

Data provided to support consistent manufacturing and control of COMIRNATY Omicron XBB.1.5 DP included:

- Validation of the updated [redacted] and [redacted] PCR assays for identity determination of the Omicron XBB.1.5 DP.
- Validation of the assay for *in vitro* expression assay of the Omicron XBB.1.5-specific S protein.
- Batch analysis for one DP lot for each of the following presentations (4 lots total):
  - 30µg/dose ready-to-use (DTU) single-dose vial (SDV)
  - 10µg/dose dilute-to-use (DTU) multi-dose vial (MDV)
  - 10µg/dose ready-to-use (RTU) single-dose vial (SDV)
  - 3µg/dose dilute-to-use (DTU) multi-dose vial (MDV)

All four lots met specification and were comparable to historical lots for COMIRNATY Original & Omicron BA.4/BA.5.

Taken together, the data and information provided in the submission support the consistent manufacturing of COMIRNATY Omicron XBB.1.5 vaccine.

STABILITY CLAIM(S)

Due to the expedited development of the Omicron XBB.1.5 vaccine, stability data for the Omicron XBB.1.5 DS and DP were not available. However, minor sequence changes to the mRNA DS are not expected to impact the stability of the Omicron XBB.1.5 DS and DP, based on extensive stability data for commercial-scale COMIRNATY vaccines (Original

Tris/Sucrose formulation and Bivalent). Therefore, there is no change to the currently approved shelf-lives for the COMIRNATY DS and the DP at the various presentations:

Drug substance

[redacted]

Drug Product

The proposed shelf-life of the Omicron XBB.1.5 DP is 18 months when formulated at 0.1 mg/mL or 12 months when formulated at 0.033 mg/mL when stored at the recommended -90 to -60°C. Short-term storage at 5 ± 3°C for up to 10 weeks is also acceptable, as long as the storage does not exceed the expiry of the DP.

SUMMARY OF VIRAL CLEARANCE / ADVENTITIOUS AGENTS DATA

The controls applied to all raw materials are identical to those used by currently approved for COMIRNATY Original & Omicron BA.4/BA.5.

SUMMARY OF KEY QUALITY ISSUES

[redacted] elements present on plasmid [redacted]

In response to Quality Clarifax dated August 4th, 2023, the Sponsor [redacted] DNA regulatory elements present on plasmid [redacted] These elements include an SV40 promoter, SV40 poly A terminator [redacted]

[redacted] Health Canada's position was provided to the sponsor in a follow-up clarifax dated August 24th, 2023. [redacted]

The presence of the SV40 promoter is not considered a concern for the following reasons:

1. The plasmid is linearized and further digested during the DS downstream manufacturing process. The current approved specification for residual DNA template (<10ng/dose) is in line with WHO recommendation concerning residual DNA fragments derived from continuous cell line (as worst case). Furthermore, the Sponsor has committed to provide additional data concerning the size of DNA fragments and residual plasmid capable of replication in bacterial cells, by December 1, 2023.
2. There is no peer-reviewed scientific literature suggesting that the SV40 promoter itself or the other non-functional elements pose a risk to human health.

Health Canada will continue to work with our international partners, seeking harmonized regulatory approach to [redacted]

ON-SITE EVALUATION (OSE)

No new manufacturing sites were proposed for Canadian supply, relative to multiple sites previously authorized through an IO or NDS-CV regulatory process for monovalent and bivalent COMIRNATY vaccines. All facilities used to manufacture COMIRNATY vaccines have been issued Establishment Licenses (EL) by Health Canada Regulatory Operations and Enforcement Branch (ROEB). Furthermore, other than minor changes to the plasmid sequence and the primers used for identity test of DS/DP, the manufacturing and control remains the same. Thus, an OSE is not required for any currently

approved facility..

LOT RELEASE EVALUATION GROUPING AND LABORATORY TESTING

Pre-Approval Group 1B Consistency Testing

The information included in the submission provides sufficient assurance that the quality attributes of COMIRNATY Omicron XBB.1.5 DP lots will be of comparable quality to material manufactured for COMIRNATY Original & Omicron BA.4/BA.5. In addition, given the expedited review timeline, consistency testing was not performed for this submission. BRDD will continue to have an oversight of this vaccine through the Lot Release Program.

Post-Approval Lot Release Evaluation

COMIRNATY Omicron XBB.1.5 should remain in Evaluation Group 3 of the Lot Release Program.

YBPR FILING REQUIREMENT

Yearly Biologic Product Reports will not be requested. BRDD will continue to have an oversight of this vaccine through the post-NOC changes and the Lot Release Program.

CERTIFIED PRODUCT INFORMATION DOCUMENT (CPID)

Due to the expedited nature of the submission, the sponsor has agreed to provide the CPID as a post-approval commitment.

PRODUCT MONOGRAPH

The relevant quality information in the last version of the Product Monograph (sequence 0006) was reviewed and found acceptable.

STAFF INVOLVED IN SUBMISSION REVIEW

Elisabeth Davis – 1st reviewer
Michael Wall – 2nd reviewer

FOREIGN REVIEW DOCUMENTATION

Method 4: The Canadian review and regulatory decision is based on a critical assessment of the Canadian data package. **No** foreign decisions or review reports were provided.

ASSOCIATED REPORTS IN SUPPORT OF REVIEW RECOMMENDATION

Report Type	Required		Number	Comment
	Yes	No		
Submission Review	☒	☐	1	Quality Review Report
DMF Review	☐	☒		
Consistency Testing	☐	☒		
OSE	☐	☒		
Other	☐	☒		

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-10-12 1:51 PM
To: [redacted]PhD
Subject: Fwd: SV40 Comirnaty

Sent from my iPhone

Begin forwarded message:

From: [redacted]@ema.europa.eu>
Date: October 12, 2023 at 1:27:02 PM EDT
To: [redacted]@fda.hhs.gov, "Smith, Dean (HC/SC)" <dean.smith@hc-sc.gc.ca>, [redacted]@fda.hhs.gov
Subject: SV40 Comirnaty

Dear All

We are going to discuss the matter of SV40 with Pfizer/Biontech as well as these alleged high level of DNA in vaccines coming from these external parties

Have you taken any action? What would be your perspective?

Many thanks and happy to discuss

Best
[redacted]

Wu, Tong (HC/SC)

From: Wu, Tong (HC/SC)
Sent: 2023-10-12 1:52 PM
To: Smith, Dean (HC/SC)
Subject: RE: SV40 Comirnaty

Great! When I get back home.

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Thursday, October 12, 2023 1:51 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Subject: Fwd: SV40 Comirnaty

Hi Tong,
Please take a look below and we can chat later today.
Dean

Sent from my iPhone

Begin forwarded message:

> **From:** [redacted]@ema.europa.eu>
> **Date:** October 12, 2023 at 1:27:02 PM EDT
> **To** [redacted]@fda.hhs.gov, "Smith, Dean (HC/SC)" <dean.smith@hc-sc.gc.ca>, [redacted]@fda.hhs.gov
> **Subject: SV40 Comirnaty**
>
> Dear All
>
> We are going to discuss the matter of SV40 with Pfizer/Biontech as well as these alleged high level of DNA in vaccines coming from these external parties
>
> Have you taken any action? What would be your perspective?
>
> Many thanks and happy to discuss
>
> Best
> [redacted]
>
> Classified as confidential by the European Medicines Agency

Wu, Tong (HC/SC)

From: Wu, Tong (HC/SC)
Sent: 2023-10-12 5:06 PM
To: Smith, Dean (HC/SC)
Subject: RE: SV40 Comirnaty
Attachments: Response to Clarifax of Aug-04-2023 (PDF).pdf; Response to Clarifax of Aug-22-2023 (PDF).pdf; Final comments.pdf

Two clarifax and one final comments.

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Thursday, October 12, 2023 1:51 PM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Subject: Fwd: SV40 Comirnaty

Hi Tong,
Please take a look below and we can chat later today.
Dean

Sent from my iPhone

Begin forwarded message:

From: [redacted]@ema.europa.eu>
Date: October 12, 2023 at 1:27:02 PM EDT
To: [redacted]@fda.hhs.gov, "Smith, Dean (HC/SC)" <dean.smith@hc-sc.gc.ca>, [redacted]@fda.hhs.gov
Subject: SV40 Comirnaty

Dear All

We are going to discuss the matter of SV40 with Pfizer/Biontech as well as these alleged high level of DNA in vaccines coming from these external parties

Have you taken any action? What would be your perspective?

Many thanks and happy to discuss

Best
[redacted]

Classified as confidential by the European Medicines Agency

Wu, Tong (HC/SC)

From: Pal, Yasmeen (HC/SC)
Sent: 2023-08-30 4:04 PM
To: [redacted]
Cc: Belisle, Luc (HC/SC); Antonio, Christopher (HC/SC); Garay, Kenneth (HC/SC)
Subject: COVID-19: NDS-CV for Comirnaty Omicron XBB.1.5 (raxtozinameran), Control # 276302 - Quality Comment

Dear [redacted]

Further to the response to Quality Clarifax #2 for Comirnaty Omicron XBB.1.5 (raxtozinameran), NDS-CV Control #276302, submitted on August 25, 2023 in sequence 0008, the Quality review team would like to provide the following comment to the sponsor. Please note that this is for information only and a formal response is not required at this time:

1. [redacted]

Should you have any questions or concerns, please do not hesitate to contact me.

Please confirm receipt of this email.

Kind regards,
Yasmeen (on behalf of Kenneth Garay)

Yasmeen Pal
Senior Regulatory Affairs Officer
Office of Regulatory Affairs
Biologic and Radiopharmaceutical Drugs Directorate
Health Products and Food Branch / Health Canada

Agente principale des affaires réglementaires
Bureau des affaires réglementaires
Direction des médicaments biologiques et radiopharmaceutiques
Direction générale des produits de santé et des aliments / Santé Canada

Email: yasmeen.pal@hc-sc.gc.ca/ Tel: 343-552-0330

COMMENT 1

1. Sequence analysis of plasmid [redacted] (XBB 1.5) indicates the presence of the regulatory region sequences of Simian Virus (SV40), located in the plasmid region [redacted]. Provide the following:

   a. [redacted]

   b. Justification for the SV40 regulatory elements in the plasmid.

RESPONSE

a. The SV40 regulatory region sequences [redacted] in the submission since this [redacted] is relevant neither for plasmid production in *E. coli* nor for production of mRNA.

b. The backbone of [redacted] contains elements for plasmid use in either bacterial or mammalian cells. Only the prokaryotic backbone elements of this plasmid are utilized for generation of the DNA starting material in *E. coli* and for mRNA production. The SV40 regulatory elements are not relevant to mRNA production since mammalian cells are not used in this process. SV40 regulatory elements are used to drive neomycin/kanamycin resistance in mammalian cells. This function is not used during production of plasmid [redacted] in *E. coli*. [redacted]

References

None

SUPPORTING DOCUMENTATION

New, Appended, or Replaced Supporting Documentation.

None

Previously Submitted Supporting Documentation

None

COMMENT 2

2. Update Table 3.2.S.2.3-1 (Functional Elements of [redacted] Omicron XBB.1.5) to include the following plasmid [redacted] (XBB 1.5) sequences:

[redacted]

RESPONSE

Table 1 below lists the non-functional elements in the backbone of plasmid [redacted]

[redacted]

2 below lists the functional elements of plasmid [redacted] These are involved in plasmid production in *E. coli* and in the mRNA production process.

[redacted]

It should be noted that the SV40 promotor/enhancer is neither an oncogene or infectious virus, which are the primary safety concerns for plasmid-related DNA carryover. Since the SV40 promotor/enhancer DNA does not contain known oncogenes, infectious agents, or regions that could lead to functional transcripts, the DNA does not present any specific safety concerns.

The SV40 regulatory elements are also not relevant to mRNA production (See Response 1). However, using theoretical calculations based on the relative size ratio of the SV40

promoter/enhancer to the full length plasmid while considering the residual DNA limit, the absolute maximum amount of SV40 promoter/enhancer DNA could be estimated to picogram quantities at a maximum dose of 30 µg per dose, which is below the 10 ng per DNA/dose recommended by WHO. This calculation is based on SV40 promoter/enhancer burden and expected reduction during RNA purification process. Based on these considerations the SV40 promoter/enhancer poses minimal safety risk for human use.

SUPPORTING DOCUMENTATION

Previously Submitted Supporting Documentation

3.2.S.2.3. Control of Materials - Source, History, and Generation of Plasmids [Omicron (XBB.1.5) Variant]

COMMENT 3

3. Concerning the residual plasmid DNA in the drug substance, provide data/information characterizing the following:

a. the size distribution of the residual DNA fragments.
b. residual intact circular plasmid.

RESPONSE

Pfizer and BioNTech acknowledge the request for additional data and/or information characterizing the size distribution of residual DNA fragments and residual intact circular plasmid. The quantitative PCR (qPCR) assay used to measure residual DNA in the mRNA drug substance is capable of detecting both linear and circular plasmid DNA. Therefore, the reported residual DNA template result encompasses all DNA that may be present in the mRNA drug substance. Original and Omicron Variant drug substance batches manufactured to date, [redacted] routinely meet the residual DNA template acceptance criterion of ≤ 330 ng DNA / mg RNA. This acceptance criterion complies with the WHO recommendation of not more than 10 ng DNA/dose, based on a maximum dose of 30 µg RNA.

Pfizer and BioNTech commit to provide additional data and/or information characterizing the size distribution of residual DNA fragments and residual intact circular plasmid by Dec 1, 2023 as the data are not readily available and will require time to generate. Please note that to date, Pfizer and BioNTech have not been requested to provide these characterization data across global markets for Original or Omicron drug substances. Thus, the commitment date is requested to complete the appropriate characterization testing and regulatory submission.

Pfizer and BioNTech remain available to discuss as needed via teleconference.

References

None

SUPPORTING DOCUMENTATION

New, Appended, or Replaced Supporting Documentation.

None

Previously Submitted Supporting Documentation

None

COMMENT 1

Health Canada is not aware of any peer-reviewed scientific evidence that would raise safety concerns over the residual SV40 regulatory elements present in the final vaccine product.

[redacted]

Please comment.

RESPONSE

[redacted]

As stated in the response to Clarifax dated 04 August 2023 (submitted on 09 August 2023, under sequence 0004), these non-functional elements are not used for the mRNA drug substance production process. [redacted]

[redacted]

As for the use of current plasmid DNA elements, considerable safety data are available. A comprehensive, multifaceted program is in place to ensure that the risk is acceptable with respect to potential viral contamination of drug substance. Additionally, the SV40 promoter/enhancer amounts in the drug product poses minimal safety risk for human use (refer to clarifax response submitted on 09 August 2023).

References

None

SUPPORTING DOCUMENTATION

None

COMMENT 2

Health Canada notes the commitment to provide additional data and/or information characterizing the size distribution of residual DNA fragments and residual intact circular plasmid. We ask that the data package also address whether the residual DNA plasmid is capable of replication in bacteria.

RESPONSE

Pfizer and BioNTech acknowledge the request above and commit to address the capability of the residual DNA plasmid of replicating in bacteria.

The commitment provided in response to Clarifax dated 04 August 2023 (submitted on 09 August 2023, under sequence 0004) related to characterizing data for size distribution of residual DNA fragments and residual intact circular plasmid is updated accordingly. The commitment date of Dec 1, 2023 remains unchanged.

References

None

SUPPORTING DOCUMENTATION

None

Wu, Tong (HC/SC)

From: Wu, Tong (HC/SC)
Sent: 2023-10-13 10:39 AM
To: Smith, Dean (HC/SC)
Subject: RE: FYI Tong : [EXTERNAL] SV40 Comirnaty

Well said.

Thanks,
tong

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Friday, October 13, 2023 10:37 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Subject: FYI Tong : [EXTERNAL] SV40 Comirnaty

Here you go!
Thanks again for leading this Tong!!
Dean

From: Smith, Dean (HC/SC)
Sent: Friday, October 13, 2023 10:36 AM
To: [redacted]@fda.hhs.gov>; [redacted]@ema.europa.eu>; [redacted]@fda.hhs.gov>
Subject: RE: [EXTERNAL] SV40 Comirnaty

Dear [redacted]

Thanks for reaching out.

In addition to dealing with the media inquires that you and [redacted] have commented on, as we mentioned briefly at a cluster meeting, there have been a couple written exchanges with Pfizer regarding the plasmid since this became public.

[redacted]

They have agreed to evaluate our request and to provide additional characterization data on plasmid DNA degradation fragment distribution, as well as replication potential in bacteria with any reaming circular plasmid that remains in the final product by Dec 1, 2023.

As you are aware the fragment size is related to the probability of integration, and the WHO guidance assumes a fragment size of generally less than 200 bp.

[redacted]

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-10-13 10:52 AM
To: Wu, Tong (HC/SC)
Subject: RE: FYI Tong : [EXTERNAL] SV40 Comirnaty

Glad you approve!
Dean

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Friday, October 13, 2023 10:39 AM
To: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: RE: FYI Tong : [EXTERNAL] SV40 Comirnaty

Well said.

Thanks,
tong

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Friday, October 13, 2023 10:37 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Subject: FYI Tong : [EXTERNAL] SV40 Comirnaty

Here you go!
Thanks again for leading this Tong!!
Dean

From: Smith, Dean (HC/SC)
Sent: Friday, October 13, 2023 10:36 AM
To: [redacted]@fda.hhs.gov>; [redacted]@ema.europa.eu>; [redacted]@fda.hhs.gov>
Subject: RE: [EXTERNAL] SV40 Comirnaty

Dear [redacted]

Thanks for reaching out.

In addition to dealing with the media inquires that you and [redacted] have commented on, as we mentioned briefly at a cluster meeting, there have been a couple written exchanges with Pfizer regarding the plasmid since this became public.

[redacted]

They have agreed to evaluate our request and to provide additional characterization data on plasmid DNA degradation fragment distribution, as well as replication potential in bacteria with any reaming circular plasmid that remains in the final product by Dec 1, 2023.

Wu, Tong (HC/SC)

From: Wu, Tong (HC/SC)
Sent: 2023-10-13 11:24 AM
To: Smith, Dean (HC/SC)
Subject: RE: [EXTERNAL] SV40 Comirnaty

Next Friday afternoon (geneva time). I can take a call. If this is not good, go ahead with Mike/Rich for this next week.

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Friday, October 13, 2023 11:11 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Subject: FW: [EXTERNAL] SV40 Comirnaty

Hi Tong next is not good for you and the week after I'm off. What is good for you. We'll also see what David comes back with.

From: [redacted]@ema.europa.eu>
Sent: Friday, October 13, 2023 11:08 AM
To: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>; [redacted]@fda.hhs.gov>; [redacted]@fda.hhs.gov>
Subject: RE: [EXTERNAL] SV40 Comirnaty

Thanks Dean and [redacted]

I think a call could be useful, not the least since right now we are receiving queries from different media and fact-checker groups also from US

An aligned position would help navigating the communication challenge

Let me know when best for you

Best
[redacted]

Classified as confidential by the European Medicines Agency

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Friday, 13 October 2023 16:36
To: [redacted]@fda.hhs.gov>; [redacted]@ema.europa.eu>; [redacted]@fda.hhs.gov>
Subject: RE: [EXTERNAL] SV40 Comirnaty

Dear [redacted]

Thanks for reaching out.

In addition to dealing with the media inquires that you and [redacted] have commented on, as we mentioned briefly at a cluster meeting, there have been a couple written exchanges with Pfizer regarding the plasmid since this became public.

De Rose, Carol (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-10-16 4:16 PM
To: Pham, Co (HC/SC)
Subject: Co RE: [EXTERNAL] SV40 Comirnaty informal

Hi Co,

Below is the email exchange I mentioned, that preceded the 30 min discussion at 8:30 AM this morning.

We are the most advanced in these exchanges with Pfizer so far, but there is now more interest in this from EMA and FDA as you and I discussed.

The highlighted text below is a summary of the HC exchange with Pfizer, that Tong's group had lead that I've worked on with them.

Dean

From [redacted]@ema.europa.eu>
Sent: Friday, October 13, 2023 11:08 AM
To: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>; [redacted]@fda.hhs.gov>; [redacted]@fda.hhs.gov>
Subject: RE: [EXTERNAL] SV40 Comirnaty

Thanks Dean and [redacted]

I think a call could be useful, not the least since right now we are receiving queries from different media and fact-checker groups also from US

An aligned position would help navigating the communication challenge

Let me know when best for you

Best
[redacted]

Classified as confidential by the European Medicines Agency

From: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Sent: Friday, 13 October 2023 16:36
To: [redacted]@fda.hhs.gov>; [redacted]@ema.europa.eu>; [redacted]@fda.hhs.gov>
Subject: RE: [EXTERNAL] SV40 Comirnaty

Dear [redacted]

Thanks for reaching out.

In addition to dealing with the media inquires that you and [redacted] have commented on, as we mentioned briefly at a cluster meeting, there have been a couple written exchanges with Pfizer regarding the plasmid since this became public.

[redacted]

They have agreed to evaluate our request and to provide additional characterization data on plasmid DNA degradation fragment distribution, as well as replication potential in bacteria with any reaming circular plasmid that remains in the final product by Dec 1, 2023.

As you are aware, the fragment size is related to the probability of integration, and the WHO guidance assumes a fragment size of generally less than 200 bp.

[redacted]

They also commented on the fact that HC was the only agency raising these issues, and we appreciate the engagement of EMA in the discussion with Pfizer.

As with [redacted] finial comment, we are also happy to join a call if helpful.

Thanks again!

Best regards,
Dean

From: [redacted]@fda.hhs.gov>
Sent: Friday, October 13, 2023 6:36 AM
To: [redacted]@ema.europa.eu>; Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>; [redacted]@fda.hhs.gov>
Subject: RE: [EXTERNAL] SV40 Comirnaty

Dear [redacted]

We are tracking the ongoing external parties' discussion on this topic, including responding to incoming inquiries.

Glad to join a call, if helpful.

Thank you.

Be well,
[redacted]

From: [redacted]@ema.europa.eu>
Sent: Thursday, October 12, 2023 1:27 PM
To: [redacted]@fda.hhs.gov>; Dean Smith <dean.smith@hc-sc.gc.ca>; [redacted]@fda.hhs.gov>
Subject: [EXTERNAL] SV40 Comirnaty

ATIA - 19(1)

Dear All

We are going to discuss the matter of SV40 with Pfizer/Biontech as well as these alleged high level of DNA in vaccines coming from these external parties

Have you taken any action? What would be your perspective?

Many thanks and happy to discuss

Best

[redacted]

Smith, Dean (HC/SC)

From: Smith, Dean (HC/SC)
Sent: 2023-10-16 3:08 PM
To: Tong Wu
Subject: RE: NC3R

Excellent Tong and thanks for the update!

Can I assume you have shared the same news with Richard I.?

On as different topic, the chat with [redacted] and [redacted] also went regarding Pfizer and the SV40 Enhance / promoter. That topic will now be on the Dec 1 cluster meeting agenda and as a updated.

I've updated Rich on this as well and I;ll send you a bit more on that and copy Rich when I can make the time.

Very glad you all had a good day!

Cheers,
Dean

From: Tong Wu <tongwu[redacted]
Sent: Monday, October 16, 2023 2:57 PM
To: Smith, Dean (HC/SC) <dean.smith@hc-sc.gc.ca>
Subject: NC3R

Hi Dean,

The NC3R discussion went very well. There is no opposition to a 3R related WHO document.

Talk to you later.
tong

Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

Kevin McKernan, Yvonne Helbert, Liam T. Kane, Stephen McLaughlin
Medicinal Genomics, 100 Cummings Center, Suite 406-L, Beverly Mass, 01915

Several methods were deployed to assess the nucleic acid composition of four vials of the Moderna and Pfizer bivalent mRNA vaccines. Two vials from each vendor were evaluated with Illumina sequencing, qPCR, RT-qPCR, Qubit™ 3 fluorometry and Agilent Tape Station™ electrophoresis. Multiple assays support DNA contamination that exceeds the European Medicines Agency (EMA) 330ng/mg requirement and the FDAs 10ng/dose requirements. These data may impact the surveillance of vaccine mRNA in breast milk or plasma as RT-qPCR assays targeting the vaccine mRNA cannot discern DNA from RNA without RNase or DNase nuclease treatments. Likewise, studies evaluating the reverse transcriptase activity of LINE-1 and vaccine mRNA will need to account for the high levels of DNA contamination in the vaccines. The exact ratio of linear fragmented DNA versus intact circular plasmid DNA is still being investigated. Quantitative PCR assays used to track the DNA contamination are described.

Introduction

Several studies have made note of prolonged presence of vaccine mRNA in breast milk and plasma (Bansal et al. 2021; Hanna et al. 2022; Castruita et al. 2023). This could be the result of the stability of N1-methylpseudouridine (m1Ψ) in the mRNA of the vaccine. Nance *et al*. depict a vaccine mRNA synthesis method that utilizes a dsDNA plasmid that is first amplified in *E.coli* prior to an *in-vitro* T7 polymerase synthesis of vaccine mRNA (Nance and Meier 2021). Failure to remove this DNA could result in the injection of spike encoded nucleic acids more stable than the modified RNA. The EMA has stated limits at 330ng/mg of DNA to RNA (Josephson 2020-11-19). The FDA has issued guidance for under 10ng/dose in vaccines (Sheng-Fowler et al. 2009).

Residual injected DNA can result in type I interferon responses and can increase the potential for DNA integration(Ulrich-Lewis et al. 2022).

Results

To assess the nucleic acid composition of the vaccines, vaccine DNA was deeply sequenced using two different methods. The first method used a commercially available New England Biolabs RNA-seq method that favored the sequencing of the RNA but still presented over 500X coverage for the unanticipated DNA vectors (Figure 1 and 2). The RNA-seq assemblies had truncated poly A tracts compared to the constructs described by Nance *et al*. The second method eliminated the RNA with RNase A treatment and sequenced only the DNA using a Watchmaker Genomics fragment library kit. The DNA focused assemblies delivered vector assemblies with more intact poly A tracts (Figure 3).

These assemblies were utilized to design multiplex qPCR and RT-qPCR assays that target the spike sequence present in both the vaccine mRNA and the DNA vector while also targeting the origin of replication sequence present only in the DNA vector (Figure 3). The assembly of Pfizer

vial 1 contains a 72bp insertion not present in the assembly of Pfizer vial 2. This indel is known for its enhancement to the SV40 promoter and its nuclear targeting signal (Dean et al. 1999) (Moreau et al. 1981).

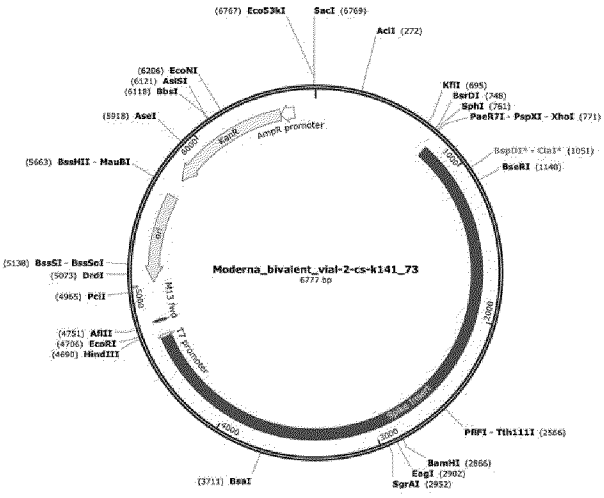


Figure 1. A Moderna vector assembly of an RNA-seq library with a spike insert (red), Kanamycin resistance gene (green) driven by an AmpR promoter and a high copy bacterial origin of replication (yellow).

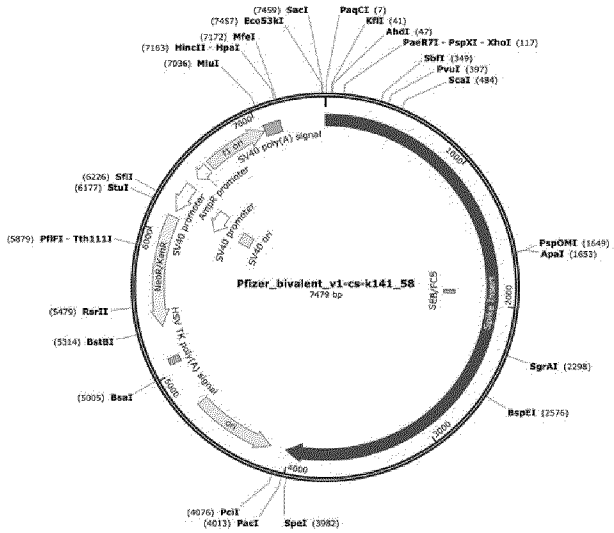


Figure 2. Pfizer bivalent vaccine assembly of the RNA-seq library. Annotated with SEB/FCS, spike insert (red), bacterial origin of replication (yellow), Neo/Kan resistance gene(green), F1 origin (yellow) and an SV40 promoter (yellow and white).

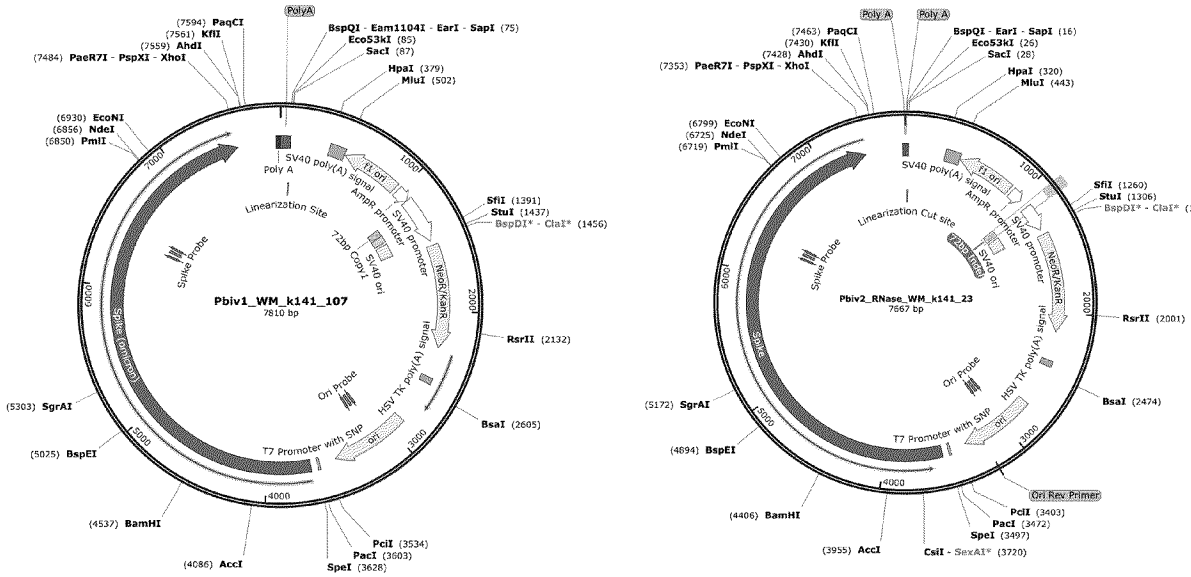


Figure 3. RNase treated vaccines were shotgun sequenced with Illumina (RNase-Seq not RNA-seq). Pfizer vectors from vial 1 (left) and vial 2 (right) contain a 72bp difference in the SV40 promoter (green and light blue annotation). qPCR assays are depicted in pink as Spike probe and Ori probe. The RNase sequencing provided better resolution over the Eam1104i linearization site and the Poly adenylation sequence. The vectors differ in the length of the polyA tail (likely sequencing artifact) and the 72bp indel.

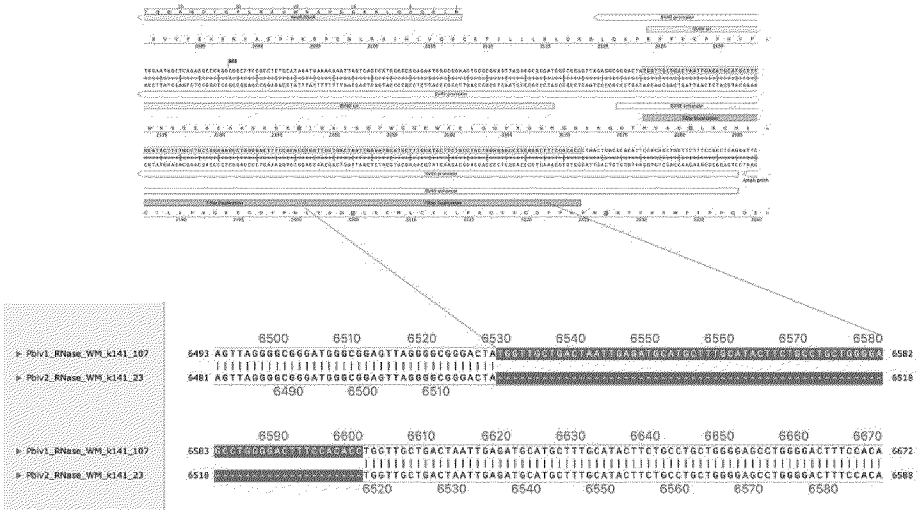


Figure 4. Local alignment of Pfizer vial 1 to Pfizer vial 2 vectors highlights the 72bp tandem duplication in blue.

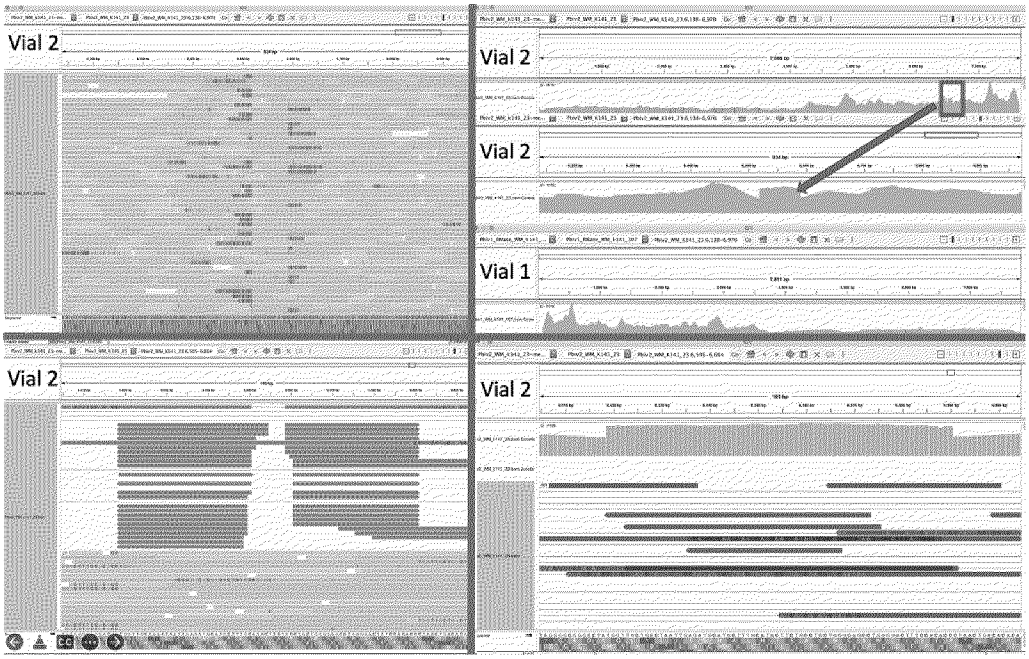


Figure 5A. Close inspection of the Integrative Genome Viewer (IGV) demonstrates the appearance of a 72bp insertion that is heteroplasmic in Pfizer vial 2. The upper left IGV view is a zoomed-out view where the colored marks depict the indel. The lower Left IGV view shows inverted paired reads as the 72bp insertion is a tandem repeat and paired reads shorter than 72bp can be mapped two different ways. Upper Right IGV view demonstrates a read coverage pile up or 'Plateau'. This occurs when the reference has one copy of the 72bp repeat and the sample has 2 copies. Note- In the upper right IGV depiction, the sequence in Vial 1 is in the opposite orientation in IGV as Vial 2. Lower right IGV view is a zoomed view of the upper right IGV screen.

Since the two Pfizer vials share the same lot number, finding a heterozygous copy number change between the two vials is unexpected. It was hypothesized that the appearance of a heteroplasmic copy number change is instead the result of the Megahit assembler collapsing what is actually two copies of the 72bp sequence into a single copy due to the insert sizes in the sequencing libraries being too short (105bp). It is noteworthy that the longer paired-end reads in the library resolve the 72bp tandem repeat.

When references have a single copy of the 72bp repeat and the sample has two copies of the repeat, reads should pile up to twice the coverage over the single copy 72bp loci as seen in Figure 5A. To test this hypothesis, we added a second 72bp sequence to the shorter plasmid assembly and observed that the reads map without artifact and no evidence of heteroplasmy (Figure 5B).

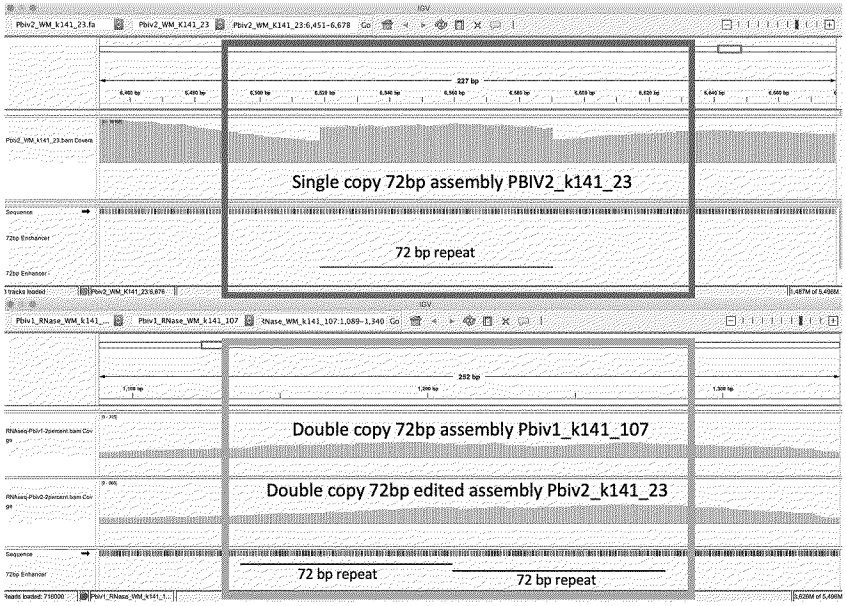
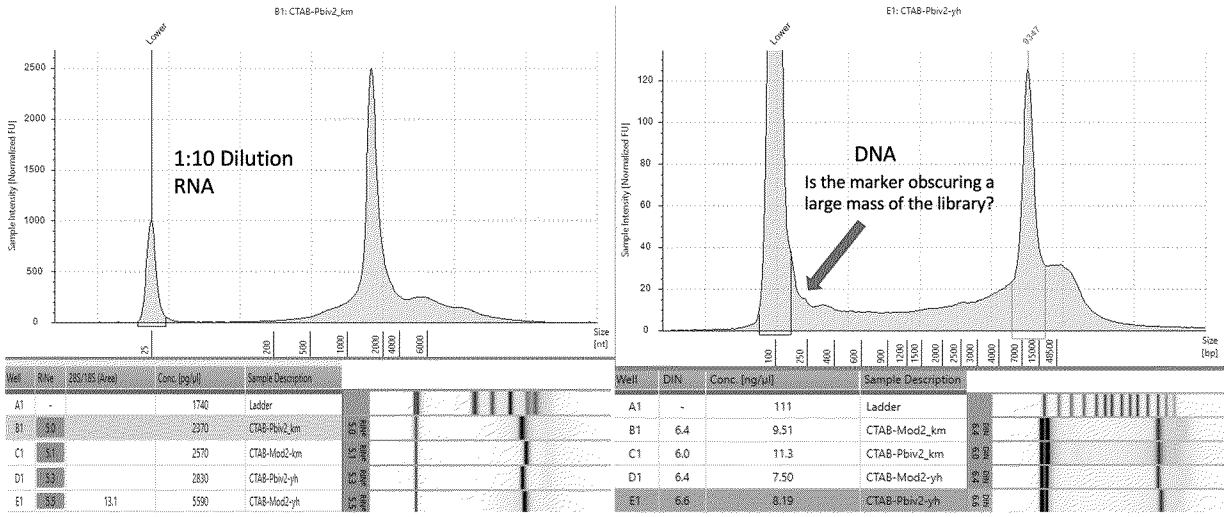


Figure 5B. IGV view of the read coverage over Pbiv2_k141_23 shows a discrete 72bp plateau in coverage (red rectangle). Editing the Pbiv2_k141_23 reference to include 2 copies of the 72bp sequence, and remapping the sequence data to this corrected sequence shows that the coverage over both vectors is more normal with no coverage plateau in Pfizer vial 2.

These data conclude that all Pfizer vectors contain a homoplastic 2 copy 72bp SV40 Enhancer associated with more robust expression and nuclear localization. The initial heteroplastic indel was an artifact of the Megahit assembler and short insert libraries. These vectors contain an SV40 Promoter, SV40 Enhancer, SV40 Origin, and an SV40 polyA signal. They do not contain the entire SV40 virus or the SV40 T-antigen.

To estimate the size of the DNA, the purified vaccines were evaluated on an Agilent Tape Station™ using DNA (genomic DNA screen tapes) and RNA based (high sensitivity RNA tapes) electrophoresis tapes.

Agilent Tape Station™ electrophoresis reveal 7.5 - 11.3 ng/µl of dsDNA compared to the 23.7 -55.9ng/µl of mRNA detected in each 300µl sample. Qubit™ 3 fluorometry estimated 1-2.8ng/µl of DNA and 21.8ng - 52.8ng/µl of RNA. There is higher fragmentation seen in the DNA electrophoresis. The total RNA levels are less than the anticipated 30ug (100ng/µl) and 100ug (200ng/µl) doses suggesting a loss of yield in DNA and RNA isolation, manufacturing variance or RNA decay with questionable cold chains.



Well	Rin	28S/18S (Area)	Conc. [pg/µl]	Sample Description
A1	-		1740	Ladder
B1	5.0		2370	CTAB-Pbiv2_km
C1	5.1		2570	CTAB-Mod2-km
D1	5.3		2830	CTAB-Pbiv2-yh
E1	5.5	13.1	5590	CTAB-Mod2-yh

Well	DIN	Conc. [ng/µl]	Sample Description
A1	-	111	Ladder
B1	6.4	9.51	CTAB-Mod2_km
C1	6.0	11.3	CTAB-Pbiv2_km
D1	6.4	7.50	CTAB-Mod2-yh
E1	6.6	8.19	CTAB-Pbiv2-yh

Figure 6. Agilent Tape Station™ electrophoresis demonstrates 23.7ng/µl – 55.9ng/µl of RNA (left). 7.5ng-11.3ng/µl are observed on DNA based Tape Station™. While the DNA electropherogram shows a peak suggestive of a full-length plasmid, this sample is known to have high amounts of N1-methylpseudouridine RNA present. DNA hybrids with N1-methylpseudouridine mRNA may provide enough intercalating dye cross talk to produce a peak. The sizing of the peak on the RNA tape on the left is shorter than expected. This may be the results of N1 methylpseudouridine changing the secondary structure or the mass to charge ratio of the DNA.

Quantitative PCR assays were designed using IDTs Primer Quest software targeting a region in the spike protein that was identical between Moderna and Pfizer spike sequences and a shared sequence in the vectors' origin of replication. This allowed the qPCR and RT-qPCR assessment of the vaccines. qPCR only amplifies DNA while RT-qPCR amplifies both DNA and RNA. Gradient qPCR was utilized to explore conditions where both targets would perform under the same cycling conditions for both RT-qPCR and PCR (gradient PCR data not shown).

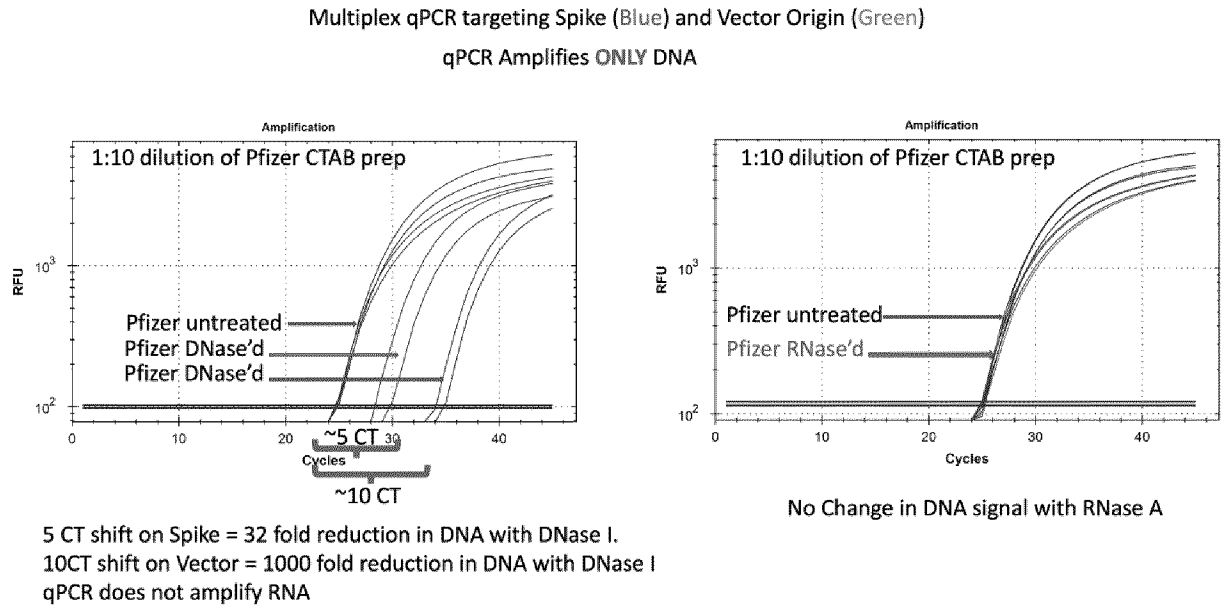


Figure 7. qPCR of Pfizer's bivalent vaccine with and without DNase I (left) and RNase A (right). Untreated mRNA demonstrates equal CTs for Spike and Vector assays as expected. Vector is more DNase I sensitive than the Spike suggesting the modRNA may inhibit nuclease activity of DNase I against complementary DNA targets. RNase A treatment doesn't alter the qPCR signal. Non Template Control (NTC) amplification produces no signal out to CT 40 with the spike assay and no signal out to CT 37 with the bacterial origin of replication assay. This background Ori CT may vary with different polymerases that are expressed in bacteria vectors containing this common Ori.

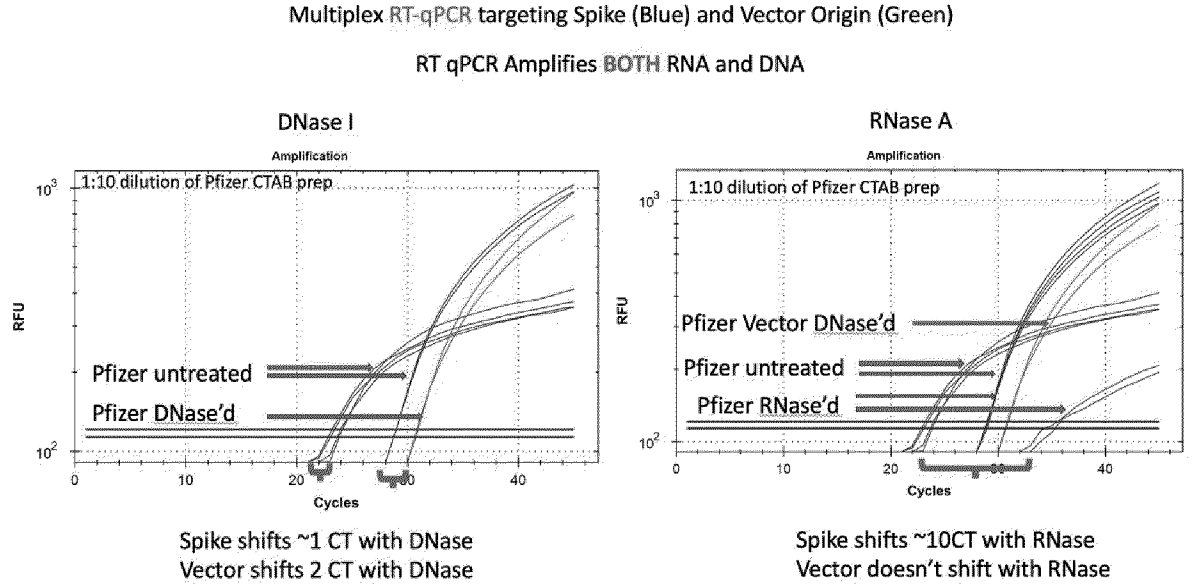


Figure 8. RT-qPCR amplifies both DNA and RNA. The untreated samples show a large CT offset with Pfizer Spike and Vector assays (Left Blue versus Green). This is anticipated as the T7 polymerization should create more mRNA over spike than over the vector. Small 1-2 CT shifts are seen with DNase I treatment. This is expected if the DNA is less than equal concentration of nucleic acid in RT-PCR. RNase treatment (Right) shows a 10 CT offset but doesn't alter the DNA vector CT.

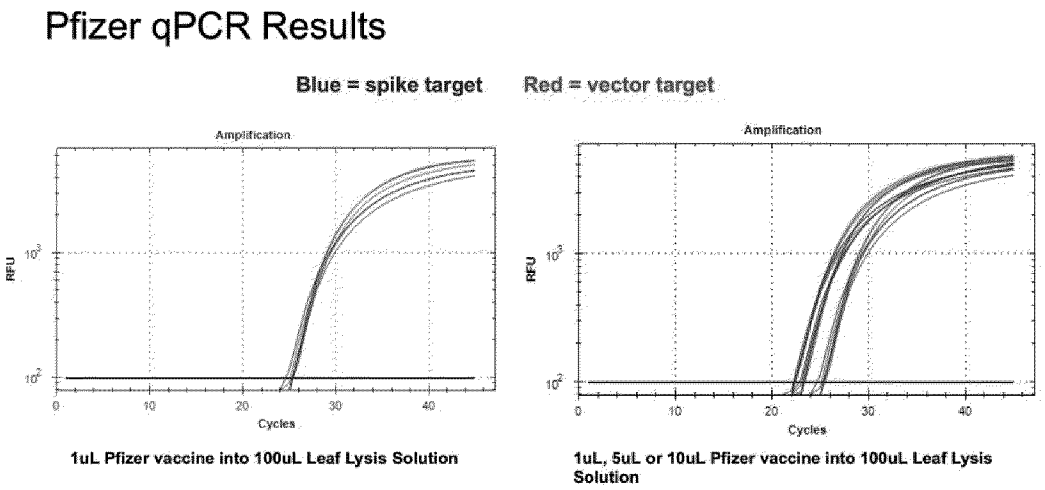


Figure 9. 1µl of the Pfizer bivalent vaccine placed in 100µl Leaf Lysis buffer for an 8 minute boil step delivers a CT of 24 for both Vector and Spike targets in qPCR (Left). Assay is responsive to 1,5,10µl of input (Right).

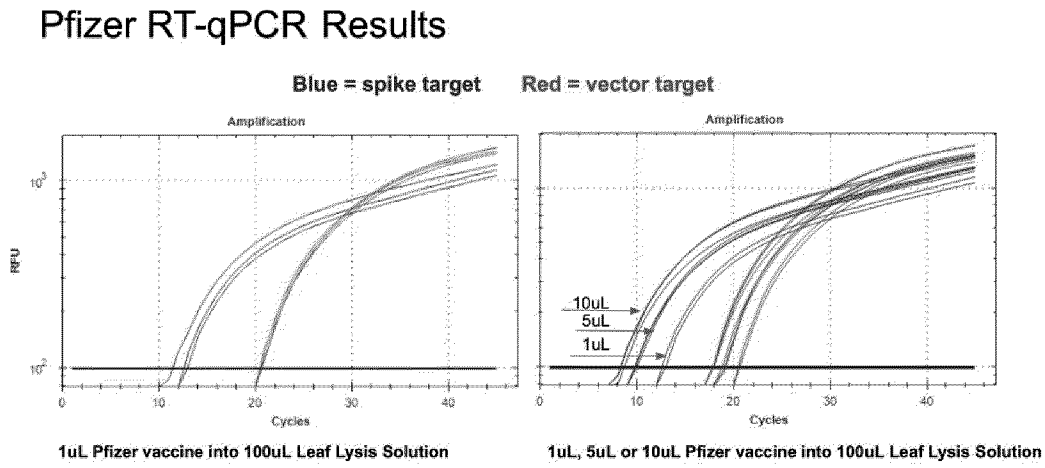


Figure 10. 1µl of the Pfizer bivalent vaccine placed in 100µl Leaf Lysis buffer for an 8 minute boil step delivers a CT of 20 and 12 for both Vector and Spike targets in RT-qPCR (Left). Assay is responsive to 1,5,10µl of input (Right).

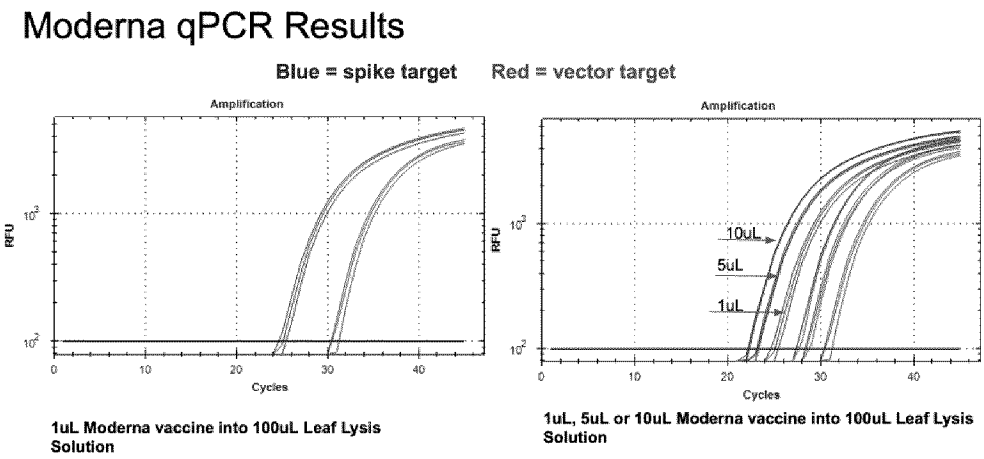


Figure 11. 1µl of the Moderna bivalent vaccine exhibits different CTs values for the spike and the vector targets (Left) with qPCR. This needs to be explored further as the assays provide equal CT scores on Pfizers' vaccines and the sequence of the amplicon is identical between the two vector origins. There are 2 mismatches in the spike amplicons between Moderna and Pfizer but none of the mismatches are under a primer or probe. The assay is responsive to 1,5,10µl of direct boil mRNA (Right).

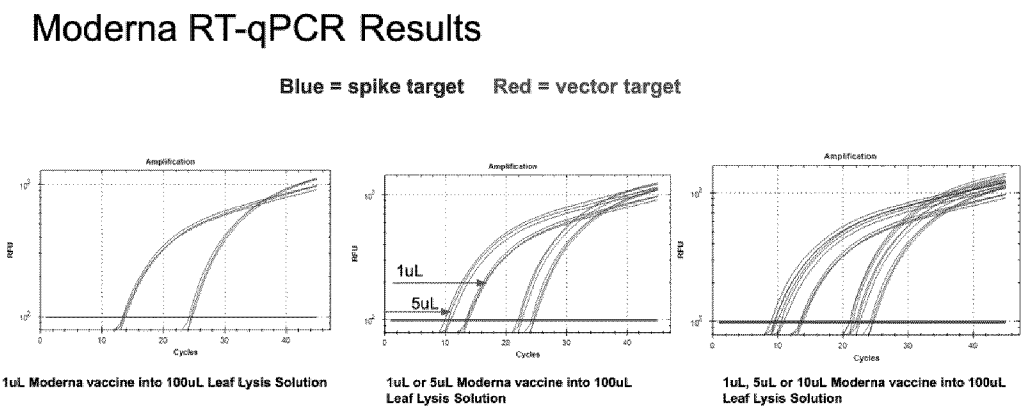


Figure 12. 1µl of the Moderna bivalent vaccine exhibits different CTs values for the spike and the vector targets (Left) with RT-qPCR. The large 10 CT shift between Spike and Vector needs to take into consideration that qPCR control shows a 5 CT offset. The boil preps can tolerate 1-10µl of vaccine (Middle and Right).

	Qubit DNA ng/µl	Qubit RNA ng/µl
Pbiv1	2.81	30.0
Pbiv2	1.47	52.8
Mod1	2.67	21.8
Mod2	1.04	49.0

Table 1. Qubit™ 3 Fluorometry estimates 1.04-2.8 ng/µl of dsDNA in the vaccines and 21.8ng-52.8ng/µl of RNA.
Synthetic templates were synthesized with IDT to build RT-qPCR standard curves to benchmark CTs to the mass of DNA in the reaction. This method uses ideal templates and fails to quantitate DNA molecules smaller than the amplicon size. As expected, this method delivers lower DNA concentration estimates than Qubit™ 3 fluorometry or Agilent Tape Station™. It also represents an ideal environment which doesn't capture the inhibition or primer depletion that can occur when large quantities of mRNA with identical sequence to your DNA target are co-present in a qPCR assay.

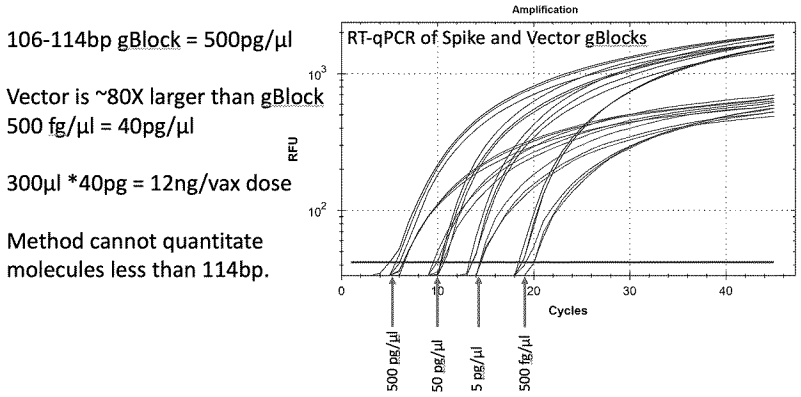


Figure 13. Two gBlocks were synthesized at IDT for Spike and Ori positive control templates used in an RT-qPCR assays. 10-fold serial dilutions were run in triplicate to correlate CT scores with picograms of DNA. The threshold is lowered from 10^2 for review of the background. CT of ~20 = 500fg/RT-qPCR reaction. Since 100bp targets only represent 1/80th of the vector DNA present as a potential contaminant, 500 fg/µl manifests in 40pg/µl of vector DNA. Any DNA that is DNase I treated and is smaller than the amplicon size cannot amplify or be quantitated with this method. This method will under quantitate DNase I treated samples compared to Qubit™ 3 or Agilent Tape Station™.

This work was further validated by testing 8 unopened Pfizer monovalent vaccines with both qPCR and RT-qPCR.

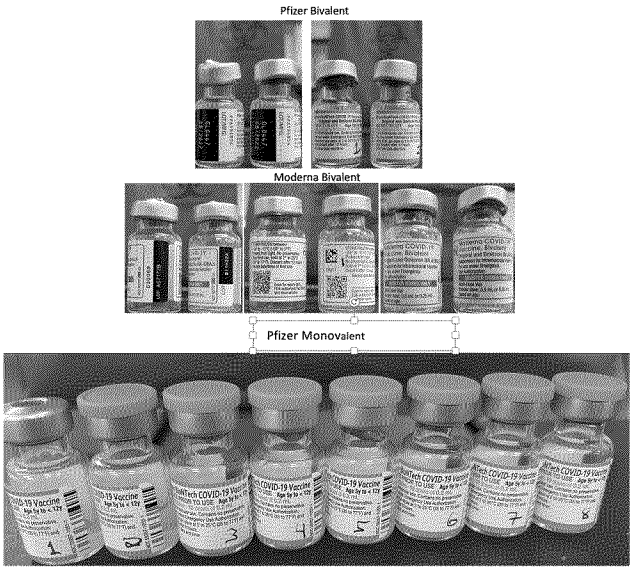


Figure 14. Moderna and Pfizer Bivalent vaccines were unopened and not expired (Top). 8 Monovalent Pfizer mRNA vaccines. These were unopened but past expiration (Bottom).

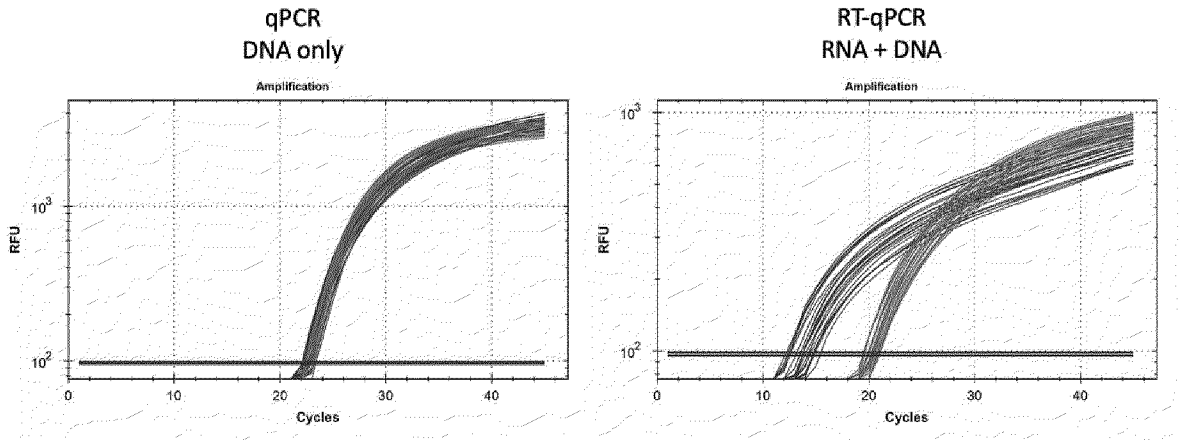


Figure 15. 1µl of vaccine boiled in 100µl of Leaf Lysis buffer was subjected to qPCR (left) and RT-qPCR (right) for Vector (red) and Spike (blue). 8 samples were tested in triplicate.

qPCR-Spike	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	23.12	22.98	22.58	22.33	22.36	22.08	22.20	22.06	0.401
Replicate 2	23.16	22.90	22.70	22.36	22.20	22.16	22.29	22.22	0.373
Replicate 3	23.22	22.84	22.59	22.29	22.44	22.26	22.29	22.11	0.366
STDEV	0.05	0.07	0.07	0.03	0.12	0.09	0.05	0.08	

qPCR: (Vector-Spike)	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	0.20	0.08	0.27	(0.00)	0.18	0.18	0.10	0.24	0.090
Replicate 2	0.16	0.22	0.29	0.11	0.18	0.12	0.03	0.13	0.079
Replicate 3	0.14	0.31	0.20	0.17	0.31	0.19	0.20	0.13	0.069
STDEV	0.03	0.11	0.05	0.09	0.08	0.04	0.08	0.06	

qPCR-Vector	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	23.33	23.06	22.85	22.32	22.54	22.26	22.30	22.30	0.411
Replicate 2	23.32	23.12	23.00	22.47	22.38	22.28	22.32	22.35	0.419
Replicate 3	23.36	23.15	22.79	22.46	22.75	22.46	22.49	22.23	0.383
STDEV	0.02	0.04	0.11	0.08	0.19	0.11	0.11	0.06	

RATIO RNA/DNA	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	1	1	1	1	1	1	1	1	0.068
Replicate 2	1	1	1	1	1	1	1	1	0.062
Replicate 3	1	1	1	1	1	1	1	1	0.056
STDEV	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1	

Table 2. CT values for Spike and Vector during qPCR (DNA only). Standard deviation for the triplicate measurements run horizontally in black font. Standard deviation for vial to vial run vertically in Red. Delta CT or (Vector CT minus Spike CT) represents the ratio of Spike to Vector DNA and should = 1.

RT-Spike	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	14.05	14.77	13.18	13.77	13.79	12.52	12.62	13.53	0.749
Replicate 2	14.29	14.74	14.38	14.82	13.78	13.82	12.57	12.38	0.925
Replicate 3	14.49	14.91	15.43	13.84	13.74	13.55	12.36	12.19	1.141
STDEV	0.22	0.09	1.12	0.59	0.02	0.69	0.14	0.72	

RT: (Vector-Spike)	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	6.74	5.93	7.20	6.40	6.51	7.31	7.33	5.97	0.570
Replicate 2	6.33	6.06	5.92	5.67	6.34	6.13	6.92	7.06	0.478
Replicate 3	6.33	6.07	5.43	6.39	6.13	6.38	7.09	7.18	0.562
STDEV	0.24	0.07	0.91	0.42	0.19	0.62	0.21	0.67	

RT-Vector	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	20.80	20.71	20.39	20.16	20.30	19.83	19.95	19.50	0.439
Replicate 2	20.62	20.80	20.30	20.49	20.12	19.96	19.49	19.45	0.499
Replicate 3	20.81	20.98	20.86	20.23	19.88	19.93	19.45	19.37	0.638
STDEV	0.11	0.14	0.30	0.17	0.21	0.07	0.28	0.07	

RATIO RNA/DNA	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	107	61	147	84	91	159	161	63	41.54
Replicate 2	80	67	61	51	81	70	121	134	29.25
Replicate 3	80	67	43	84	70	83	136	145	34.79
STDEV	15.5	3.3	55.8	19.2	10.4	47.9	20.3	44.6	

Table 3. CT values for Spike and Vector during RT-qPCR (RNA+DNA). Ratio of RNA:DNA ranges from 43:1 To 161:1. EMA allowable limit is 3030:1. This is 18-70 fold over the EMA limit.

Discussion

Multiple methods highlight high levels of DNA contamination in the both the monovalent and bivalent vaccines. While the Qubit™ 3 and Agilent Tape Station™ differ on their absolute quantification, both methods demonstrate it is orders of magnitude higher than the EMAs limit of 330ng DNA/ 1mg RNA. qPCR and RT-qPCR confirms the relative RNA to DNA ratio. An 11-12 CT offset should be seen between Spike and Vector RT-qPCR signals to represent a 1:3030 contamination limit (2^11.6 = 3100). Instead, we observe much smaller CT offsets (5-7 CTs) when looking at qPCR and RT-qPCR data with these vaccines. It should be noted that Qubit™ 3 and Agilent methods stain all DNA in solution while qPCR measures only amplifiable molecules without DNase I cut sites between the primers. The further apart you space the qPCR primers, the fewer Qubit™ 3 and Agilent detectable molecules will amplify. The primers used in this study are 106bp and 114bp apart, thus any molecules that are DNase I cut below this length will be undercounted with the qPCR methods relative to more general dsDNA measurements from Qubit™ 3 or Agilent Tape Station™.

This also implies that qPCR standard curves using 100% intact synthetic DNA standards will amplify more efficiently and thus undercount the total digested DNA contamination. For example, standard curves with 106-114bp synthetic templates provide CTs under 20 in the picogram range (not low nanogram range) suggesting large portions of the library are smaller than the minimum amplifiable size. Pure standards also do not contain high concentrations of modified mRNA with identical sequence which could serve as a competitive primer sink or inhibitor to qPCR methods.

Alternatively, the Qubit™ 3 and the Agilent Tape Station™ could be inflating the DNA quantification due to intercalating dye cross talk with N1-methylpseudouridine RNA. For this reason, we believe the ratio we observed when these molecules are more scrupulously interrogated with polymerases specific for each template type in qPCR and RT-qPCR is a more relevant metric. The EMA metric is also stated as such a ratio.

This also brings into focus if these EMA limits took into consideration the nature of the DNA contaminants. Replication competent DNA should arguably have a more stringent limit. DNA with mammalian promoters or antibiotic resistance genes may also be of more concern than just random background *E.coli* genomic DNA from a plasmid preparation (Sheng-Fowler et al. 2009). Background *E.coli* DNA was measured with qPCR and had CT over 35.

There has been a healthy debate about the capacity for SARs-CoV-2 to integrate into the human genome(Zhang et al. 2021). This work has inspired questions regarding the capacity for the mRNA vaccines to also genome integrate. Such an event would require LINE-1 driven reverse transcription of the mRNA into DNA as described by Alden *et al*. (Alden et al. 2022). dsDNA contamination of sequence encoding the spike protein wouldn't require LINE-1 for Reverse Transcription and the presence of an SV40 nuclear targeting signal in Pfizer's vaccine vector would further increase the odds of integration. This work does not present evidence of genome integration but does underscore that LINE-1 activity is not required given the dsDNA levels in these vaccines. The nuclear localization of these vectors should also be verified.

Prior sequencing of the monovalent vaccines from Jeong *et al.* only published the consensus sequence (Dae-Eun Jeong 2021). The raw reads for this project are not available and should be scrutinized for the presence of vector sequence.

Given these vaccines exceed the EMA limits (330ng/mg DNA/RNA) with the Qubit™ 3 and Agilent data and these data also exceed the FDA limit (10ng/dose) with the more conservative qPCR standard curves, we should revisit the lipopolysaccharide (LPS) levels. Plasmid contamination from *E.coli* preps are often co-contaminated with LPS. Endotoxins contamination can lead to anaphylaxis upon injection (Zheng et al. 2021).

A limitation of this study is the unknown provenance of the vaccine vials under study. These vials were sent to us anonymously in the mail without cold packs. RNA is known to degrade faster than DNA and it is possible poor storage could result in faster degradation of RNA than DNA. RNA as a molecule is very stable but in the presence of metals and heat or background ubiquitous RNases, it can degrade very quickly. All of the monovalent vaccines in this study are past the expiration date listed on the vial suggesting more work is required to understand the DNA to RNA ratios in fresh lots. The bivalent vaccines were not expired. The expiration dates for various vaccine lots have been continually extended by the manufacturers and used in patients. The publication of these qPCR primers may assist in surveying additional lots with more controlled supply chains. Studies evaluating vaccine longevity in breast milk or plasma may benefit from vector DNA surveillance as this sequence is unique to the vaccine and may persist longer than mRNA.

While the sequencing delivered full coverage of the plasmid backbones, it is customary to assemble plasmids from DNase I fragmented libraries. These methods have not discerned the ratio of linear versus circular DNA in the vials. While plasmid DNA is more competent and stable, linear DNA may have higher genome integration risks.

The intercalating dyes used in the Qubit™ 3 and Agilent systems are known to have low fluorescent cross talk with DNA and RNA but it is unknown to what degree N1-methylpseudouridine alters the specificity of these intercalating dyes. As a result, we have relied on the CT offsets between RT-qPCR and qPCR with the vector and spike sequence as the best relative assessment of the EMA ratio-metric regulation. These qPCR and RT-qPCR reagents

may be useful in tracking these contaminants in vaccines, blood banks or patient tissues in the future.

Methods
Purifying the mRNA from the LNPs

LiDs/SPRI purification

100µl of each vial was sampled (1/3rd to 1/5th of a dose)

- 5µl of 2% LiDs was added to 100µl of Vaccine to dissolve LNPs
- 100µl of 100% Isopropanol
- 233µl of Ampure (Beckman Genomics)
- 25µl of 25mM MgCl2 (New England Biolabs)

Samples were tip mixed 10X and incubated for 5 minutes for magnetic bead binding. Magnetic Beads were separated on a 96-well magnet plate for 10 minutes and washed twice with 200µl of 80% EtOH. The beads were left to air dry for 3 minutes and eluted in 100µl of ddH20. 2µl of eluted sample was run on an Agilent Tape Station™.

CTAB/Chloroform/SPRI purification of Vaccines

Some variability in qPCR performance was noted with our LiDs/SPRI purification method of the vaccines. This left some samples opaque and may represent residual LNPs in the purification. A CTAB/Chloroform/SPRI isolation was optimized to address this and used for further qPCR and Agilent electrophoresis. Briefly, 300µl of Vaccine was added to 500µl of CTAB (MGC solution A in SenSATIVAx MIP purification kit. #420004). The sample was then vortexed and heated for 5 minutes at 37°C. 800µl of chloroform was added, vortexed and spun at 19,000 rpms for 3 minutes. The top 250µl of aqueous phase was collected and added to 250µl of solution B and 1ml of magnetic binding buffer. Samples were vortexed and incubated for 5 minutes and magnetically separated. The supernatant was removed and the beads washed with 70% Ethanol two times. Samples were finally eluted in 300µl of MGC elution buffer.

Simple boil preparation for evaluating vaccine qPCR.

This boil prep process simply takes 1-10µl of the vaccine and dilutes it into a PCR compatible leaf lysis buffer and heats it (Medicinal Genomics part number 420208).

- 65°C for 6 minutes
- 95°C for 2 minutes

Library Construction for Sequencing

50µl of each 100µl sample was converted into RNA-Seq libraries for Illumina sequencing using the NEB NEBNext UltraII Directional RNA library Kit for Illumina (NEB#E7760S).

To enrich for longer insert libraries the fragmentation time was reduced from 15 minutes to 10 minutes and the First strand synthesis time was extended at 42°C to 50 minutes per the long insert recommendations in the protocol.

No Ribo depletion or PolyA enrichment was performed as to provide the most unbiased assessment of all fragments in the library. The library was amplified for 16 cycles according to the manufacturers protocol. A directional library construction method was used to evaluate the single stranded nature of the mRNA. This is an important quality metric in the EMA and TGA disclosure documents as dsRNA (>0.5%) can induce an innate immune response. dsRNA content is often estimated using an ELISA. Directional DNA sequencing offers a more comprehensive method for its estimation and was previously measured and 99.99% in Jeong et al. It is unclear how this may vary lot to lot or within the new manufacturing process for the newer bivalent vaccines.

RNase A treatment of the Vaccines

RNase A cleaves both uracils and cytosines. N1-methylpseudouridine is known to be RNAse-*L* resistant but RNase A will cleave cytosines which still exist in the mRNAs. This leaves predominantly DNA for sequencing. Vaccine mRNA that was previously sequenced and discussed here, was treated at 37°C for 30 minutes with 10µl of 20 Units/µl Monarch RNase A from NEB. The RNase reaction was purified using 1.5X of SenSATIVAx (Medicinal Genomics #420001). Sample were eluted in 20µl ddH20 after DNA purification. 15µl was used for DNA sequencing.

DNase treatment of the vaccines

50µl of CTAB purified vaccine was treated at 37°C for 30 minutes with 2µl DNase I and 6µl of DNase I buffer (Grim reefer MGC#420143). 2.5µl of LiDs Lysis buffer was added to stop the DNase reaction. Reactions were purified using 60µl 100% Isopropanol, 140µl Ampure, 15µl MgCl2. Magnetic beads were tip mixed 10 times, left for 5 minutes to incubate, magnetically separated and then washed twice with 80% EtOH.

Whole genome shotgun of RNase'd Vaccines.

15µl of the DNA was converted into sequence ready libraries using Watchmakers Genomics WGS library construction kit. This kit further fragments the DNA to smaller sizes making fragment length in the vaccines difficult to predict.

Qubit™ 3 Fluorometry

Qubit™ 3 fluorometry was performed using Biotum AccuBlue RNA Broad Range kit (#31073) and Biotum AccuGreen High Sensitivity dsDNA Quantitation Kit (#31066) according to the manufacturers instructions.

E.coli qPCR

Medicinal Genomics PathoSEEK™ E.coli Detection assay (#420102) was utilized according to the manufacturers instructions.

qPCR and RT-qPCR Spike Assay

- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Forward
- >AGATGGCCTACCGGTTCA
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Reverse
- >TCAGGCTGTCCTGGATCTT
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Probe
- >/56-FAM/CGAGAACCA/ZEN/GAAGCTGATCGCCAA/3IABkFQ/

qPCR and RT-qPCR Vector Origin Assay

- MedGen_Vax-vector_Ori_Forward
- >CTACATACCTCGCTCTGCTAATC
- MedGen_Vax-vector_Ori_Reverse
- GCGCCTTATCCGGTAACTATC
- MedGen_Vax-vector_Ori_Probe
- /5HEX/AAGACACGA/ZEN/CTTATCGCCACTGGC/3IABkFQ/

Elute primer to 100uM according to IDT instructions.

Make 50X primer-probe mix.

1. 25µl 100uM Forward Primer
2. 25µl 100uM Reverse Primer
3. 12.5µl 100uM Probe
4. 37.5µl nuclease free ddH20.

Use 15µl of this mixture in the **qPCR master mix** setup seen below. (0.5µl primer/probe per reaction)

Use 10µl of this mixture in the **RT-qPCR master mix** setup seen below.

Medicinal Genomics Master Mix kits used

1. https://store.medicinalgenomics.com/qPCR-Master-Kit-v3-200-rxns

2. https://store.medicinalgenomics.com/pathoseek-rt-qpcr-master-kit

Reaction setup for 30 reactions of qPCR

- 114µl Enzyme Mix (green tube)
- 24µl Reaction Buffer (blue tube)
- 246µl nuclease free ddH20
- 15µl of Primer-Probe set Spike
- 15µl of Primer-Probe set Ori

Use 13.8µl of above MasterMix and 5µl of purified sample (1µl Vax DNA/RNA + 4µl ddH20 if CT <15)

Reaction setup for 34 reactions of RT-qPCR

- 200µl Enzyme mix
- 96µl nuclease free ddH20
- 20µl RNase Inhibitor (purple tube)
- 4µl DTT (green tube)
- 10µl Primer-Probe set Spike
- 10µl Primer-Probe set Ori

10µl of MasterMix and 1µl of Vax DNA/RNA

Medicinal Genomics MIP DNA Purification Kit used

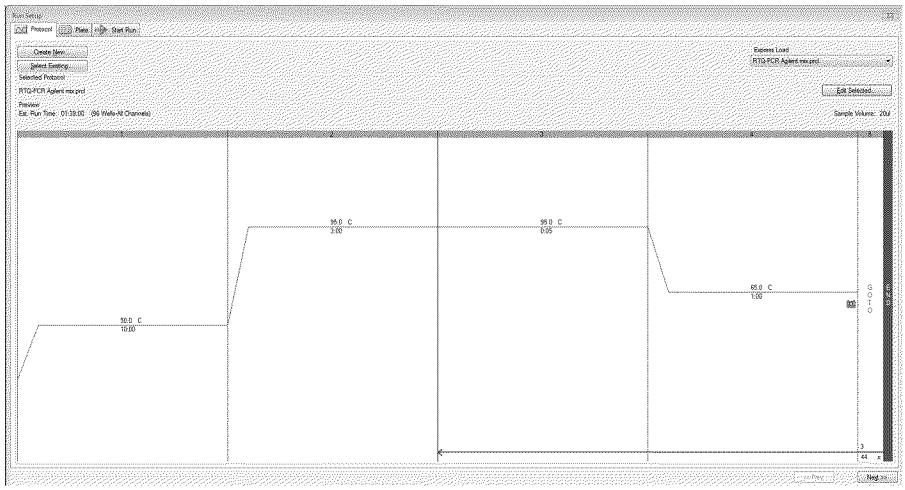
1. https://store.medicinalgenomics.com/SenSATIVAx-DNA-Extraction-Kit-200-reactions_2

he CTAB/Chloroform/SPRI based DNA/RNA isolation methods are described above.

Cycling conditions

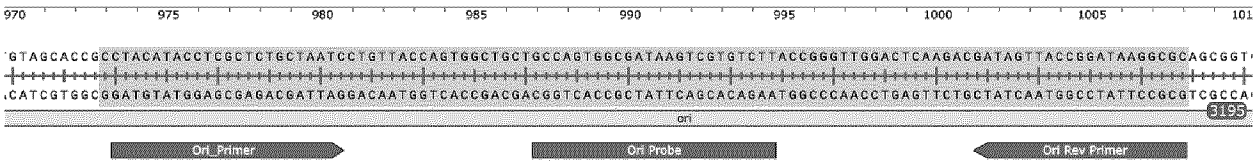
These conditions work for both qPCR and RT-qPCR. Note: The 50°C RT step can be skipped with qPCR. The MGC qPCR MasterMix kits used have a hot start enzyme which are unaffected by this 50°C step. For the sake of controlling RNA to DNA comparisons, we have put qPCR and RT-qPCR assays on the same plate and run the below program with the RT step included for all samples.

Cycling Conditions used for qPCR and RT-qPCR

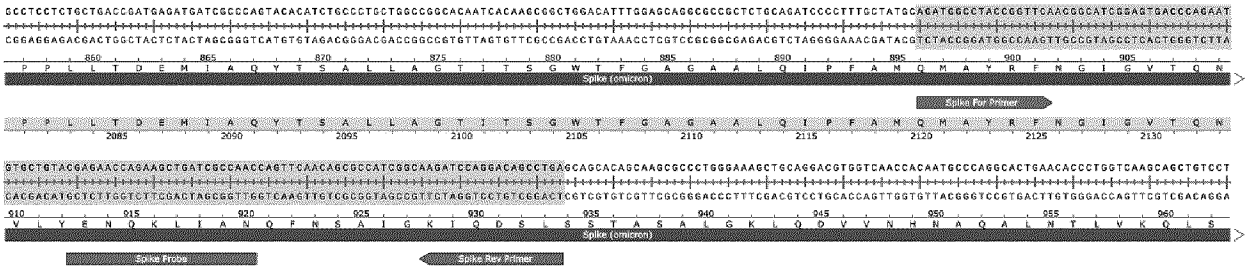


Sequences of amplicons for gBlock Positive Controls. Ori = 106bp, Spike = 114bp.

Ori target



Spike target



Sequencing Data

Raw Illumina Reads RNA-seq

- Pfizer Bivalent Vial 1 Forward reads
- Pfizer Bivalent Vial 1 Reverse reads
- Pfizer Bivalent Vial 2 Forward reads
- Pfizer Bivalent Vial 2 Reverse reads

- Moderna Vial 1 Forward reads
- Moderna Vial 1 Reverse reads
- Moderna Vial 2 Forward reads
- Moderna Vial 2 Reverse reads

Read files are run through sha256 (Hash and stash) and etched onto the DASH blockchain. The sha256 hash of the read file is spent into the OP_RETURN of an immutable ledger. If the hash of the file doesn't match the hash in these transactions, the file has been tampered with.

- Pfizer Vial 1 Forward hash
- Pfizer Vial 1 Reverse hash
- Pfizer Vial 2 Forward hash
- Pfizer Vial 2 Reverse hash
- Moderna Vial 1 Forward hash
- Moderna Vial 1 Reverse hash
- Moderna Vial 2 Forward hash
- Moderna Vial 2 Reverse hash

Megahit Assemblies

- Pfizer Vial 1
- Pfizer Vial 2
- Moderna Vial 1
- Moderna Vial 2

Illumina Reads mapped back to Megahit Assemblies

- Pfizer Vial 1 BAM File. Index File
- Pfizer Vial 2 BAM File. Index File
- Moderna Vial 1 BAM File. Index File
- Moderna Vial 2 BAM File. Index File

Q30 Filtered Illumina Reads (use these for transcriptional error rate estimates)

FastQ-Filter download: usage> fastq-filter -e 0.001 -o output.fastq input.fastq

- Pfizer bivalent Vial 1 Forward Reads
- Pfizer bivalent Vial 1 Reverse Reads
- Pfizer bivalent Vial 2 Forward Reads

- Pfizer bivalent Vial 2 Reverse Reads
- Moderna bivalent Vial 1 Forward Reads
- Moderna bivalent Vial 1 Reverse Reads
- Moderna bivalent Vial 2 Forward Reads
- Moderna bivalent Vial 2 Reverse Reads

Q30 BAM files. Q30 Reads mapped against Megahit assemblies

- Pfizer Vial 1 q30-BAM file. Index File
- Pfizer Vial 2 q30-BAM file. Index File
- Moderna Vial 1 q30-BAM file. Index File
- Moderna Vial 2 q30-BAM file. Index File

IGVtools error by base on q30 reads

Fields = Position in contig, Positive stand (+)A, +C, +G, +T, +N, +Deletion, +Insertion, Negative strand -A, -C, -G, -T, -N, -Deletion, -Insertion

- Moderna Vial 1
- Moderna Vial 2
- Pfizer Vial 1
- Pfizer Vial 2

Analysis pipeline

Reads were demultiplexed and processed with

- Trimgalore - Removes Illumina Sequencing adaptors.
- Megahit- assembles reads into contigs.
- Megahit for SARs-CoV-2
- Samtools- generates BAM files for viewing in IGV.
- Samtools stats used to calculate outie reads.
- BWA-mem- Short read mapper used to align reads back to the assembled references.
- SnapGene software- (www.snapgene.com)- Used to visualize and annotate expression vectors
- IGV- Integrated Genome Viewer used to visualize Illumina sequencing reads.

RNase Treated Libraries-BAM files

contig specific BAM files were created using samtools

samtools view -h input.bam contig_name -O BAM > contig.bam; samtools index contig.bam;

Samtools stats run on a each contig in each assembly.

```
for out_prefix in `ls *.sort.bam | perl -pe "s/.sort.bam//"`; do mkdir -p ${out_prefix}-samtools-stats; for contig in `samtools view -H ${out_prefix}.sort.bam  | grep "^@SQ"  | cut -f 2 | perl -pe "s/SN\://"`; do echo "Now calculating stats for ${contig}/$out_prefix..."; samtools stats ${out_prefix}.sort.bam $contig > ${out_prefix}-samtools-stats/${contig}-samtools-stats.txt; done; done
```

- Pbiv1_RNase_WM_k141_107.fa
- Pbiv1_RNase_WM_k141_107.bam
- Pbiv1_RNase_WM_k141_107.bam.bai
- Pbiv2_RNase_WM_k141_23.fa
- Pbiv2_RNase_WM_k141_23.bam
- Pbiv2_RNase_WM_k141_23.bam.bai

Author contributions

KJM- constructed the sequencing libraries, designed the qPCR assays, ran Qubit™ 3s and Agilent Tape Station™ and performed the analysis, drafted the manuscript.
YH-Optimized DNA isolations, Tape Station™ and qPCR results.
SM, LTK- assisted in demultiplexing and trimming the reads and assembly troubleshooting

Conflicts of interest- Authors of this paper are employees of Medicinal Genomics which manufacturers some of the qPCR and DNA isolation kits used in this study.

Acknowledgements.
We'd like to thank Lei Zhang for organizing the sequencing outsourcing. We'd like to thank Jessica Rose, Sabine Hazan, Jikkyleaks, Pathogenetics, Steve Massey, Valentine Bruttel, Lynn Fynn, Sasha Letypova, David Wiseman and Pharmacoconuts for helpful comments on this topic.

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De Rose, Carol (HC/SC)

From: Wu, Tong (HC/SC)
Sent: 2023-10-26 10:51 AM
To: Pham, Co (HC/SC)
Cc: Laflamme, Nancy (HC/SC); De Rose, Carol (HC/SC); Isbrucker, Richard (HC/SC); Siggers, Richard (HC/SC); Wall, Michael (HC/SC)
Subject: RE: some of the technical questions

Hi Co,

Please see the response below to the questions. Rich Siggers/Mike will explain in detail.
tong

From: Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>
Sent: Thursday, October 26, 2023 10:14 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Isbrucker, Richard (HC/SC) <richard.isbrucker@hc-sc.gc.ca>
Cc: Laflamme, Nancy (HC/SC) <nancy.laflamme@hc-sc.gc.ca>; De Rose, Carol (HC/SC) <carol.derose@hc-sc.gc.ca>
Subject: FW: some of the technical questions

Hi Team;
I would like to have a meeting with you at 11h00 today (I'm in the office) regarding a summary and response to this issue.

Thanks
C

From: van Oosten, Marianne (HC/SC) <marianne.vanoosten@hc-sc.gc.ca>
Sent: Thursday, October 26, 2023 9:55 AM
To: Anoop, Poovadan (HC/SC) <poovadan.anoop@hc-sc.gc.ca>; Colapinto, Cynthia (HC/SC) <cynthia.colapinto@hc-sc.gc.ca>; Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>
Subject: RE: some of the technical questions

Great! If you can set up a quick call with Co (and us) as well, I can ask a few questions and make sure I completely understand the answers to these questions. Then I can take another run at the response. ASAP would be lovely.

Thanks!
Marianne

Marianne van Oosten
(she | elle)
Senior Communications Strategist / *Conseillère principale en communications stratégiques*
Health Canada / *Santé Canada*
marianne.vanoosten@hc-sc.gc.ca/ Cell: 613-854-9275

From: Anoop, Poovadan (HC/SC) <poovadan.anoop@hc-sc.gc.ca>
Sent: Thursday, October 26, 2023 9:53 AM
To: van Oosten, Marianne (HC/SC) <marianne.vanoosten@hc-sc.gc.ca>; Colapinto, Cynthia (HC/SC)

<cynthia.colapinto@hc-sc.gc.ca>
Subject: some of the technical questions

Hi Marianne,
I know you were taking notes but here is what I noted from Supriya.

- What is the role of the SV40 promoter enhancer sequence?
  Response: SV40 promoter enhancer is widely used to drive gene expression in mammalian cells. However, SV40 promoter enhancer serves no purpose in the manufacturing of Pfizer COVID-19 vaccines.
- Was it unexpected to find this sequence in the finished product?
  Response: Yes, because Pfizer did not identify the presence of SV40 promoter enhancer on the plasmid template used to produce mRNA, in their original filing.

And I think to answer this further, we should also have as background:

- Has this sequence been seen in low levels in other vaccines that use the SV40 promoter?
  Response: To the best of our knowledge, this sequence has never been used in the manufacture of other vaccines. Therefore, not present in any vaccines currently approved in Canada.
- Why did the purification process not eliminate this particular fragment in the finished product?
  Response: It is challenging to eliminate all DNA template in the down stream purification process. However, the removal of this sequence from the DNA plasmid used as starting material can eliminate this particular fragment.

Thank you,
anoop

From: Siggers, Richard (HC/SC)
Sent: 2023-10-26 10:30 AM
To: Wu, Tong (HC/SC); Pham, Co (HC/SC)
Cc: Wall, Michael (HC/SC)
Subject: RE: some of the technical questions

Hi Co,

If acceptable with you and Tong, Mike and I can come to your office at 11am.

Rich

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Thursday, October 26, 2023 10:27 AM
To: Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>
Cc: Isbrucker, Richard (HC/SC) <richard.isbrucker@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>
Subject: RE: some of the technical questions

Sorry Co,

I am at home today.
tong

From: Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>
Sent: Thursday, October 26, 2023 10:25 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Cc: Isbrucker, Richard (HC/SC) <richard.isbrucker@hc-sc.gc.ca>; Siggers, Richard (HC/SC) <richard.siggers@hc-sc.gc.ca>
Subject: RE: some of the technical questions

Hi Tong;
I appreciate the clarification to excuse Richard Isbrucker on this.

My apologies Richard for including you outside of your divisional work.

Tong: I have cc'd Richard Siggers on this and would like to meet you and Richard Siggers at 11h00. In my office if you are in LCDC.

Thanks
C

From: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>
Sent: Thursday, October 26, 2023 10:23 AM
To: Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>
Cc: Isbrucker, Richard (HC/SC) <richard.isbrucker@hc-sc.gc.ca>
Subject: RE: some of the technical questions

Hi Co,

This is our division's file. Richard Siggers and Mike were involved (Dean as well), and Richard Isbrucker was not. Richard Siggers will be acting for me next week. Would you like to include Siggers in the meeting?

Thanks,
tong

From: Pham, Co (HC/SC) <co.pham@hc-sc.gc.ca>
Sent: Thursday, October 26, 2023 10:14 AM
To: Wu, Tong (HC/SC) <tong.wu@hc-sc.gc.ca>; Isbrucker, Richard (HC/SC) <richard.isbrucker@hc-sc.gc.ca>
Cc: Laflamme, Nancy (HC/SC) <nancy.laflamme@hc-sc.gc.ca>; De Rose, Carol (HC/SC) <carol.derose@hc-sc.gc.ca>
Subject: FW: some of the technical questions

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Subject: RE: some of the technical questions

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Marianne van Oosten
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Senior Communications Strategist / *Conseillère principale en communications stratégiques*
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Subject: some of the technical questions

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- Has this sequence been seen in low levels in other vaccines that use the SV40 promoter?
- Why did the purification process not eliminate this particular fragment in the finished product?

Thank you,
anoop

De Rose, Carol (HC/SC)

From: Poulin, Manon L (HC/SC)
Sent: 2023-10-30 9:36 AM
To: Fortin, Nathalie (HC/SC); De Rose, Carol (HC/SC)
Cc: Pham, Co (HC/SC); BRDD Correspondence / Correspondance DMBR Correspond (HC/SC)
Subject: Heads Up! Order Paper Question Q-1954

Importance: High

Good morning CVCTB,

I hope you all had a restful weekend.

This is a Heads Up. Written Question Q-1954 appeared on today's Notice Paper. An official tasking will follow shortly with the template and deadline. I do advise that you start drafting your response to these questions. You can provide links to the requestor with the information in your response.

Merci
Manon

Q-1954 — October 27, 2023 — Mr. Carrie (Oshawa) — With regard to Health Canada's (HC) approval of the Pfizer/BioNTech COVID-19 vaccines:
(a) did Pfizer disclose that Process 1 vaccine formula was used during the original trial to obtain their safety and efficacy data while Process 2 was invoked following Interim Order to massively upscale production of vaccine doses whereby DNA was cloned into a bacterial plasmid vector for amplification in Escherichia coli (E. coli) before linearization with the possibility of potential residual DNA;
(b) was HC aware of the quantum of linearized DNA fragments present in each dose of the Pfizer vaccine prior to releasing the vaccine to Canadians, and, if so, what was the amount of acceptable residual DNA per vaccine dose and the method used to measure it;
(c) if the response to (b) is negative, has HC since confirmed the quantum of linearized DNA per vaccine dose per mRNA manufacturer, and, if so, what method was used;
(d) do the risks of residual DNA meet HC's standards for transfected foreign DNA;
(e) did Pfizer and BioNTech disclose to HC the presence of the Simian Virus 40 (SV40) promoter-enhancer-ori used to amplify the production of Spike mRNA in the DNA plasmid used to produce the mRNA;
(f) has HC confirmed the presence of SV40 sequences in the Pfizer vaccine, and, if so, is the amount of SV40 within safe limits and how was it tested;
(g) if the response to (f) is negative, when and who will conduct the study to confirm the presence of SV40 and by what method;
(h) how were contaminants and impurities addressed throughout the regulatory process for both Pfizer/BioNTech and Moderna products;
(i) are further studies planned to investigate how these contaminants and impurities will impact human subjects given transfection for both products, and, if so, who will conduct the investigation and when will it be conducted;
(j) is HC considering regulating these products as gene therapy products; and
(k) how does HC plan to inform those Canadians who received the mRNA products about the adulteration of these products, specifically SV40 in Pfizer and heightened levels of DNA plasmids in both Pfizer and Moderna products, to ensure fully informed consent?

Q-1954 — 27 octobre 2023 — M. Carrie (Oshawa) — En ce qui concerne l'approbation des vaccins contre la COVID-19 de Pfizer-BioNTech par Santé Canada (SC) :

a) Pfizer a-t-il révélé que la formule de vaccin du Processus 1 a été utilisée au cours de l'essai initial en vue de fournir des données relatives sa sécurité et à son efficacité, alors que la formule du Processus 2 a été invoquée après l'arrêté d'urgence pour augmenter massivement la production de doses de vaccin, l'ADN étant cloné dans un vecteur plasmidique bactérien pour être amplifié dans la bactérie Escherichia coli (E. coli) avant la linéarisation, avec la possibilité d'un ADN résiduel;

b) SC avait-il connaissance de la quantité de fragments d'ADN linéarisés présents dans chaque dose du vaccin Pfizer avant d'inoculer le vaccin aux Canadiens, et, le cas échéant, quelle était la quantité d'ADN résiduel acceptable par dose de vaccin et quelle était la méthode utilisée pour la mesurer;

c) si la réponse en b) est négative, SC a-t-il depuis confirmé le quantum d'ADN linéarisé par dose de vaccin et par fabricant d'ARNm, et, le cas échéant, quelle fut la méthode utilisée;

d) les risques liés à l'ADN résiduel sont-ils conformes aux normes de SC relatives à l'ADN étranger transfecté;

e) Pfizer et BioNTech ont-ils divulgué à SC la présence de la séquence promotrice-amplificatrice-ori du virus Simien 40 (SV40) utilisée pour amplifier la production de l'ARNm de la protéine du spicule dans le plasmide d'ADN utilisé pour produire l'ARNm;

f) SC a-t-il confirmé la présence de séquences de SV40 dans le vaccin de Pfizer, et, le cas échéant, la quantité de SV40 se situe-t-elle dans les limites de sécurité établies et comment a-t-elle été mesurée;

g) si la réponse en f) est négative, quand et qui réalisera l'étude pour confirmer la présence du SV40 et quelle sera la méthode utilisée;

h) comment avons-nous abordé la question des contaminants et des impuretés tout au long du processus réglementaire pour les produits de Pfizer/BioNTech et de Moderna;

i) d'autres études sont-elles prévues pour évaluer les effets de ces contaminants et impuretés sur les sujets humains ayant subi une transfection pour les deux produits et, le cas échéant, qui mènera l'enquête et quand sera-t-elle menée;

j) SC envisage-t-il de réglementer ces produits à titre de produits de thérapie génique;

k) comment SC prévoit-il informer les Canadiens qui ont reçu les produits à base d'ARNm de leur adultération, en particulier de la présence de SV40 dans les produits Pfizer et de niveaux élevés de plasmides d'ADN dans les produits Pfizer et Moderna, afin de permettre un consentement pleinement éclairé?

De Rose, Carol (HC/SC)

From: Scolli, Tania (HC/SC) on behalf of BRDD Correspondence / Correspondance DMBR Correspond (HC/SC)
Sent: 2023-10-31 10:32 AM
To: Pham, Co (HC/SC); Fortin, Nathalie (HC/SC); De Rose, Carol (HC/SC)
Cc: Poulin, Manon L (HC/SC); Anoop, Poovadan (HC/SC)
Subject: OFFICIAL TASKING >>>>>> Written Question / Question écrite Q-1954
Attachments: SofC-All Organizations (Blank)-ENG .docx; Q-1954 HC-Response.docx

Good morning,

As a follow up to Manon's email from yesterday, please see below the official tasking for this Written Question.

BF to DGO: **3 pm on November 8th.**

Thanks!

Tania Scolli
(she | elle)

Acting Administrative Officer, Director General's Office / Biologic and Radiopharmaceutical Drugs Directorate / Health Products and Food Branch
Health Canada / Government of Canada
tania.scolli@hc-sc.gc.ca / Tel: (343) 552-1100

Adjointe administrative par interim, bureau du directrice générale / Direction des médicaments biologiques et radiopharmaceutiques / Direction générale des produits de santé et des aliments
Santé Canada / Gouvernement du Canada
tania.scolli@hc-sc.gc.ca / Tél: (343) 552-1100

Health Canada Santé Canada

Order Paper Question (Written Question) Q-1954 was tasked by PCO.

We are tasking: **BRDD**

The wording of the written question is as follows:

Q-1954 — October 27, 2023 — Mr. Carrie (Oshawa) — With regard to Health Canada's (HC) approval of the Pfizer/BioNTech COVID-19 vaccines:
(a) did Pfizer disclose that Process 1 vaccine formula was used during the original trial to obtain their safety and efficacy data while Process 2 was invoked following Interim Order to massively upscale production of vaccine doses whereby DNA was cloned into a bacterial plasmid vector for amplification in Escherichia coli (E. coli) before linearization with the possibility of potential residual DNA;
(b) was HC aware of the quantum of linearized DNA fragments present in each dose of the Pfizer vaccine prior to releasing the vaccine to Canadians, and, if so, what was the amount of acceptable residual DNA per vaccine dose and the method used to measure it;
(c) if the response to (b) is negative, has HC since confirmed the quantum of linearized DNA per vaccine dose per mRNA manufacturer, and, if so, what method was used;
(d) do the risks of residual DNA meet HC's standards for transfected foreign DNA;

(e) did Pfizer and BioNTech disclose to HC the presence of the Simian Virus 40 (SV40) promoter-enhancer-ori used to amplify the production of Spike mRNA in the DNA plasmid used to produce the mRNA;
(f) has HC confirmed the presence of SV40 sequences in the Pfizer vaccine, and, if so, is the amount of SV40 within safe limits and how was it tested;
(g) if the response to (f) is negative, when and who will conduct the study to confirm the presence of SV40 and by what method;
(h) how were contaminants and impurities addressed throughout the regulatory process for both Pfizer/BioNTech and Moderna products;
(i) are further studies planned to investigate how these contaminants and impurities will impact human subjects given transfection for both products, and, if so, who will conduct the investigation and when will it be conducted;
(j) is HC considering regulating these products as gene therapy products; and
(k) how does HC plan to inform those Canadians who received the mRNA products about the adulteration of these products, specifically SV40 in Pfizer and heightened levels of DNA plasmids in both Pfizer and Moderna products, to ensure fully informed consent?

Q-1954 — 27 octobre 2023 — M. Carrie (Oshawa) — En ce qui concerne l'approbation des vaccins contre la COVID-19 de Pfizer-BioNTech par Santé Canada (SC) :
a) Pfizer a-t-il révélé que la formule de vaccin du Processus 1 a été utilisée au cours de l'essai initial en vue de fournir des données relatives sa sécurité et à son efficacité, alors que la formule du Processus 2 a été invoquée après l'arrêté d'urgence pour augmenter massivement la production de doses de vaccin, l'ADN étant cloné dans un vecteur plasmidique bactérien pour être amplifié dans la bactérie Escherichia coli (E. coli) avant la linéarisation, avec la possibilité d'un ADN résiduel;
b) SC avait-il connaissance de la quantité de fragments d'ADN linéarisés présents dans chaque dose du vaccin Pfizer avant d'inoculer le vaccin aux Canadiens, et, le cas échéant, quelle était la quantité d'ADN résiduel acceptable par dose de vaccin et quelle était la méthode utilisée pour la mesurer;
c) si la réponse en b) est négative, SC a-t-il depuis confirmé le quantum d'ADN linéarisé par dose de vaccin et par fabricant d'ARNm, et, le cas échéant, quelle fut la méthode utilisée;
d) les risques liés à l'ADN résiduel sont-ils conformes aux normes de SC relatives à l'ADN étranger transfecté;
e) Pfizer et BioNTech ont-ils divulgué à SC la présence de la séquence promotrice-amplificatrice-ori du virus Simien 40 (SV40) utilisée pour amplifier la production de l'ARNm de la protéine du spicule dans le plasmide d'ADN utilisé pour produire l'ARNm;
f) SC a-t-il confirmé la présence de séquences de SV40 dans le vaccin de Pfizer, et, le cas échéant, la quantité de SV40 se situe-t-elle dans les limites de sécurité établies et comment a-t-elle été mesurée;
g) si la réponse en f) est négative, quand et qui réalisera l'étude pour confirmer la présence du SV40 et quelle sera la méthode utilisée;
h) comment avons-nous abordé la question des contaminants et des impuretés tout au long du processus réglementaire pour les produits de Pfizer/BioNTech et de Moderna;
i) d'autres études sont-elles prévues pour évaluer les effets de ces contaminants et impuretés sur les sujets humains ayant subi une transfection pour les deux produits et, le cas échéant, qui mènera l'enquête et quand sera-t-elle menée;
j) SC envisage-t-il de réglementer ces produits à titre de produits de thérapie génique;
k) comment SC prévoit-il informer les Canadiens qui ont reçu les produits à base d'ARNm de leur adultération, en particulier de la présence de SV40 dans les produits Pfizer et de niveaux élevés de plasmides d'ADN dans les produits Pfizer et Moderna, afin de permettre un consentement pleinement éclairé?

PCO templates are attached which require completion:

- **Q-1954 – Ministry of Inquiry Form (attached)**

- ~~**PCO instructions and template**~~ **(none)**
- **Statement of Completeness (attached)**
- Costing amount (please send in email along with response - $60/hr, $0.39/word for translation)

As a reminder, in completing your responses, we ask you ensure that:

- the financial data be verified by your Branch Senior Financial Officer (BSFO).
- Health Canada and the Agencies search all records (paper and electronic) as well as all public sources of information such as, but not limited to, the Public Accounts and Departmental Reports on Plans and Priorities and Departmental Performance Reports.
- Health Canada and the Agencies clearly indicate in their Statement of Completeness (SOC) the efforts undertaken to respond to the question.
- Health Canada and the Agencies verify with Communications to ensure all messagings are the same.
- Health Canada and the agencies should verify response provided is consistent with *Access to Information Act* requests, Library of Parliament request or any pro-active disclosures.

Thank you ! Merci !

Name of organization
(HC-branch / agency)

STATEMENT OF COMPLETENESS
RESPONSE TO WRITTEN QUESTION, Q-xx

Name of Parliamentarian: x
Constituency: x
Date of Inquiry: x

A) 1. Briefly describe records, analysis and consultations on which the proposed response is based:

Lead official: x

2. Were publicly available documents used to draft the response? If yes, please list titles and dates below:

Yes	**No**

B) Describe any limitations, considerations and/ or data quality statements that apply to this response (include applicable section of Access to Information Act):

Yes	**No**

C) Have information or documents relevant to this response been disclosed publicly, in any manner (e.g. access to information request or request from the Library of Parliament)? If yes, please identify relevant documents and explain any differences in the proposed response.	**Yes**	**N/A**

Attestation:

As the Designated Senior Official for **(name of HC-branch / agency)** attest that the information contained in the proposed response, based on the records and limitations described in this Statement of Completeness, is accurate and as complete as possible.

Signature	Date

Name of Designated Senior Official	Title

Government of Canada / Gouvernement du Canada

INQUIRY OF MINISTRY
DEMANDE DE RENSEIGNEMENT AU GOUVERNEMENT

PREPARE IN ENGLISH AND FRENCH MARKING "ORIGINAL TEXT" OR "TRANSLATION"
PRÉPARER EN ANGLAIS ET EN FRANÇAIS EN INDIQUANT "TEXTE ORIGINAL" OU "TRADUCTION"

QUESTION NO./Nº DE LA QUESTION	BY / DE	DATE
Q-1954	Mr. Carrie (Oshawa)	October 27, 2023

Reply by the Minister of Health
Réponse du ministre de la Santé

Signed by the Honourable Mark Holland

PRINT NAME OF SIGNATORY
INSCRIRE LE NOM DU SIGNATAIRE

SIGNATURE
MINISTER OR PARLIAMENTARY SECRETARY
MINISTRE OU SECRÉTAIRE PARLEMENTAIRE

QUESTION

With regard to Health Canada's (HC) approval of the Pfizer/BioNTech COVID-19 vaccines: (a) did Pfizer disclose that Process 1 vaccine formula was used during the original trial to obtain their safety and efficacy data while Process 2 was invoked following Interim Order to massively upscale production of vaccine doses whereby DNA was cloned into a bacterial plasmid vector for amplification in Escherichia coli (E. coli) before linearization with the possibility of potential residual DNA; (b) was HC aware of the quantum of linearized DNA fragments present in each dose of the Pfizer vaccine prior to releasing the vaccine to Canadians, and, if so, what was the amount of acceptable residual DNA per vaccine dose and the method used to measure it; (c) if the response to (b) is negative, has HC since confirmed the quantum of linearized DNA per vaccine dose per mRNA manufacturer, and, if so, what method was used; (d) do the risks of residual DNA meet HC's standards for transfected foreign DNA; (e) did Pfizer and BioNTech disclose to HC the presence of the Simian Virus 40 (SV40) promoter-enhancer-ori used to amplify the production of Spike mRNA in the DNA plasmid used to produce the mRNA; (f) has HC confirmed the presence of SV40 sequences in the Pfizer vaccine, and, if so, is the amount of SV40 within safe limits and how was it tested; (g) if the response to (f) is negative, when and who will conduct the study to confirm the presence of SV40 and by what method; (h) how were contaminants and impurities addressed throughout the regulatory process for both Pfizer/BioNTech and Moderna products; (i) are further studies planned to investigate how these contaminants and impurities will impact human subjects given transfection for both products, and, if so, who will conduct the investigation and when will it be conducted; (j) is HC considering regulating these products as gene therapy products; and (k) how does HC plan to inform those Canadians who received the mRNA products about the adulteration of these products, specifically SV40 in Pfizer and heightened levels of DNA plasmids in both Pfizer and Moderna products, to ensure fully informed consent?

REPLY / RÉPONSE

ORIGINAL TEXT / TEXTE ORIGINAL [X]

TRANSLATION / TRADUCTION [ ]

Health Canada

Government of Canada / Gouvernement du Canada

INQUIRY OF MINISTRY
DEMANDE DE RENSEIGNEMENT AU GOUVERNEMENT

PREPARE IN ENGLISH AND FRENCH MARKING "ORIGINAL TEXT" OR "TRANSLATION"
PRÉPARER EN ANGLAIS ET EN FRANÇAIS EN INDIQUANT "TEXTE ORIGINAL" OU "TRADUCTION"

QUESTION NO./Nº DE LA QUESTION	BY / DE	DATE
Q-1954	M. Carrie (Oshawa)	Le 27 octobre 2023

Reply by the Minister of Health
Réponse du ministre de la Santé

Signé par l'honorable Mark Holland

PRINT NAME OF SIGNATORY
INSCRIRE LE NOM DU SIGNATAIRE

SIGNATURE
MINISTER OR PARLIAMENTARY SECRETARY
MINISTRE OU SECRÉTAIRE PARLEMENTAIRE

QUESTION

En ce qui concerne l'approbation des vaccins contre la COVID-19 de Pfizer-BioNTech par Santé Canada (SC) : a) Pfizer a-t-il révélé que la formule de vaccin du Processus 1 a été utilisée au cours de l'essai initial en vue de fournir des données relatives sa sécurité et à son efficacité, alors que la formule du Processus 2 a été invoquée après l'arrêté d'urgence pour augmenter massivement la production de doses de vaccin, l'ADN étant cloné dans un vecteur plasmidique bactérien pour être amplifié dans la bactérie Escherichia coli (E. coli) avant la linéarisation, avec la possibilité d'un ADN résiduel; b) SC avait-il connaissance de la quantité de fragments d'ADN linéarisés présents dans chaque dose du vaccin Pfizer avant d'inoculer le vaccin aux Canadiens, et, le cas échéant, quelle était la quantité d'ADN résiduel acceptable par dose de vaccin et quelle était la méthode utilisée pour la mesurer; c) si la réponse en b) est négative, SC a-t-il depuis confirmé le quantum d'ADN linéarisé par dose de vaccin et par fabricant d'ARNm, et, le cas échéant, quelle fut la méthode utilisée; d) les risques liés à l'ADN résiduel sont-ils conformes aux normes de SC relatives à l'ADN étranger transfecté; e) Pfizer et BioNTech ont-ils divulgué à SC la présence de la séquence promotrice-amplificatrice-ori du virus Simien 40 (SV40) utilisée pour amplifier la production de l'ARNm de la protéine du spicule dans le plasmide d'ADN utilisé pour produire l'ARNm; f) SC a-t-il confirmé la présence de séquences de SV40 dans le vaccin de Pfizer, et, le cas échéant, la quantité de SV40 se situe-t-elle dans les limites de sécurité établies et comment a-t-elle été mesurée; g) si la réponse en f) est négative, quand et qui réalisera l'étude pour confirmer la présence du SV40 et quelle sera la méthode utilisée; h) comment avons-nous abordé la question des contaminants et des impuretés tout au long du processus réglementaire pour les produits de Pfizer/BioNTech et de Moderna; i) d'autres études sont-elles prévues pour évaluer les effets de ces contaminants et impuretés sur les sujets humains ayant subi une transfection pour les deux produits et, le cas échéant, qui mènera l'enquête et quand sera-t-elle menée; j) SC envisage-t-il de réglementer ces produits à titre de produits de thérapie génique; k) comment SC prévoit-il informer les Canadiens qui ont reçu les produits à base d'ARNm de leur adultération, en particulier de la présence de SV40 dans les produits Pfizer et de niveaux élevés de plasmides d'ADN dans les produits Pfizer et Moderna, afin de permettre un consentement pleinement éclairé?

REPLY / RÉPONSE

ORIGINAL TEXT / TEXTE ORIGINAL [ ]
TRANSLATION / TRADUCTION [X]

Santé Canada