EMA/128110/2024
Committee for Medicinal Products for Human Use (CHMP)

Type II variation assessment report

Invented name: Zoonotic Influenza Vaccine Seqirus

Procedure No. EMEA/H/C/006375/II/0001

Common name: zoonotic influenza vaccine (H5N8) (surface antigen, inactivated, adjuvanted)

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Status of this report and steps taken for the assessment			
Current step	**Description**	**Planned date**	**Actual Date**
☐	Start of procedure	11 Dec 2023	11 Dec 2023
☐	ETF discussion	12 Jan 2024	12 Jan 2024
☐	CHMP Rapporteur Assessment Report	15 Jan 2024	15 Jan 2024
☐	CHMP members comments	17 Jan 2024	17 Jan 2024
☐	Updated CHMP Rapporteur Assessment Report	19 Jan 2024	19 Jan 2024
☐	Start of written procedure	n/a	n/a
☐	1st Request for supplementary information	25 Jan 2024	25 Jan 2024
	Submission of responses	30 Jan 2024	30 Jan 2024
☐	Start of procedure	31 Jan 2024	31 Jan 2024
☐	CHMP Rapporteur Assessment Report	07 Feb 2024	07 Feb 2024
☐	CHMP members comments	12 Feb 2024	12 Feb 2024
☐	Updated CHMP Rapporteur Assessment Report	15 Feb 2024	16 Feb 2024
☐	2nd Request for supplementary information	22 Feb 2024	22 Feb 2024
☐	Submission of responses	27 Feb 2024	27 Feb 2024
☐	Restart	28 Feb 2024	28 Feb 2024
☐	CHMP Rapporteur Assessment Report	6 March 2024	09 Mar 2024
☐	CHMP members comments	11 March 2024	11 March 2024
☐	Updated CHMP Rapporteur Assessment Report	14 March 2024	n/a
☒	Opinion	21 March 2024	21 March 2024

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1. Background information on the procedure

Pursuant to Article 16 of Commission Regulation (EC) No 1234/2008, Seqirus S.r.l. submitted to the European Medicines Agency on 23 November 2023 an application for a variation.

The following changes were proposed:

Variation requested		Type	Annexes affected
B.I.a.5.a	B.I.a.5.a - Changes to the Active Substance of a seasonal, prepandemic or pandemic vaccine against human influenza - Replacement of the strain(s) in a seasonal, prepandemic or a pandemic vaccine against human influenza	Type II	I, IIIA, IIIB and A

Type II, B.I.a.5.a - zoonotic strain change from A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG 23) (clade 2.2.1) to A/Astrakhan/3212/2020 (H5N8)-like strain (CBER-RG8A) (clade 2.3.4.4b)

The requested variation proposed amendments to the Summary of Product Characteristics, Labelling, Package Leaflet and Annex A.

GMP inspections

No request for GMP inspection is deemed necessary in connection to this procedure.

GLP/GCP inspections

No request for GLP inspections is deemed necessary in connection to this procedure.

2. Overall conclusion and impact on the benefit/risk balance

The "Zoonotic Influenza Vaccine Seqirus" is a monovalent influenza avian vaccine (egg-based, surface antigen, inactivated, MF59C.1 adjuvanted) containing purified Hemagglutinin (HA) and Neuraminidase (NA) surface antigens from the influenza avian virus A/turkey/Turkey/1/2005 (H5N1) like strain (NIBRG-23) clade 2.2.1. The H5N1 "Zoonotic Influenza Vaccine Seqirus" received the CHMP positive opinion on 14 September 2023 as an Informed Consent procedure aiming to duplicate the existing Marketing Authorisation for the already authorised H5N1 zoonotic vaccine "Aflunov", also based on A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) of clade 2.2.1.
Zoonotic influenza vaccines are intended for immunisation in the context of an outbreak of zoonotic influenza viruses with pandemic potential and when there is anticipation of a possible pandemic due to the same or similar influenza strain.

Given the evolution of reassortant highly pathogenic avian influenza (HPAI) viruses (H5N1) and since an increasing number of avian influenza spillover into mammals with a limited number human cases have been registered recently, a strain update for the "Zoonotic Influenza Vaccine Seqirus" has been considered appropriate to more adequately target the currently circulating clade of the HPAI H5 virus and, thus, to strengthen the pandemic preparedness capabilities.

Therefore, as agreed with the EMA and the ETF and on the basis of influenza surveillance data and monitoring of HPAI virus outbreaks from ECDC and of development and availability of A(H5) Candidate Virus Vaccine (CVV) as published by the WHO, it was agreed that the CVV for a novel zoonotic vaccine with the greatest potential coverage against the avian viruses of concern, which are currently of clade 2.3.4.4b, would be based on antigenic prototype strain: **A/Astrakhan/3212/2020 (H5N8) (clade 2.3.4.4b**).
It is noted that the proposed strain update (H5N1/Clade 2.2.1→ H5N8/Clade 2.3.4.4b), where the NA antigen subtype changes but the H5 subtype is unchanged, is not clearly addressed by the current GLs on Influenza Vaccines (*neither Quality nor Non-clinical and Clinical Modules*).

However, with the support of the EMA/ETF, it was agreed with the MAH that the procedure would be supported pre-approval by quality data only (*and immunogenicity non-clinical data in Ferret*) **with no generation of clinical data**.

In the attempt to further streamline the availability of the updated H5N8 zoonotic vaccine, within the proposed regulatory strategy, an accelerated timetable for the assessment was also agreed and, concerning the main initial supporting data package (i.e. quality data), a 2-step submission approach was exceptionally allowed in order to be flexible with the MAH that experienced significant and repeated delays in the availability of the Single-radial-immunodiffusion (SRID) reagents.
In detail, the following main quality documents were initially submitted to support the present Type II strain update procedure:

Step-1 (*data evaluated at the first assessment round*):
- Seeds lots passage history and "temporary" release certificates for Working Virus Seed (WVS)
- Gene Sequencing Data on WVS, supporting seeds identity. Antigenic HA identity at seed level, as per regulatory requirements, unsuccessful at WHO/CC laboratories
- Active Substance (AS) – H5N8 manufacturing process optimization data, "preliminary" characterization report, 3-month stability (real time)
- Finished Product (FP) – composition, batch formula, stability protocol, adventitious agent risk assessment.

Step-2 (*data evaluated at the second round of assessment*)
- Virus seed "full" release certificate
- AS – "final" characterisation report, SRID verification report, batch analysis data
- FP – SRID verification report, formulated bulk and batch analysis data, stability data

Despite several of the concerns raised at the first round of assessment (Step 1 LoQ, i.e. 1st RSI) were adequately addressed by the MAH, a number of outstanding issues remained or were raised following evaluation of the Step 2 data package as well as Step 1 responses to LoQ (informal RSI). In particular the confirmed absence of adequate real time product stability data led to a Major Objection in this regard.
Considering the relevance of the procedure and the accelerated timetable, as agreed with the EMA and the MAH, the preliminary Assessment Report relative to Step 2, with the informal RSI / interim list of outstanding issues (ref. Sec. 11), was shared with the MAH for a quick response slot (with no formal adoption of a new LoOI).
Moreover, as recommended by ETF and agreed with the EMA, the Rapporteur Team requested the opinion of the Biologics Working Party (BWP) during the February 2024 plenary meeting, with main achievements and recommendations summarised as follows (*pending the finalisation of the BWP minutes*):

- General endorsement of the Rapp AR conclusions
- Prior to consider whether a 12 months shelf-life can be granted for the Finished Product, BWP

recommended that a stability data package needs to be evaluated pre-approval, including at least full results from accelerated and stressed stability studies for the new H5N8 strain, along with a detailed comparative analysis with respect to the accelerated/stressed stability results collected so far for the current H5N1 zoonotic vaccine. All available real-time stability data on the FP should be provided, and a detailed comparative analysis of H5N8 AS and FP real-time stability results should be carried out against the corresponding data for the current H5N1 zoonotic vaccine (AS/FP) at the relevant time-points.

- All data relevant to the CVV should be available pre-approval and should include as a minimum: official CBER documentation supporting the acceptability of the CVV of the Zoonotic Influenza vaccine (including details on the reverse genetics construct and how the plasmid expressing the HA of A/Astrakhan H5 without the multibasic trait has been obtained), safety tests performed *in vivo* and *in vitro*, a safety risk assessment linked to the absence of the intravenous pathogenicity test in chickens, certification of the suitability of the cell line used for the rescue of the reverse genetics-derived CVV, updated risk assessment on the potential risks of adventitious agents in the new H5N8 vaccine

On this premise and after the highly accelerated evaluation of the additional responses and supporting documents received on February 13th, to the informal RSI, the outcome of the overall assessment of the quality data package submitted by the MAH, can be summarised as follows.

The Major objection raised on the stability data package in support of the shelf-life claim for both AS and FP was considered not resolved, and further outstanding issues were raised to evaluate whether a 12 months shelf-life might be granted (2nd RSI). At the previous assessment round, the main reasons and pending uncertainties related to stability issues for the H5N8 Zoonotic vaccine were the following:

- According to the Guideline on Influenza vaccines – Quality module (*EMA/CHMP/BWP/310834/2012*), a minimum of 6 months real time stability data need to be provided for a pre-pandemic/zoonotic vaccine both for the AS and the FP. Moreover, the GL requires that for a new strain, all manufacturing and quality data (including stability data) should be provided.
- At the preceding assessment round, the submitted stability data for the H5N8 zoonotic vaccine were not sufficient.

- No direct comparison of the degradation profile of the H5N8 strain with that of the H5N1 A/turkey/Turkey strain, using the actual data, was provided neither at real-time nor at accelerated/stressed conditions.
- The use of statistical extrapolation models described in ICH Q5E is notoriously not applicable to predict the shelf life of a biological product, in particular an inactivated vaccine, since the degradation rate of a product with a complex and not entirely defined matrix may, and often follows, non-linear patterns. For this reason, the ICH Q5C, that requires real time stability data, applies instead;
- Due to the high degree of amino acid differences (more than 8%) between the current (H5N1) and the new (H5N8) strains, it is not possible to assume a stability profile similar to that of the current strain, just because they belong to the same H5 subtype. In literature, over 50 HA residues across the HA protein have been shown to affect HA stability in several subtypes, including the H5 one.
- Due to the lack of an historical stability database for H5 strains comparable to that existing for the seasonal strains as well as considering the significant differences existing between the current H5N1 A/turkey/Turkey and the new H5N8 A/Astrakhan/3212/2020 hemagglutinins, the expectation of applying automatically the shelf-life (24-month) authorised for Aflunov to the

updated H5N8 Zoonotic vaccine, cannot be supported from both a scientific and regulatory point of view. It was also noted that for the seasonal vaccines only 12 months stability is envisaged.

In conclusion, summarising the first RSI (and interim RSI) assessment: a certain degree of flexibility with respect to requirements of applicable guidelines was applied, as a consequence of the public health relevance and urgency of the present procedure. In order to a support a 12-month shelf-life indication for the Finished Product, based on a comparison with the real-time and accelerated/stressed stability profile of NIBRG-23 H5N1 vaccine, as defined in both early and most recent stability studies, at the same time points and conditions was requested for both H5N8 zoonotic vaccine AS/FP. In addition, since very limited information was provided on the newly proposed CVV that, being an influenza virus strain produced by Reverse Genetics derivation from a highly pathogenic precursor (H5), would require a number of additional information, at least official documents from CBER describing the preparation of the CVV and the safety tests performed and certifying the suitability of the cell line used for the rescue of the reverse genetics-derived CVV were requested pre-approval, as also recommended by BWP. Moreover, a risk assessment duly justifying the absence of the IVPT test in chickens was requested and, to complete the minimum data package on the working seeds, the MAH was requested to provide sequence data confirming the stable removal of the highly pathogenic trait at the cleavage site of the HA protein on two additional harvests or, if duly justified, formally commits to provide such data post-approval.

Following the above assessment, as agreed with EMA, an additional round of RSI (*2nd RSI round*) with accelerated timetable and a two-step submission approach was agreed to give the opportunity to the MAH to address the remaining outstanding issues. Within this conclusive round of assessment (2nd RSI), the MAH has provided all the **stability** data available so far for the H5N8 Active Substance (and for the H5N8 FP as well as comparative data vs the current H5N1 AS/FP.

The comparability study and the actual data provided so far indicate a similar stability profile between the H5N1 and H5N8 AS batches under all the conditions (real-time/accelerated) for the Active Substance. Therefore, the assignment of a 24-month storage time for the H5N8 AS could be considered overall acceptable, although applying some flexibility considering the relevance of the procedure.

As regards the H5N8 Finished Product, even with extremely limited real-time stability data , a 12-month shelf life can be overall supported based on the accelerated/stressed stability data provided. If the ongoing stability studies will show stability equivalence between H5N1 and H5N8 FP batches at real-time conditions, through a due comparability analysis vs H5N1 batches, the MAH could apply for an extension of the shelf-life for FP via a variation procedure.

All the further pending information from CBER on the newly proposed **CVV** have been submitted within the 2nd RSI response document, while the MAH committed to provide post-approval sequence data confirming the stable removal of the highly pathogenic trait at the cleavage site of the HA protein on two additional harvests.

In conclusion, from the quality point of view, the proposed H5N8 strain update for the Zoonotic Influenza Vaccine Seqirus **is now approvable**, provided that the MAH would submit the due updates (*ref. Section 3.2.S.3 – Characterization and 3.2.P.8 Stability FP)*, including documents submitted via Eudralink on March 5th 2024, **within the closing sequence**. Moreover, the Quality **Recommendations** listed in Section 16 of the present assessment report should be duly fulfilled. A Letter of Undertaking detailing the Recommendations has been provided by the applicant.

To support the strain update variation, findings from a non-GLP ferret study considered "proof of concept" by the MAH, were provided.

The following pseudoviruses expressing HA and NA homologous (A/Astrakhan/3212/2020 (H5N8) clade 2.3.4.4b) and heterologous proteins to Zoonotic Influenza Vaccine Seqirus H5N8, were used as antigens:

- A/turkey/Turkey/1/2005 (H5N1) clade 2.2.1
- A/Hubei/1/2010 (H5N1) clade 2.3.2.1a
- A/duck/Bangladesh/19097/2013 (H5N1) clade 2.3.2.1a
- A/duck/Bangladesh/17D1012/2018 (H5N1) clade 2.3.2.1a
- A/American wigeon/South Carolina/22-000345-001/2021 (H5N1) clade 2.3.4.4b
- A/Ezo red Fox/Hokkaido/1/2022 (H5N1) clade 2.3.4.4b
- A/chicken/Ghana/AVL-76321VIR7050-39/2021 (H5N1) clade 2.3.4.4b
- M2 IDCDC-RG78 UC (H5N1) clade 2.3.4.4b
- A/duck/Vietnam/NCVD-1584/2012 (H5N1) clade 2.3.2.1c
- A/Guangdong/18SF020/2018 (**H5N6**) clade 2.3.4.4h

Antigenicity equivalence between pseudovirus and CVV is considered acceptable.
Immunogenicity was evaluated using a standard HI assay. Results demonstrate that 1 or 2 doses of 12.5 µg (approx the clinical dose if the overage is considered) 3-week apart of the monovalent clade 2.3.4.4b "Zoonotic influenza vaccine Seqirus" (CBER-RG8A A/Astrakhan/3212/2020-like strain MF59C.1-adjuvanted vaccine) is immunogenic (by Haemagglutination Inhibition assay) against homologous H5N8 strain and heterologous H5N1 strains A/American wigeon/South Carolina/22-000345-001/2021 and A/Ezo red Fox/Hokkaido/1/2022 (H5N1) both within the same clade 2.3.4.4b of the vaccine. Immunogenicity was quite persistent 7 weeks after the second dose. A single-dose vaccination induced lower but still significant levels of HI antibodies.
No cross reactivity was detected (GMT < 1:10) for heterologous pseudovirus strain A/chicken/Ghana/AVL-76321VIR7050-39/2021 (H5N1) although within the same clade 2.3.4.4b of the vaccine.
No cross-reactivity was observed against pseudovirus H5 strains outside the 2.3.4.4b clade.
HAI results from the ferret study LC-07, #0154-23, were reported in the sub-section in SmPC section 5.1.
Although the recently published references cited by the MAH demonstrate the ability of HAI to correlate with protection, the MN assay results could provide relevant complementary info on neutralising activity also considering the absence of any clinical data and of protection (challenge) study. Thus, upon request the MAH also provided NAb results which mostly correlate with HAI results.

With regards to the present strain update variation, the MAH proposed a revised and "broader" version of the indication than that approved for Aflunov/"Zoonotic Influenza Vaccine H5N1", suggesting a wording that defines influenza A virus based solely on HA and deleting reference to NA (i.e. Active immunisation of adults against H5 subtype influenza A viruses (see section 5.1) vs against H5N8 subtype of Influenza A virus).
The MAH's proposal was discussed with the ETF and agreed since the vaccine update is intended for protection against H5 viruses from clade 2.3.4.4b matching the current circulating strains, and thus making to reference to N8 not relevant.
In the absence of clinical data, results from ferret study are considered relevant to the definition of clinical efficacy of the "Zoonotic influenza vaccine Seqirus" and support the proposed wording of indication. However, some concerns were raised regarding the potential cross-reactivity against former H5N1 circulating clades and other influenza A(H5) currently circulating clades (e.g. 2.3.2.1).
To guide an appropriate use of the vaccine according to subtype clade, cross reference to SmPC section 4.4 has been introduced in the indication and the existing sub-section "Cross-reactivity immunity" adequately amended to reflect the limited data on cross-immunogenicity against different H5N1 circulating clades other than 2.3.4.4b.

The MAH has also confirmed to submit post-approval, supportive clinical data generated using H5N8 and H5N6-cell-based vaccines. In order to ensure the transferability of the immunogenicity data from cell-based to egg-based vaccines, consideration should be given to whether antigenicity equivalence is confirmed between the working seeds of the 2 vaccines (cell and egg based).
Finally, since currently the SmPC contains a mix of information coming from H5N1 vaccine (Aflunov: A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) (clade 2.2.1) + A/Vietnam/1194/2004 (H5N1) (clade 1), and from H5N8 vaccine (that is only a minority), a simplification of the SmPC focusing on information relevant to the use of the H5N8 was implemented in order to improve readability.

In conclusion the variation is approvable and the benefit-risk balance of the H5N8 "Zoonotic Influenza Vaccine Seqirus", can be considered **positive**.

3. Recommendations

Based on the review of the submitted data, this application regarding the following change:

Variation requested		**Type**	**Annexes affected**
B.I.a.5.a	B.I.a.5.a - Changes to the AS of a seasonal, prepandemic or pandemic vaccine against human influenza - Replacement of the strain(s) in a seasonal, prepandemic or a pandemic vaccine against human influenza	Type II	I, IIIA, IIIB and A

Type II, B.I.a.5.a - zoonotic strain change from A/turkey/Turkey/1/2005 (H5N1) like strain (NIBRG 23) (clade 2.2.1) to A/Astrakhan/3212/2020 (H5N8)-like strain (CBER-RG8A) (clade 2.3.4.4b)

☒ is recommended for approval.

Amendments to the marketing authorisation

In view of the data submitted with the variation, amendments to Annex(es) I, IIIA, IIIB and A are recommended.

4. EPAR changes

The table in Module 8b of the EPAR will be updated as follows:

Scope

Please refer to the Recommendations section above

Summary

Not applicable

For more information, please refer to the Summary of Product Characteristics.

The SmPC sections 1, 2, 3, 4.1,4.2, 4.3, 4.4, 4.5, 4.6, 4.8, 5.1, 5.3, 6.3 and 6.6, have been updated

as follows:

To include the updated vaccine strain: A/Astrakhan/3212/2020 (H5N8)-like strain (CBER-RG8A) (clade 2.3.4.4b) and to rationalise the text in relation to the updated H5N8 strain.

Annex A, Labelling and PL have been updated accordingly.

Annex: Rapporteur’s assessment comments on the type II variation

5. Introduction

A zoonosis is an infectious disease that spreads from animals to humans. Zoonotic influenza vaccines are intended for active immunisation in the context of an outbreak of zoonotic influenza viruses with pandemic potential, including use in specific groups like veterinarians or laboratory personnel, and when there is anticipation of a possible pandemic due to the same or similar influenza strain.

Only two egg-based zoonotic influenza vaccines are currently authorised in EU, both from the same marketing authorization holder (MAH) Seqirus S.r.l.: Aflunov approved in 2010 and "Zoonotic Influenza Vaccine Seqirus" recently approved. Legal status for both vaccines is: medicinal product subject to medical prescription. The Cell-based zoonotic influenza vaccine Celldemic (surface antigen, inactivated, MF59C.1-adjuvanted) based on A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) from Seqirus S.r.L., received positive opinion in February.

The "Zoonotic Influenza Vaccine Seqirus" is a monovalent avian influenza vaccine (surface antigen, inactivated, MF59C.1-adjuvanted) containing purified Hemagglutinin (HA) and Neuraminidase (NA) surface antigens from the avian influenza virus A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) of clade 2.2.1. The "Zoonotic Influenza Vaccine Seqirus" received the CHMP positive opinion on 14 September 2023 as an Informed Consent procedure under Article 10(c) of Directive 2001/83/EC aiming to duplicate the existing Marketing Authorisation for the already authorised H5N1 zoonotic vaccine Aflunov also based on A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) of clade 2.2.1. Being authorised as duplicate license of the H5N1 zoonotic vaccine Aflunov, the "Zoonotic Influenza Vaccine Seqirus" license fully cross-refers to the up-to-date quality, non-clinical and clinical data of the original dossier of the zoonotic vaccine Aflunov, as further modified through all the post-approval Aflunov changes which have been assessed and authorised until duplicate license granting. Indeed, when initially approved, Aflunov contained A/Vietnam/1194/2004 (clade 1) influenza H5N1 strain and was called pre-pandemic influenza vaccine.

The reason behind the choice of an Informed Consent application procedure is that Seqirus intends to continue the marketing of the currently authorised Aflunov H5N1 vaccine and, in parallel, "to introduce an alternative strain which is effective against the currently circulating H5N1 2.3.4.4b clade." The strain selected by the MAH as antigenic prototype to develop a Candidate Vaccine Virus (CVV) is A/H5N8 - A/Astrakhan/3212/2020 (CBER-RG8A) clade 2.3.4.4b. Therefore, as previously agreed with the EMA and the Emergency Task Force (ETF), following duplicate license granting, within the present procedure the MAH is submitting a Type II variation to introduce a strain change for the "Zoonotic Influenza Vaccine Seqirus" to more adequately target the currently circulating clade of the highly pathogenic avian influenza (HPAI) H5 virus, while maintaining the same strain for the current Aflunov license.

With the present type II variation, the MAH intended to change the wording of indication in section 4.1 of the SmPC:

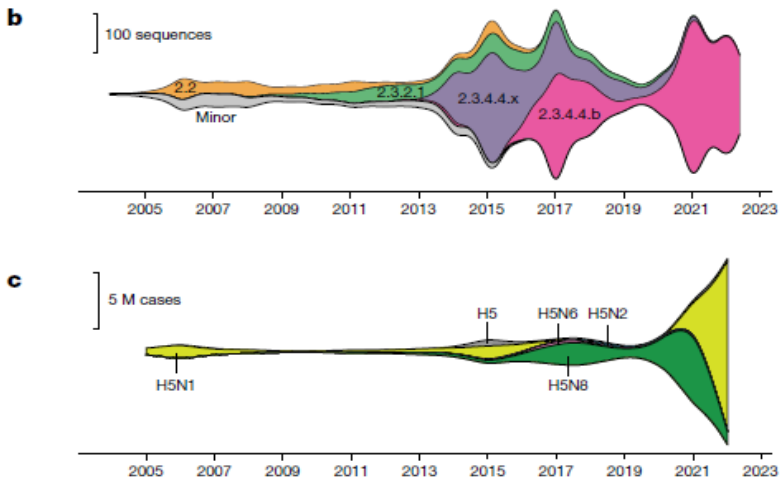
Present	**Proposed**
"Zoonotic Influenza Vaccine Seqirus" based on A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) of clade 2.2.1	"Zoonotic Influenza Vaccine Seqirus" based on A/Astrakhan/3212/2020 (H5N8)-like strain (CBER-RG8A) of clade 2.3.4.4b
4.1 Therapeutic indications Active immunisation against **H5N1** subtype of Influenza A virus. This indication is based on immunogenicity data from healthy subjects from the age of 18 years onwards following administration of two doses of the vaccine containing H5N1 subtype strain (see sections 4.4 and 5.1).	**4.1 Therapeutic indications** Active immunisation of adults against **H5** subtype influenza A virus**s** (see section 5.1).

Zoonotic Influenza Vaccine Seqirus should be used in accordance with official recommendations.	The vaccine should be used in accordance with official recommendations.

Currently, Zoonotic Influenza Vaccine Seqirus is indicated for immunisation of adults and elderly (18 years of age and above) with a course of 2 IM doses of 0.5 ml (7.5 micrograms HA) each administered 3 weeks apart.

Aflunov was developed to protect against a zoonotic influenza viral strain closely matched to strains circulating in avian populations at the time of submission, via early vaccination during pre-pandemic stages (e.g. to reduce mortality in exposed subjects in those countries where infections are occurring). Moreover, the zoonotic vaccine may also help reducing the chance of the emergence of a reassortant pandemic strain by vaccinating those (e.g. veterinarians, poultry workers, operators involved in the manufacturing of vaccines with pandemic-like strains, laboratory workers) at high risk of infection from both avian and human viruses.

As described by Xie R et al. (Nature, 2023) the scale of Highly pathogenic avian influenza (HPAI) H5 outbreaks in wild birds has escalated beyond Asia since 2014, driven by the emergence of H5 HA clade 2.3.4.4 viruses with several NA subtypes including H5N2, H5N6 and H5N8 (collectively H5Nx). From 2016, outbreaks in wild birds were repeatedly caused by clade 2.3.4.4b H5N8 viruses that originated in China. Most recently, a reassortant HPAI H5N1 virus, which evolved from clade 2.3.4.4b viruses, has almost entirely replaced the formerly dominant (from 2014–2021) clade 2.3.4.4b H5N8 viruses (see Figure below). Based on GISAID data, an HPAI virus H5N1 subtype of different clade 2.3.2.1, has been sporadically identified in Asia (*https://gisaid.org/phylogeny-influenza/influenza-h5nx/*)



From Xie et al., Nature 2023
b, Temporal changes in HPAI H5 HA clade prevalence estimated using sample collection dates of sequences submitted to the GISAID and NCBI Influenza Virus Resource databases from January 2004 to June 2022.
c, Temporal changes in HPAI H5Nx subtype prevalence estimated using observation dates of all reported cases submitted to the WOAH from January 2005 to January 2022.

Since the first detection of zoonotic transmission of HPAI A(H5N1), limited clusters of human cases have occurred, but no sustained human-to-human transmission has been observed. Zoonotic transmission to humans from infected birds occurs either directly or through environmental contamination. The risk for occupationally or otherwise exposed groups to avian influenza-infected birds or mammals according to the World Health Organization (WHO) is assessed as 'low to moderate'.

Overall, from 2003 to 2023, a total of 878 human cases for avian influenza A(H5N1) were reported to the WHO, with a fatality rate of 52% (Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2023 *https://cdn.who.int/media/docs/default-source/influenza/h5n1-*

human-case-cumulative-table/cumulative-number-of-confirmed-human-cases-for-avian-influenza-a(h5n1)-reported-to-who--2003-2023.pdf?sfvrsn=74bc4d1_1&download=true).

With regards to infections due to H5N1 clade 2.3.4.4b viruses, since December 2021, the WHO has reported a few human infections (8): 2 cases, United Kingdom 3 cases, United States 1 case, Vietnam 1 case, Ecuador 1 case, Chile 1 case. The severity of the disease has varied widely from asymptomatic, mild to severe, with fatality. Most patients had exposure to infected poultry, except for the Chilean case; however, highly pathogenic H5 outbreaks were reported in the vicinity of the patient's residency. In July 2023 the European Centre for Disease Prevention and Control (ECDC) stated that, currently, avian influenza virus A(H5Nx) transmission to humans remains a rare event, because despite the high number of exposure events due to the large outbreaks in poultry and wild birds since 2020, no symptomatic human infection due to avian influenza A(H5Nx) has been reported from EU/EEA countries (Public health situation for avian influenza A(H5) viruses *https://www.ecdc.europa.eu/en/infectious-disease-topics/z-disease-list/avian-influenza/threats-and-outbreaks/situation-ah5*). The detection of Influenza A(H5N1) virus in two asymptomatic poultry farm workers in Spain in 2022 was finally classified as suspected environmental contamination. The recent global shift in the ecology of H5N1 HPAI, and avian influenza spillover into mammals (Venkatesan P et al., Lancet Microbe 2023) both raise concerns and prompt pandemic preparedness. Thus, the Food and Agriculture Organization of the United Nations (FAO), the WHO, and the World Organisation for Animal Health (WOAH) urge actions against the ongoing avian influenza outbreaks in animals that continue to pose risk to humans. The acquisition of adaptive mutations in mammals warrants continuous monitoring of H5N1 clade 2.3.4.4b viruses for the presence of mutations that could potentially increase their pandemic risk for humans.

Thus, to strengthen pandemic preparedness activities, a strain update for the "Zoonotic Influenza Vaccine Seqirus" has been considered appropriate as agreed with the EMA and the ETF. During interactions of the MAH with the EMA/ETF on the basis of influenza surveillance data and monitoring of HPAI virus outbreaks from ECDC and of development and availability of A(H5) CVV as published by the WHO (*https://cdn.who.int/media/docs/default-source/influenza/cvvs/cvv-zoonotic---northern-hemisphere-2022-2023/h5-non-h5n1_cvv_20220225.pdf?sfvrsn=8f360e05_9*), it was agreed that the CVV for a novel zoonotic vaccine with the greatest potential coverage against the avian viruses of concern which are currently of clade 2.3.4.4b, would be based on antigenic prototype strain **A/Astrakhan/3212/2020 (H5N8).** The CVV identified is **CBER-RG8A A/Astrakhan/3212/2020 (clade 2.3.4.4b).**

According to the MAH, the H5 influenza human cases from which genetic sequence information is available (n=6) were caused by clade 2.3.4.4b viruses. However, data available from the GISAID's EpiFlu Database, show that since 2020 human infections have been caused by further 3 different A (H5) clades of the A/goose/Guangdong/1/1996-lineage, namely:

- 2.3.2.1c (Laos, 2020 and Cambodia, 2023),
- 2.3.2.1a (India, 2021),

- 2.3.4.4h (China, 2020–2021)

	A/Turkey/Turkey/1/2005 (H5N1) 2.2.1	A/duck/Bangladesh/17D1012/2018 (H5N1) 2.3.2.1a	A/duck/Bangladesh/19097/2013 (H5N1) 2.3.2.1a	A/duck/Vietnam/NCVD-1584/2012 (H5N1) 2.3.2.1c	A/Hubei/1/2010 (H5N1) 2.3.2.1a	A/Guangdong/18SF020/2018 (H5N6) 2.3.4.4h	A/AmericanWigeon/SouthCarolina/AH0195145/2021 (H5N1) 2.3.4.4b	A/chicken/Ghana/AVL-763_21VIR7050-39/2021 (H5N1) 2.3.4.4b	A/Astrakhan/3212/2020 (H5N8) 2.3.4.4b	A/Ezo red fox/Hokkaido/1/2022 (H5N1) 2.3.4.4b
A/Turkey/Turkey/1/2005 (H5N1) 2.2.1	100	93.62	93.44	93.62	94.5	90.96	91.86	92.02	92.38	92.2
A/duck/Bangladesh/17D1012/2018 (H5N1) 2.3.2.1a	93.62	100	98.94	97.34	98.05	90.96	91.67	91.84	92.2	92.2
A/duck/Bangladesh/19097/2013 (H5N1) 2.3.2.1a	93.44	98.94	100	97.7	98.05	90.43	91.13	91.67	91.67	91.84
A/duck/Vietnam/NCVD-1584/2012 (H5N1) 2.3.2.1c	93.62	97.34	97.7	100	98.23	90.6	91.31	91.49	91.84	92.02
A/Hubei/1/2010 (H5N1) 2.3.2.1a	94.5	98.05	98.05	98.23	100	91.13	91.67	91.84	92.2	92.38
A/Guangdong/18SF020/2018 (H5N6) 2.3.4.4h	90.96	90.96	90.43	90.6	91.13	100	93.09	93.26	93.62	93.44
A/AmericanWigeon/SouthCarolina/AH0195145/202	91.86	91.67	91.13	91.31	91.67	93.09	100	99.11	99.47	99.29
A/chicken/Ghana/AVL-763_21VIR7050-39/2021	92.02	91.84	91.67	91.49	91.84	93.26	99.11	100	99.65	99.47
A/Astrakhan/3212/2020 (H5N8) 2.3.4.4b	92.38	92.2	91.67	91.84	92.2	93.62	99.47	99.65	100	99.82
A/Ezo red fox/Hokkaido/1/2022	92.2	92.2	91.84	92.02	92.38	93.44	99.29	99.47	99.82	100

Figures above, from Module 4, ferret study report

Moreover, as agreed with the EMA/ETF, the proposed strain update would be supported by a data package, mostly based on a quality data aligned with that usually provided for the annual update of seasonal influenza vaccines and compiled according to the requirements of the EMA Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012, July 2017) and the CMDh Best Practice Guide on Fast Track Procedure for the Annual Update of Human Influenza Vaccines (CMDh/290/2013/Rev.2, March 2017).

Indeed, it is noted that the proposed strain update, where the NA antigen subtype changes whereas the H5 subtype is unchanged compared to the previous vaccine, is not explicitly foreseen in the Guideline on Influenza Vaccines - Non-clinical and Clinical Module (EMA/CHMP/VWP/457259/2014) which currently states for changes on strain composition of zoonotic influenza vaccines the following (the same applies for section 4.1.2.2 of the Guideline on Influenza Vaccines – Quality Module):

5.3.2. Requirements for applications to change vaccine strain composition

It may become necessary to replace the zoonotic strain that was in the vaccine at the time of the MA by another zoonotic strain if, for example, there are data indicating low or negligible cross-reactivity and cross-protection against drift variants. Two scenarios could occur that have different implications for data requirements as follows:

a) Replacement of the strain in the authorised vaccine with a different strain of the same subtype (e.g. supplanting the original H5N1 with another H5N1 clade). In this case the MAH may submit a strain change variation that includes only the manufacturing and quality data related to the new strain (see the Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012)), if appropriately justified. However, whenever feasible, it is recommended that the new version of the vaccine is administered to subjects who previously received the initial vaccine to assess the degree of cross-priming, although such data may be submitted after the strain change variation has been approved.

b) Replacement of the HA/NA subtype (e.g. supplanting the original H5N1 strain with an H7N7 strain). In this case advice should be sought from competent regulatory authorities on the data requirements, but in principle immunogenicity and safety studies are required.

As discussed during the Innovation Task Force meeting and with the ETF, provided that the HA subtype does not change from the original registered HA subtype, submission of manufacturing and quality data related to the new strain should be sufficient for the zoonotic strain change. Currently, the NA content is not controlled during vaccine development and manufacturing: notably, being the NA only controlled for identity, batch to batch variability in terms of amount of NA in each vaccine lot cannot be excluded. During the clinical development of influenza vaccine, NA immunogenicity is not routinely measured (section 6.1.1 of the GL on influenza vaccines - non-clinical and clinical module: "Applicants may consider evaluating anti-neuraminidase NA antibodies at least in randomly selected subset."). Although both the two major glycoproteins on the virus surface elicit immune response against influenza virus infection, HA is immunodominant contributing to reduction in virus shedding while NA antibody titers are associated with reduction of disease severity and with heterologous protection since NA specific antibodies bind to domains that are well conserved within a subtype (Eichelberger M et al., Curr Opin Immunol, 2018).
Therefore, on the basis that vaccine platform is well known, the change in NA is not expected to affect the antigenicity and immunogenicity of the HA component of the vaccine which remains the same as in the approved formulation, and the update in composition is required with some urgency given the episodic resurgence of HPAI H5 virus since 2021, EMA/ETF agreed with the MAH's proposal not to generate any clinical data with the updated H5N8 egg-based zoonotic influenza A vaccine pre-approval, but to provide the following post-approval clinical data coming from:

I) the US trial (NCT05874713, V205_01) evaluating the cell-based MF59-adjuvanted vaccine A/Astrakhan/3212/2020 (H5N8c) and performed to support BARDA's pandemic preparedness programme. This study includes immunogenicity and safety data in adults and elderly receiving 2 doses of MF59-adjuvanted H5N8c vaccine as well as heterologous A/Guangdong/18SF020/2018 (clade 2.3.4.4h) H5N6c vaccine.

II) the US trial (NCT05422326, V89_18E1) evaluating the cell-based MF59-adjuvanted A/Guangdong/18SF020/2018 H5N6c (clade 2.3.4.4h) vaccine. The study investigates whether 2 priming doses of MF59-adjuvanted H5N1 cell culture-derived vaccine (H5N1c) followed by 1 or 2 booster vaccinations with a MF59-adjuvanted H5N6 cell culture derived vaccine (H5N6c) 3 weeks apart elicit immune responses to the antigens used for priming (H5N1) and boosting (H5N6) after first and second heterologous booster vaccination.

III) the Enhanced Passive Safety Surveillance (EPSS) when at least one Member State implements vaccination for a sufficient number of individuals. This will be an adapted seasonal EPSS approach

(safety surveillance information and call-in contacts) to ensure an early and rapid monitoring of the reactogenicity of the updated H5N8 vaccine (Zoonotic influenza vaccine).

The proposed regulatory strategy for strain change from H5N1 to H5N8, in terms of content of the dossier and reduced timeline, has been mostly agreed with EMA/ETF. As anticipated, besides quality data to be submitted in 2 steps, the MAH submitted immunogenicity and cross reactivity data obtained in a ferret study by using Astrakhan H5N8 vaccine to support the current strain update variation.

6. Quality aspects

The MAH (Seqirus S.r.l.) has submitted a Type II variation for the strain change of the Zoonotic Influenza Vaccine Seqirus (submission Step 1), as detailed below:

• Current strain:

o A/turkey/Turkey/1/2005 (**H5N1**)-like strain (NIBRG-23) (**clade 2.2.1**)

• Proposed strain:

o A/Astrakhan/3212/2020 (**H5N8**)-like strain (CBER-RG8A) (**clade 2.3.4.4b**)

Due to delays in the availability of the SRID reagents, as agreed with the Agency, the MAH is making a 2-step submission, with several documents (*indicated in orange*) being provided in a second submission concurrent with updated assessment report finalization. For the same reason, in the stability data been presented in section 3.2.S.7.3 the potency assay (by SRID) has been performed using non-homologous reagents. Furthermore, working seed has been submitted to both CBER and the WHO collaborating centre in Melbourne for HA identity testing and both centres were not able to complete the antigenic HA identity test by hemagglutination inhibition (HAI) on the seeds and recommended Seqirus to rely on HA and NA genetic sequence to demonstrate the identity of the seeds.

In detail, the following quality package documents have been already submitted (*submission **Step 1***) or will be provided (*submission **Step 2***) to support the proposed change:

Module 2

- 2.3 Quality Overall Summary – Addendum
- 2.3.S.2 Manufacture
- 2.3.S Stability
- 2.3.P.1 Description and composition of the Finished Product
- 2.3.P.2 Pharmaceutical Development
- 2.3.P.3 Manufacture
- 2.3.P.8 Stability

Module 3

Active Substance

- 3.2.S.2.2 Description of Manufacturing Process and Process Controls – Strain Specific Changes
- 3.2.S.2.3 Control of Materials-Eggs
  Attachment 3.2.S.2.3-1 SPF (specific pathogen free) eggs CoA
- 3.2.S.2.3 Control of Materials - Seeds
  Attachment 3.2.S.2.3-1 Mycoplasma testing report for A/Astrakhan/3212/2020
- 3.2.S.2.3-2 Passage history for A/Astrakhan/3212/2020
  Attachment 3.2.S.2-3 gene sequencing for A/Astrakhan/3212/2020
  Attachment 3.2.S.2-4 Seed lot reports for A/Astrakhan/3212/2020

- 3.2.S.2.5 Process Validation and/or Evaluation
  Attachment 3.2.S.2.5-1 Inactivation Technical Report for A/Astrakhan/3212/2020
  Attachment 3.2.S.2.5-2 Splitting efficiency Technical Report for A/Astrakhan/3212/2020
  Attachment 3.2.S.2.5-3 Zonal Mapping Report for A/Astrakhan/3212/2020
- 3.2.S.2.6 Manufacturing Process Development

(*interim report Step 1* – final report ***Step 2*** *submission*)

- 3.2.S.3. Characterization
Monovalent Characterisation Report - A/Astrakhan/3212/2020

- 3.2.S.4.1 Specification (for information only)
- 3.2.S.4.2 Analytical procedure – Neuraminidase Identity

(***Step 2*** *submission*)

3.2.S.4.3 Validation of Analytical Procedures - HA ID	**Updated for the A/Astrakhan/3212/2020 strain***
3.2.S.4.4 Batch Analyses	**Data for the A/Astrakhan/3212/2020** • **A/Astrakhan/3212/2020 Seed information*** • **A/Astrakhan/3212/2020 MPH batch analysis***
3.2.S.5 Reference Standards	• **Updated A/Astrakhan/3212/2020:** • **Reagent Qualification Reports – H5N8***

- 3.2.S.7.1 Stability Summary and Conclusions
- 3.2.S.7.2 Post Approval Stability
  Attachment 3.2.S.7.2 Stability Protocol for A/Astrakhan/3212/2020
- 3.2.S.7.3 Stability Data
  Attachment 3.2.S.7.3 Interim Stability Report MPH

<u>Finished Product</u>

- 3.2.P.1 Description and Composition of the Finished Product
- 3.2.P.2.2 Finished Product
- 3.2.P.3.2 Batch Formula
- 3.2.P.5.1 Specifications
  Attachment 3.2.P.5.1-1 Summary Protocol Template for Influenza Vaccine

(***Step 2*** *submission*)

3.2.P.5.3 Validation of Analytical Procedures	**Updated for A/Astrakhan/3212/2020:** • **SRID Verification***
3.2.P.5.4 Batch Analysis	**Updated with data from the Drug Product data for A/Astrakhan/3212/2020***

- 3.2.P.8.2 Post Approval Stability
  Attachment 3.2.P.8.2-1 Stability protocol for A/Astrakhan/3212/2020 Finished Product

(***Step 2*** *submission*)

3.2.P.8.3 Stability Data	**Available stability data on A/Astrakhan/3212/2020 product***

Appendices

- 3.2.A.2 Adventitious Agents

Attachment 3.2.A.2 Adventitious Agents Risk Assessment

Below is provided a summary of the quality data package submitted at **Step 1** and relative assessment.

Step 2 quality data package and relative assessment has been also included in the body of the present assessment report.

ACTIVE SUBSTANCE

Description of Manufacturing Process and Process Controls – Strain Specific Changes (*3.2.S.2.2*)

The Manufacture and sterile filtration of the Monovalent Pooled Harvest *(MPH_Active Substance*) is performed at Seqirus.
The MAH has provided a 3.2.S.2.2 section including a listing of the manufacturing steps that may require strain specific modification due to the introduction of the new strain CBER-RG8A A/Astrakhan/3212/2020 (H5N8 subtype), as well as a general outline of the studies used to investigate the strain specific conditions, and the specific modifications required.
Details relative to the manufacturing steps affected by the strain change are summarised below.

CVV
No information on the CVV used is provided in this section.

Seed
Seed Passage histories and release certificates of the working seeds have been provided in Section 3.2.S.2.3 "Seed". Batch analysis will be provided at Step 2 submission in Section 3.2.S.4.4.

Production Eggs
No strain specific changes are introduced at this step.

Virus cultivation
The optimum parameters are defined.

Harvest and Clarification of Allantoic Fluid
No strain specific changes are introduced at this step.

Virus inactivation
The inactivation step was validated according to the Ph. Eur. Monograph for a minimum of multiple production egg harvests for the A/Astrakhan/3212/2020, CBER-RG8A Influenza strain.
The relevant inactivation report is provided in *att-32s25-inactivation-H5N8*.

Ultracentrifugation (Purification)
The ultracentrifugation step is performed to purify the virus particles from the egg contaminant according to the approved manufacturing process. Upon introduction of the new H5N8 strain, strain specific studies were performed in order to optimize yield and contaminant removal.
The strain specific parameters determined are listed in the summary table below and were recommended for use:

The zonal mapping report is provided in *att-32s25-zonalmapping-H5N8*.

Diafiltration
No strain specific changes have been introduced at this step.

Haemagglutinin and Neuraminidase Solubilisation
Whole virus is split in order to release HA and NA. The split test is performed in order to determine the optimum reagent concentration as well as the optimum mixing times, to use for each strain in order to achieve maximum splitting.
The strain specific parameters determined following introduction of the A/Astrakhan/3212/2020, CBER-RG8A Influenza strain are provided in a*tt-32s25-splitting-H5N8*.

Adsorption and Sterile filtration
No strain specific changes are introduced at this step.

Optimization of the reference standards for the SRID test
An optimization of the reference standards (antigen and antiserum) used for the SRID test is planned.
The reagent qualification report for the A/Astrakhan/3212/2020, CBER-RG8A strain will be provided at the *Step 2* submission.

Control of materials *(3.2.S.2.3*)

Eggs
Specified pathogen free (SPF) embryonated eggs are used to propagate virus seed material during the manufacture of master and working seeds. Embryonated SPF eggs are supplied by approved vendors. The SPF status of the flock is established according to the Ph. Eur. Monograph Vaccina ad Usum Veterinarium, chapter 5.2.2 'Chicken Flocks free from Specified Pathogens for the Production and Quality Control of Vaccines'.
To support the present H5N8 strain update for the Zoonotic Influenza Vaccine Seqirus, the MAH has submitted a 3.2.S.2.3 Control of Materials – Eggs relative to the H5N1 vaccine Aflunov with attached an example of the certification accompanying each batch (*att-32S23-spfeggcert*). The provided certificate refers to a hatch that underwent the last sampling for control.

Seed lots
The following information has been provided in support of the introduction of the new H5N8 A/Astrakhan/3212/2020 strain:

- Mycoplasma testing report for A/Astrakhan/3212/2020 (*att-32s23-myco-h5n8*)

Within the validation report n. *R/0099/09/23*, the MAH has provided the Certificates of analysis relative to Mycoplasma testing according to EP 2.6.7. performed on influenza Working Virus Seed (WVS) A/ Astrakhan/3212/2020 , demonstrating the absence of mycoplasmas from both working seed lots and showing that the A/ Astrakhan/3212/2020 strain is not inhibitory to the growth of mycoplasma species.

- Seed Passage Histories (*att-32s23-pass-hist-h5n8*):

Information on the egg passage history of master and working seeds has been provided.
The Master seed was manufactured after passaging from the original RG reassortant, and working seeds have been manufactured from it . The former is used for the manufacture of the current vaccine lots, with a supplemental lot as back-up.

- Genetic Sequencing (*att-32s23-seq-h5n8*)

As previously mentioned, for the A/Astrakhan working seeds, both the WHO collaborating center in Melbourne, Australia and CBER attempted the HA identity by the HAI antigenic method, however, the HAI test could not be completed on the seeds as the available post-infection ferret sera displayed poor specificity in their assays. The advice from the WHO collaborating centre was to rely on genetic sequencing data for HA identity. Therefore, no identity test report has been provided within the present submission and, for the purposes of the A/Astrakhan/3212/2020 seed, HA identity has been determined using the genetic sequencing data to confirm that the working seeds are genetically identical to the CBER CVV for the strain.

Highly pathogenic H5 and H7 strains are genetically modified to remove the high pathogenic trait by deleting the polybasic cleavage site of HA protein. The absence of this specific region needs to be verified by comparison to the wild-type strain.

Genome Sequencing Data (GSD) for the Working Virus Seed of the H5N8 strain CBER-RG8A A/Astrakhan/3212/2020 have been provided in the Technical Report n. , including information provided by the WHO Collaborating Centre for Reference and Research on Influenza (VIDRL) Peter Doherty Institute for Infection & Immunity, Melbourne, Australia.

The HA and NA gene sequences were compared against the reference CVV.

Live frozen samples of both WVS lots were sent for antigenic testing to the above mentioned WHO Collaborating Centre, who performed the genetic material amplification and sequencing of samples and provided both FASTA files and the sequence alignment with the appropriate CVV from the GISAID reference database.

The HA gene characterization of both WVSs samples identified a W(T/A) to T change at position 1043 compared to the CVV nucleotide sequence. The mutation resulted in the presence of Isoleucine (I) at position 348 (position 332 without signal peptide) of the WVSs instead of a mixed of Isoleucine (I) and Lysine (K) seen in the CVV.

Concerning NA gene sequencing results, sequences were determined to be identical to the reference strain.

Seed lot testing

Batch analysis of the two WVSs has been provided in *control-of-materials-att-seed-release-h5n8* report. HA identity was planned to be confirmed by an antigenic method in the first instance. However, in the case of the A/Astrakhan working seeds, both the WHO collaborating centrein Melbourne, Australia and CBER were unable to rise ferret sera with sufficient specificity in their antigenic assay. As previously mentioned, the advice from the WHO collaborating centre was to rely on genetic sequence data for HA identity. Therefore, in place of the HA identity test report, a gene sequence report comparing the HA and NA sequences of the WVS and those of the CVV has been provided.

All other tests foreseen for WVS release (i.e. HA identity via gene sequencing, NA Identity, Mycoplasmas, Sterility, Infectivity Titre) have been provided in ”temporary” release certificates for both WVS . "Full" release certificates for the WVS, including the reagent dependant NAI test result, will be provided at the Step 2 submission. Testing results for the master seed lot are not included in the documentation submitted.

Process validation and/or evaluation specific to H5N8 A/Astrakhan/3212/2020 *(3.2.S.2.5)*

Inactivation Characterisation

The inactivation step has been validated on multiple production egg harvests of the H5N8 strain A/Astrakhan/3212/2020, CBER-RG8A reassortant.

Detailed results have been provided in the Technical report.

Viral inactivation (by the egg safety test) and the kinetics of inactivation (by the infectivity test) of the A/Astrakhan/3212/2020, CBER-RG8A reassortant have been evaluated during full scale manufacture of batches **manufactured** using a Working Seed batch .
The inactivation parameters applied and validated for the full-scale manufacture of the H5N8, A/Astrakhan/3212/2020, CBER-RG8A reassortant were those recommended following a review of a successful pilot scale study (2023_08) performed in May 2023.
Inactivation was performed on concentrated allantoic fluid.
Egg safety testing was performed on samples taken after inactivation.
Infectivity testing was performed on samples at multiple timepoints before inactivation, and at a single timepoint post inactivation .

Splitting Efficiency

Verification of the Split Test Parameters to samples of whole virus of H5N8 strain is presented in a technical report entitled "Verification of the Split Test Parameters to Samples of Whole Virus of H5N8 Strain A/Astrakhan/3212/2020, CBER-RG8A Reassortant".
The optimum quantity to allow complete splitting of the virus vaccine strains and optimal HA recovery is determined by the 'split test' prior to application to production lots.
The split test has been performed on samples from multiple full-scale batches of the H5N8 strain A/Astrakhan/3212/2020, CBER-RG8A reassortant manufactured in 2023. The batches were manufactured using WS (derived from MS).
Splitting conditions are established from a review of historical data to determine the theoretical HA titre of a batch based upon the total protein content (f = Ratio between protein concentration and HA of Whole Virus). The new strain splitting parameters were recommended from a review of those used for the manufacture of the previous pre-pandemic strain H5N1.

The following tests were performed on each sample:

- Purity, based on capillary electrophoresis analysis
- Neuraminidase (NA) enzymatic activity
- Haemagglutinin (HA) content
- Protein content by BCA

The conditions are defined on the basis of results obtained in the batches for each concentration on purity as determined by CE analysis, NA enzymatic activity, HA content, Protein Concentration and % specific purity (i.e., (HA Titre / protein conc. %).

Ultracentrifugation

The ultracentrifugation step is performed to concentrate the allantoic fluid (AF) and to purify the virus particles from the egg contaminant as per the approved manufacturing process. Upon introduction of the new H5N8 strain, strain specific studies were performed in order to optimize yield and contaminant removal. The strain specific parameters are presented in *att-32s25-zonalmapping-H5N8*.
The exact migration of the virus and non-target proteins within the sucrose gradient is known to differ between strains of influenza virus. Mapping of the Active Substance Downstream zonal centrifugation process stage was performed on the multiple batches of the H5N8 A/Astrakhan/3212/2020, CBER-RG8A reassortant . Full-scale batches were manufactured using Working Seed (derived from Master Seed). The initial zonal mapping exercise was executed using routine collection parameters for zonal processing as validated for Agrippal platform.
Multiple fractions are typically identified in the sucrose gradient, some of which are Waste fractions and not collected. The peak fractionis collected, pH adjusted and stored at 2-8ºC before being further processed.

Zonal mapping takes mutliple sample fractions from the output of the zonal centrifugation to provide a higher resolution on the distribution of the inactivated bulk fluid (IBF) components across the sucrose gradient. The zonal fractions from multiple batches were pooled to form the equivalent of Production Fractions using the routine collection parameters.
Multiple Production Fractions from each batch were analysed by SDS-PAGE, HA content by SRID using non-homologous SRID reagents, ovalbumin by ELISA and total protein. The use of non-homologous SRID reagents is acceptable.
Visual assessment of the SDS-PAGE gel indicated that HA was present predominantly in the peak fraction for all batches.
For multiple batches fractions were analysed by SDS-PAGE to show the migration of HA and non-target protein in the sucrose gradient. Visual assessment of the SDS-PAGE gel was made to determine appropriate optimised parameters for peak fraction collection. Results indicated that collection parameters for the peak were optimal, therefore there was no requirement to adjust the collection parameters.
The results met the acceptance criteria of elimination of ovalbumin over this process step No further adjustment to the zonal collection parameters was required.

Manufacturing Process Development *(3.2.S.2.6)*
There is no change to the product specification or manufacturing process as part of this strain change introducing an alternative strain (A/H5N8 - A/Astrakhan/3212/2020).
The new master seed and subsequent working seeds are manufactured using the currently approved manufacturing process.
The resultant MPH and Finished Product batches are manufactured using the currently approved manufacturing process.

Characterization *(3.2.S.3)*

Step 1 submission
No updated 3.2.S.3. Section, with detailed characterization data supporting the introduction of A/H5N8 - A/Astrakhan/3212/2020 an alternative strain for the Zoonotic Influenza Vaccine Seqirus, has been provided at the *Step 1* submission phase.
As agreed in the pre-submission phase, detailed characterization data on monovalent pool harvest (MPH) containing the new H5N8 strain A/Astrakhan/3212/2020, CBER-RG8A reassortant will be provided at the Step 2 submission phase.
However, on request of the Rapporteur during the *Step 1* assessment phase, an interim report "Monovalent Characterisation Report - A/Astrakhan/3212/2020" has been provided with the following data.
The characterization study has been performed on multiple MPH of A/Astrakhan manufactured. It includes:
A) Product Quality Specification (*PQS*) testing of the multiple batches. Some of the results are not available yet as the assays are still ongoing, however those already submitted meet the specification.

HA Content and Purity has been analysed by SDS-PAGE, de-glycosylated, in addition to the CE analysis which is part of the PQS. Both techniques confirm that the manufacturing process effectively removes viral proteins (matrix protein and nucleoprotein). The proportion of HA to total protein has been determined for all batches. In the characterization report several studies have been included, which in fact are part of the strain-specific documentation provided with this variation procedure.
HA and NA genetic and amino acid sequencing of the working seeds is in fact addressed. Sequencing has been performed also on End-of-production sample, in a separate study. Results of this study are not provided and the report itself has not been submitted. Similarly, sucrose gradient ultracentrifugation

strain specific parameters have been defined in *process-validation-att-zonalmap-h5n8,* as part of the process validation.
NA characterization of the monovalent bulks includes, in addition to the determination of NA presence and subtype as part of the PQS, the quantitation of the NA activity on all bulks tested. The method is validated for pass/fail criteria, but this activity data is being presented for characterisation only.
Dynamic light scattering (DLS) has been performed to determine the particle size distribution in the monovalent bulks and compare it against the H5N1 control limits, as no previous data for H5N8 strain is available. All batches showed the z-average particle size met the specification.
No human bocavirus was detected in the Working Seed or resultant monovalent bulks.

Step 2 submission
The characterisation report on multiple monovalent pooled harvests of A/Astrakhan/3212/2020 CBER-RG8A has been finalised with the inclusion of all the results of the Product Quality Specifications. All tested parameters meet specifications. The corresponding section of the dossier has been updated accordingly.

Specification *(3.2.S.4.1)*

There have been no updates to the Active Substance specification as a consequence of the proposed change. Section 3.2.S.4.1 "Active Substance Specification" has not been included in the submission package.

Analytical procedure – Neuraminidase Identity *(3.2.S.4.2)*
There have been no updates to the NA-ID method as a consequence of the proposed change and Section 3.2.S.4.2 "Analytical Procedures - NA ID" has not been included in the submission package.

As agreed in the pre-submission phase, the following updated CTD Sections will be provided at the *Step 2* submission phase, due to delays in the availability of the SRID reagents:

3.2.S.4.3 Validation of Analytical Procedures - HA ID	**Updated for the A/Astrakhan/3212/2020 strain***
3.2.S.4.4 Batch Analyses	**Data for the A/Astrakhan/3212/2020** • **A/Astrakhan/3212/2020 Seed information*** • **A/Astrakhan/3212/2020 MPH batch analysis***
3.2.S.5 Reference Standards	• **Updated A/Astrakhan/3212/2020:** • **Reagent Qualification Reports – H5N8***

Therefore, the following sections of the assessment will be compiled at a later stage (***Step 2***):

Validation of Analytical Procedures- HA ID *(3.2.S.4.3) - (Step 2 submission)*
The section has been updated by including the information on the introduction of the new strain A/Astrakhan/3212/2020.
The verification of the SRID method performed for A/Astrakhan/3212/2020 included the following criteria:

- ✓ Repeatability
- ✓ Intermediate Precision
- ✓ Accuracy
- ✓ Linearity
- ✓ Specificity
- ✓ Robustness

The SRID verification report for the A/Astrakhan/3212/2020 strain has been provided in *att32s43-SRD-verification-H5N8.*
The qualification report provided details the study carried out to verify that the Parallel line (PLA) SRID method is suitable for the routine quality control potency testing of Agrippal samples or equivalent containing A/Astrakhan/3212/2020 CBER-RG8A surface antigens.
This study was completed using TGA antigen 2023/144B with CBER antiserum H5-AB-2313. The concentrations of these reagents were defined in the optimisation of reagents study (report R/0158/12/23.

Batch analysis *(3.2.S.4.4)*

(Step 2 submission)

In Section 3.2.S.4.4.4 "Introduction of A/Astrakhan/3212/2020" batch analysis data have been provided for:

- Master Seed
- Working Seed
- Monovalent Pooled Harvest manufactured from working seed.

Batch analysis data are all in compliance with the approved specifications,

Reference Standard *(3.2.S.5) (**Step 2** submission)*

Reagent Qualification *(3.2.S.5.3)*

The antigen/antiserum reagents used for the A/Astrakhan/3212/2020 (CBER-RG8A) strain have been qualified. The reagent qualification report has been provided in *att-32s5-reagentqualification-H5N8*.
The Optimization Report of the batch of Antigen and Antiserum and optimization of MPH dilution for the use with the H5N8 strain has been provided.
Batch of antigen and antiserum provided by TGA and CBER are the following:

- ✓ Antigen lot – 2023/144B – by Therapeutic Goods Administration (TGA)
- ✓ Antiserum lot – H5-AB-2313 – by Center for Biologics Evaluation and Research (CBER)

The antigen lot 2023/144B was reconstituted in 0.5ml of distilled water. The recommended concentration of antiserum lot H5-AB-2313, as supplied by CBER, is 26-30µl/ml of agarose to be used for the testing of TGA antigen containing approximately 30µgHA/ml. A range of antiserum concentrations was tested.

The optimization verification was performed using the selected volumes of antiserum. Multiple replicates were performed for both Matrix 1 and Matrix 2 .
The test results were compliant with the acceptance criteria: the CV of all replicates for each batch must be ≤10%.
Antisera Volume has been selected for routine testing for Matrix-1 and antisera volume has been selected for routine testing for Matrix-2.

Stability *(3.2.S.7)*

Stability Summary
Following the introduction of new H5N8 subtype A/Astrakhan/3212/2020, multiple MPH batches have been placed on stability to support the strain update.
Data has been provided in *att-32s73-stability-data-H5N8.*
At the time of *Step 1* submission, data has been obtained using non-homologous SRID reagents.

Stability protocol

The stability protocol, aiming to support the introduction of the H5N8 strain, has been provided in *att-32s72- stability-protocol-H5N8*.

The protocol includes Zoonotic Influenza Vaccine Monovalent, Formulated Bulk and Fill Material manufactured in 2023 and details the requirements to be met in order to generate stability data for Zoonotic influenza vaccine monovalent, formulated bulk and filled Finished Product, manufactured by using the A(H5N8) A/Astrakhan/3212/2020 strain, CBER-RG8A reassortant.

The objective of the study is to provide data to support a 24-Month holding time for the monovalent material, the formulated bulk and a 24-month shelf life for the packed Finished Product (FP).

The H5N8 A/Astrakhan/3212/2020 monovalent stability samples are sterile filtered and stored in Bio Process Containers (BPCs) representative of those used in production. The formulated bulk stability samples are stored in BPC representative of BPCs used during routine production. The formulated fill material is filled as Finished Product vaccine in Pre-Filled Syringes (PFS) and samples are taken from packed final product at Seqirus.

Monovalent material will be placed on to stability under real time conditions to determine the real time stability of the Astrakhan monovalent bulk. All monovalent batches will also be placed onto accelerated stability at both Relative Humidity (RH) and temperature conditions for a period of multiple days.

Formulated bulk material will be placed onto stability for multiple months under real time conditions only to determine the real time stability of the Zoonotic influenza vaccine formulated bulk.

The filled material will be held on stability for multiple months at the recommended storage temperature to determine the real time stability of the vaccine. Accelerated studies will be performed to obtain preliminary data on the stability profile and to obtain additional information on the material in support of any potential temperature excursions.

The stability study program includes information on control time points and the relevant sampling and testing windows.

The stability-indicating parameter is the potency of the product as determined by the SRID assay. The assay uses strain-specific antibodies incorporated into agarose gel to determine the concentration of Influenza HA in the vaccine and, therefore, confirms the identification of the strain.

According to MAH, conditioning studies are no longer required on FP material. However, a description of the conditional studies conducted on the Finished Product is provided in Section 3.1. A conditioning study is where the samples from the chosen batch are pre-stressed in a stability chamber to represent the maximum processing time permitted through inspection, packaging and shipment preparation prior to being placed onto stability study.

The total conditioning time is over 100 hours, therefore once samples are shipped the time out of refrigeration (TOR) data is obtained and this TOR value is subtracted from the over hours which for the remaining time the samples are placed into the stability chamber.

Stability testing timetable and testing requirements have been provided for MPH and FP real time and accelerated stability.

The Formulated Bulk Real Time Stability will include BPCs each filled to total study allocation.

Tests and specifications used to evaluate the quality and stability characteristics of Zoonotic influenza monovalent, formulated bulk and filled (FP) material have been reported.

Stability Data

Data of the batches that have been placed in stability in support of the introduction of A/Astrakhan/3212/2020 strain have been provided in *att-32s73-stability-data-H5N8* .

At the *Step 1* submission phase, the stability data under real time conditions have been presented for Monovalent product. HA results for A/Astrakhan/3212/2020 MPH are available. Trend data are not available at this time. The study is ongoing.

FINISHED PRODUCT

Description and composition *(3.2.P.1)*

Section 3.2.P.1 "Description and Composition of Finished Product" has been updated to reflect that an alternative strain (A/H5N8 - A/Astrakhan/3212/2020 CBER-RG&A) is being introduced in the Finished Product composition and that each 0.5 ml dose of the Zoonotic Influenza Vaccine Seqirus has the following composition:

Ingredients	Quantity per dose	Function	Reference to Standards
Active Ingredient			
Influenza virus surface antigens (haemagglutinin and neuraminidase), H5N8.	≥ 7.5 µg HA	active ingredient	Ph.Eur.
Adjuvant			
squalene	9.75 mg	oil phase	Ph. Eur.
polysorbate 80	1.175 mg	surfactant	Ph.Eur.
sorbitan trioleate	1.175 mg	surfactant	Ph.Eur.
sodium citrate dihydrate	0.66 mg	buffer	Ph.Eur.
citric acid monohydrate	0.04 mg	buffer	Ph.Eur.
Other Ingredients			
sodium chloride		isotonic aid	Ph.Eur.
potassium chloride		buffer	Ph.Eur.
potassium dihydrogen phosphate		buffer	Ph.Eur.
disodium phosphate dihydrate		buffer	Ph.Eur.
magnesium chloride hexahydrate		stabiliser	Ph.Eur.
calcium chloride dihydrate		stabiliser	Ph.Eur.
water for injections		diluent	Ph.Eur.

Formulation Development *(3.2.P.2.2)*

Section 3.2.P.2.2 "Formulation development" has been updated to highlight that, in 2023, an alternative strain (A/H5N8 - A/Astrakhan/3212/2020 CBER-RG&A) is being introduced which is effective against the currently circulating H5N1 2.3.4.4b clade.

Batch formula (*3.2.P.3.2*)

The manufacturing formula included the Section 3.2.P.3.2 is an example of the type of calculation performed.

To achieve the required HA content in the Finished Product, a calculation is made for each batch of Final Bulk to be formulated, starting with the amount of HA in the Monovalent Pooled Harvests to be added. Since the HA content of each batch of Monovalent Pooled Harvest is different, the volume calculation for each one varies based on the HA potency at release. The exact amounts of the other components are then calculated according to a defined formula.

To allow for the inherent variability within the potency assay method a suitable overage for antigen content is included. The actual overage applied will take into account the stability profile for the strain included in the formulation, and to allow for the inherent variability within the potency assay method.

Specifications (*3.2.P.5.1)*

There have been no updates to the Finished Product specification as a consequence of the proposed change. Section 3.2.P.5.1 "Finished Product Specification" has been included in the submission package. The section has been updated only with reference to the attachment "*specifications-att-lrp - Lot Release Protocol Template*", providing a summary protocol for production and testing of Zoonotic Influenza Vaccine.

Stability (*3.2.P.8*)

As already described and commented in AR section 3.2.S.7, a stability protocol detailing the requirements to be met in order to generate stability data for Zoonotic influenza vaccine monovalent, formulated bulk and filled Finished Product (PFS) manufactured by using the A(H5N8) A/Astrakhan/3212/2020 strain, CBER-RG8A reassortant, has been provided.
A CTD Section "Post-approval Stability Protocol and Stability Commitment (3.2.P.8.2)", referring to Stability of Fluad H5N1 and including reference to *att-32p82-stability-protocol-zoonotic*, has been also provided.
Within the scope of this *Step 1* submission, no stability data (neither under real time nor accelerated conditions) have been submitted for the Zoonotic Influenza Vaccine (H5N8) Finished Product.
In fact, the available stability data for the H5N8 Zoonotic influenza vaccine (PFS), supporting the introduction of the new strain A/H5N8 - A/Astrakhan/3212/2020 CBER-RG&A are not available at this time and will be provided at the *Step 2* submission phase.

As agreed in the pre-submission phase, the following updated CTD Sections will be provided at the *Step 2* submission phase, due to delays in the availability of the SRID reagents:

3.2.P.5.3 Validation of Analytical Procedures	**Updated for A/Astrakhan/3212/2020:** • **SRID Verification***
3.2.P.5.4 Batch Analysis	**Updated with data from the Drug Product data for A/Astrakhan/3212/2020***

Therefore, the following sections of the assessment will be compiled at a later stage (Step 2):

Validation of Analytical Procedures- SRID Verification *(3.2.P.5.3) (**Step 2** submission)*
The section has been updated by including the reference to the attachment *att32p53-srid-verification,* provided for the Strain Specific requalification of the SRID method for the A/Astrakhan/3212/2020 strain. Verification Report on the suitability of the SRID method for the routine quality control potency testing of Finished Product containing A/Astrakhan surface antigens has been provided.
Studies were performed to test repeatability, linearity, intermediate precision, accuracy and robustness of the method.
For SRID test of A/Astrakhan/3212/2020 H5N8 strain, antigen code 2023/144B and antiserum code HS-Hb-2313 were supplied by TGA and CBER, respectively. The reagent optimization report was provided in *att-32s5-reagentqualification-H5N8*.

Assay repeatability was performed for the verification of the plate-to-plate precision of the method under the same operating conditions over a short period of time. The lab scale blend was tested by a three-plate assay on one occasion across five concentrations. Lab scale blends were produced for the multiple concentrations of the strain tested. Lab scale blends were produced following instruction found in attachment 2 of the report (not available).

Intermediate precision was verified by assessing the precision of the method when performed at two different sites, on different days, by two different analysts per site. The house blend was tested on multiple days and by multiple analysts giving multiple results. The plates were read by the multiple analysts.
The acceptance criteria were met.

Accuracy was evaluated across the range of the assay by calculating the percentage recovery of reference antigen spike. The test was performed at multiple concentrations across the range of the assay. The spiked and unspiked samples were set up by one analyst and processed in parallel.
Lab scale blends were produced for the multiple concentrations of the strain tested.
Spiked samples were prepared by diluting antigen with PBS resulting in the multiple concentrations this was then mixed with the corresponding unspiked samples forming the final spiked sample.
Linearity was verified to confirm the ability of the method to produce test results that are directly proportional to the concentration of HA in the sample. The test was performed using theoretical potencies and potencies taken from repeatability and using Excel to calculate Pearson correlation coefficient (Pearson's R).

Robustness of the method was verified to ensure that the assay was capable of withstanding variation in the reagents.
One set of standard and the blend sample was treated with zwittergent and incubated for more than 20minutes . Sample and standard were then inoculated following dilution and again at two times post incubation. The % difference was calculated for the potency results obtained from each time point compared to minimum zwittergent contact time.

Batch analysis *(3.2.P.5.4)*

Step **2** submission
Batch analyses results for the first formulated bulk batches of Zoonotic Influenza Vaccine have been provided in the subsection 3.2.P.5.4.7 "Batch Analysis for Introduction of zoonotic Influenza Vaccine (A/Astrakhan/3212/2020 strain)".

All the results were in compliance with the approved specifications.

APPENDICES

Adventitious Agents Safety Evaluation *(3.2.A.2)*

Within the present submission, the MAH has provided the 3.2.A.2 "ADVENTITIOUS AGENTS SAFETY EVALUATION" for the Zoonotic Influenza Vaccine Seqirus, updated to include among the Appendices the *att-32a2-adventious agents RA-143499* "Quality Risk assessment for the clearance of adventitious agents in Agrippal platform products".
In fact, as declared in Section 3.2.A.2.2, Seqirus has completed a risk assessment, covering all seasonal and pandemic/pre-pandemic influenza vaccines manufactured and detailing all testing and clearance of adventitious agents.
The MAH claims that the adventitious agents risk assessment is updated by periodic review or as required based on manufacturing or external requirements.
As declared in the revision history of the risk assessment provided, the report dated April 2023 has been updated and Monkeypox virus has been included in the evaluation.

7. Non-clinical aspects

In support of this strain update variation, the MAH submitted a new non-GLP "proof of concept" immunogenicity and tolerability study of Zoonotic Influenza Vaccine Seqirus H5N8 in ferrets. All non-clinical data submitted previously at the time of Aflunov approval using A/Vietnam/1194/2004 (clade 1) and A/turkey/Turkey/1/2005 (clade 2.2.1), are still applicable and unchanged.

7.1. Methods – analysis of data submitted

The immunogenicity of a single-dose or two-dose vaccination with a 3-week interval was evaluated in ferrets using standard hemagglutination inhibition (HAI) assay.
During the assessment, the MAH also provided results for the neutralization assays presented as geometric mean titers (GMT) and standard deviations (SDs).
The following pseudoviruses expressing H5NX antigens were used: PS HIV-1-H5N8-M2 Astrakhan (CBER-RG8A), PS HIV-1 H5N1 turkey/Turkey (NIBRG-23), and PS HIV-1 H5N1-M2 IDCDC-RG78 (which presumably derives from A/American wigeon/South Carolina/22-000345-001/2021, even if it is not clearly stated in Materials section). No information is provided on the antigenic characterization of the pseudoviruses.
Tolerability of the vaccines was evaluated by moribundity/mortality, detailed physical examinations, and clinical observations; injection site reactogenicity; body weights; body temperatures and injection site reactions (Draize Score) were assessed at each immunization time point: before the dose, at 24 hours, 48 hours, and 72 hours.

Monovalent zoonotic influenza vaccine Seqirus CBER-RG8A A/Astrakhan/3212/2020 contained at least 24.9 µg HA/mL.

Table 2: Study design[1]

Group[2]		Vaccination[3]		No of animals	Dose (ml)	Concentration (µg/ml)
		D0	D20			
1	Prime-Boost	Zoonotic Influenza Vaccine Seqirus	Zoonotic Influenza Vaccine Seqirus	10	0.5	25 µg
2	Prime only	Zoonotic Influenza Vaccine Seqirus	N/A	10	0.5	25 µg
3	Control	PBS	N/A	3	0.5	0

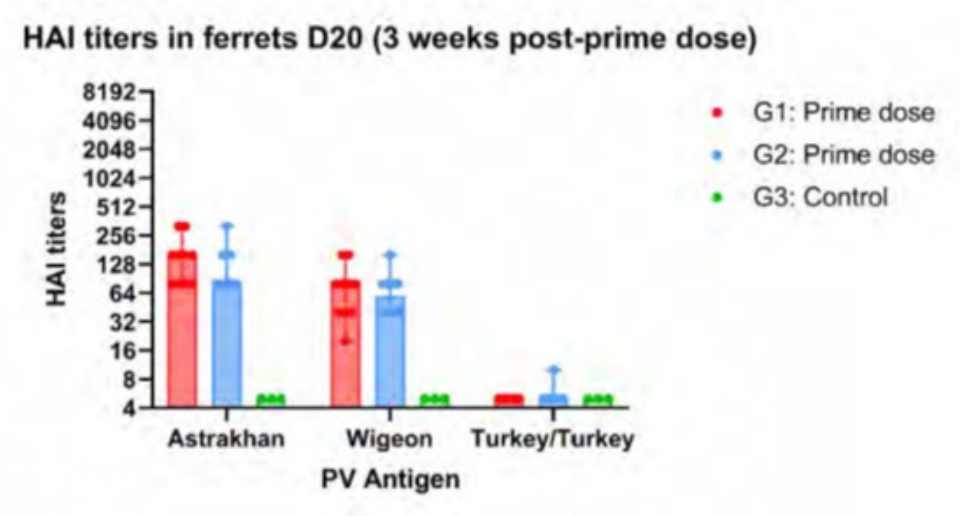
There were 4 blood draws, with serum prepared from the blood for subsequent bioanalysis, being pre-bled on day -7, a day prior to the second dosing (D20), four weeks after the boost (D49) and on the termination day (D70).
Data were analyzed using two-way analysis of variance (ANOVA) with Tukey's multiple comparisons test. The GMT was plotted as a bar graph, and individual titers were plotted as points to show the distribution.

7.2. Results

PRE-VACCINATION SCREENING HAI TITERS
All samples were negative against H3N2, B Yamagata, B Victoria, and H5N8 antigens. One ferret sera sample from group 2 tested positive to H1N1 with a titer of 1:80 HAI.

POST-PRIME DOSE HAI TITERS 20 days post-prime dose administration

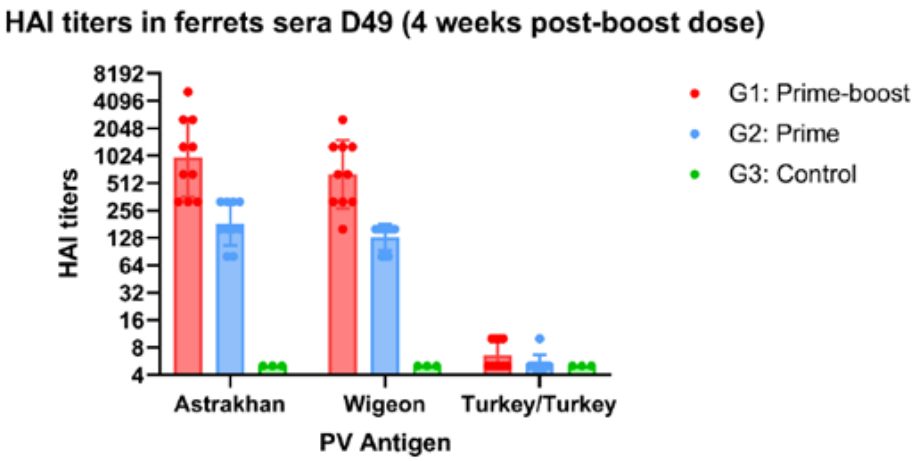
At 20 days post-prime dose administration, all vaccinated ferrets showed HAI GMT of around 1:125.5 against the A/Astrakhan/3212/2020 and 1:62.8 against A/American wigeon/South Carolina/22-000345-001/2021 antigen. Titers against the more distantly related A/turkey/Turkey/1/2005 were below the initial sera dilution of 1:10.



POST-BOOST DOSE HAI TITERS 28 days post-boost dose administration

Administration of a booster dose 3-weeks after a prime dose increased the HAI GMT against the homologous antigen by a factor of 5.2 when compared to the single-dose group (Group 2), 1:970 HAI vs 1:183.8 GMT, respectively.

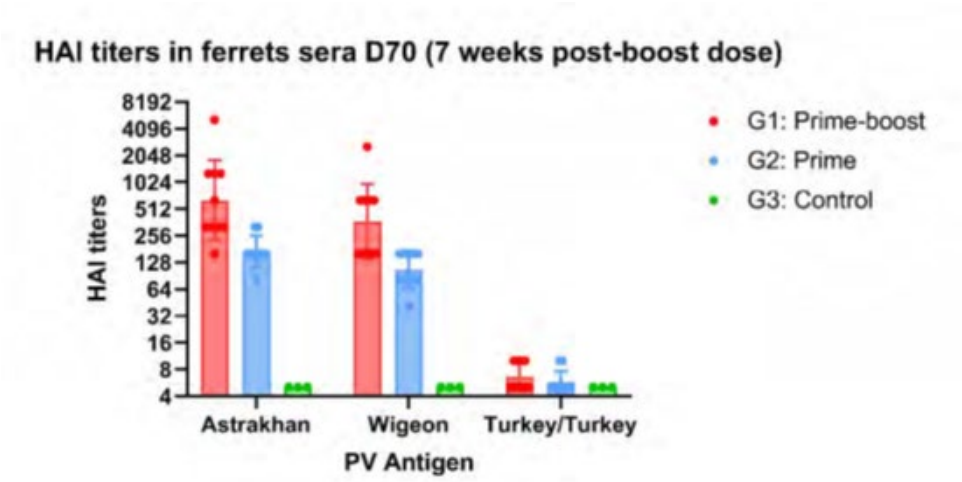
A similar pattern was observed when the closely related A/American wigeon/South Carolina/22-000345-001/2021 H5N1 antigen was used, with GMT titers of 1:640 post-boost vs 1:130 HAI post-single dose. No significant titers were observed when the more distantly related antigen A/turkey/Turkey/1/2005 H5N1 was utilized as antigen (GMT <1:10).

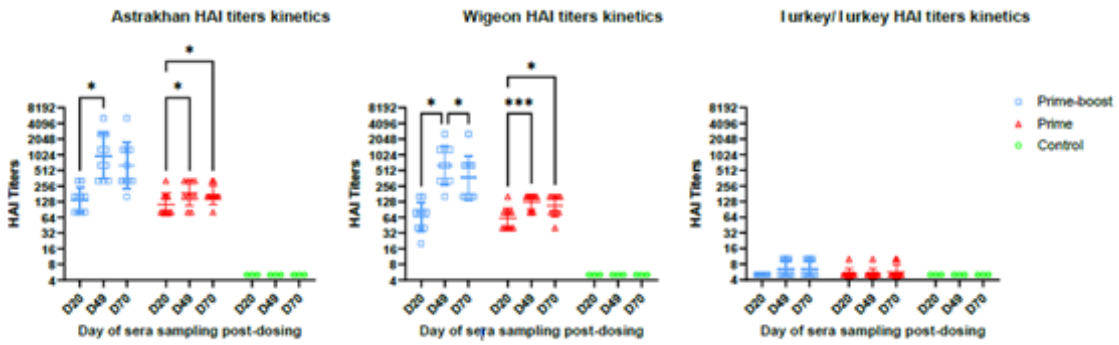


PERSISTENCE OF HAI TITERS AND KINETICS POST PRIME AND BOOST DOSE

Ferret sera was evaluated for HAI antibodies 10 weeks after a prime dose (G2) and 7 weeks post-boost to measure the persistence of circulating antibodies in blood. A slight decrease GMT against the homologous antigen was observed in the boosted and primed animals (1:640 vs 1:171.5, respectively); however, differences were not statistically different. A similar pattern was observed when the closely

related A/American wigeon/South Carolina/22-000345-001/2021 H5N1 antigen was used, with GMT titers of 1:367.6 post-boost vs 1:105.6 HAI post-single dose. No significant titers were observed when the more distantly related antigen A/turkey/Turkey/1/2005 H5N1 was utilized as antigen (GMT <1:10).





DISCUSSION OF FINDINGS

A single-dose vaccination 12.5 µg of Zoonotic influenza vaccine Seqirus CBER-RG8A A/Astrakhan/3212/2020 induced significant levels of HAI antibodies (GMT 1:125.5) 20 days post-administration that bound to the homologous A/Astrakhan/3212/2020 pseudovirus.

Serum antibody titers increased slightly from day 20 to 49 in the single-dose group (GMT 183.8). Also, ferrets vaccinated using a prime-boost strategy in a 21-day interval had ~5.2-fold higher HAI titers than animals that received a single dose (1:970 vs. 1:183.8). Titers were sustained with a slight decrease at 10 weeks post-prime and at 7 weeks post-boost dose with GMT of 1:640 and 1:171.5, respectively. Seropositivity of one ferret against seasonal H1N1 during pre-screening did not seem to affect its seroconversion following vaccination with the zoonotic influenza vaccine Seqirus H5N8 since titers were within the range of other immunized animals.

Antibody binding of two additional H5 strains, including A/turkey/Turkey/1/2005, which is an H5N1 strain of the 2.2.1 clade, and A/American wigeon/South Carolina/22-000345-001/2021 of 2.3.4.4b clade, which is more representative of currently circulating highly pathogenic influenza H5N1 strains in The Americas, was also tested. Vaccinated ferrets produced significant levels of antibodies that bound to the heterologous A/American wigeon/South Carolina/22-000345-001/2021 H5N1 pseudovirus antigen with a GMT of 1:130, and a similar boosting effect was observed following the second dose, with a GMT of 1:640. HAI titers seemed to be sustained in primed and boosted ferrets 10 and 7 weeks of postdosing, respectively, although slightly reduced (GMT of 1:367 and 1:105.6). Conversely, no significant antibodies raised against the most distantly related strain in HA, A/turkey/Turkey/1/2005 H5N1, was observed.

This lack of reactivity might be due to substantial substitutions in the antigenic and receptor binding sites of A/Astrakhan/3212/2020 and A/turkey/Turkey/1/2005 strains.
The Zoonotic influenza H5N8 vaccine was well-tolerated in ferrets. Only 5 out of 20 ferrets experienced a transient injection site reaction (very slight erythema, barely perceptible) after the first dose, which entirely resolved within 24 hours. No clinical signs, weight loss, or fever were reported throughout the study.

Assessors' comment

The study was non-GLP and carried out in research settings. As indicated in section "4.2. Requirements for applications to change vaccine strain composition" of the Guideline on Influenza Vaccines - Non-clinical and Clinical Module (ref. EMA/CHMP/VWP/457259/2014) "For inactivated vaccines, immunogenicity and protection studies in animals could support a strain change application in case human immunogenicity data are not available." Only immunogenicity assessment in ferret study was carried out. Ferrets (Mustela putorius furo) are naturally susceptible to infection with human A and B influenza viruses and have been widely used as a model for influenza virus pathogenesis and immunity studies. Since no clinical data exist with H5N8 "Zoonotic Influenza Vaccine Seqirus", the lack of protection/challenge ferret study should be justified. Although the vaccine platform is well known, there may be uncertainties on the impact of different and new for human NA8 on antigenicity/immunogenicity/protection of the HA component of the vaccine.

The MAH has performed the study using pseudoviruses for HAI assay obtained constructing chimeric viral vectors expressing the HA and NA of 3 H5 strains. The reverse genetic CVVs corresponding to the antigens of interest (NIBRG-23 and CBER-RG8A) or from which the pseudoviruses are derived (IDCDC-RG78) were all available at the time of the study. The MAH is asked to elaborate on the antigenic equivalence between the pseudoviruses and the reference reverse genetic CVVs.

Results from the ferret study demonstrate that the monovalent clade 2.3.4.4b "Zoonotic influenza vaccine Seqirus" (CBER-RG8A A/Astrakhan/3212/2020 MF59C.1-adjuvanted vaccine) is immunogenic in ferrets against homologous H5N8 strain and heterologous currently circulating H5N1 strain A/American wigeon/South Carolina/22-000345-001/2021 of the same 2.3.4.4b clade. Persistence of immunogenicity at Day 70 (7 weeks after the second dose) was higher for homologous H5N8 strain vs H5N1 strain (1:640 vs 1:368 GMT, respectively).

However, it is noted that in ferrets no significant immune response was elicited vs A/turkey/Turkey/1/2005 H5N1 virus of 2.2.1 clade that, according to data provided by the MAH, shows a lower aminoacid sequence identity in the HA gene with the H5N8 A/Astrakhan/3212/2020 virus of clade 2.3.4.4b than what observed for the other heterologous strain. Moreover, currently circulating clades 2.3.2.1 (Asia) were not tested (see clinical section and OC on wording of indication).

Serological analyses were carried out by the HI test, but did not include, in parallel, an evaluation of the antibody responses by the MN assay, capable of detecting the full range of functional antibodies raised by the vaccine antigens (possibly including also those raised by the NA), compared to the HI assay which would detect only the subset of antibodies targeting the antigenic epitopes overlapping or in close proximity to the HA receptor binding site. For the clinical immunogenicity evaluation, the EMA/CHMP/VWP/457259/2014 Guideline considers it essential that neutralizing antibody titres are determined in all studies. Considering the absence of a ferret challenge study, the MAH should justify the reason for the absence of that analysis. It is also noted that in the ferret challenge studies 765-N106857 and 673-N106850 submitted at the time of the initial marketing authorisation, MN assay was carried out. While the vaccination schedule used in ferrets mimics the clinical one (2 doses administered 3 weeks apart), doses administered in ferrets contained about 2-fold higher amount of HA vs the clinical dose (12.5 ug ferret vs 7.5 ug clinical). No dose-range testing of vaccine content of HA was performed. The MAH should justify the choice of the selected H5N8 vaccine dose used in ferret study in relation to

the dose used with H5N1 in previously performed ferret studies (see below). The clinical relevance of the H5N8 vaccine dose used in ferret study should be discussed.
At the time of approval, Aflunov contained A/Vietnam/1194/2004 (H5N1)-like strain (NIBRG-14) (clade 1) influenza strain. Clinical trials supporting the approval of Aflunov and its duplicate "Zoonotic Influenza Vaccine Seqirus H5N1" were carried out, respectively, with zoonotic influenza vaccines H5N1 A/Vietnam/1194/2004 (clade 1) and A/turkey/Turkey/1/2005 (clade 2.2.1).
Non-clinical immunogenicity/protection data from the initial marketing authorisation of Aflunov included the following 3 ferret challenge studies:

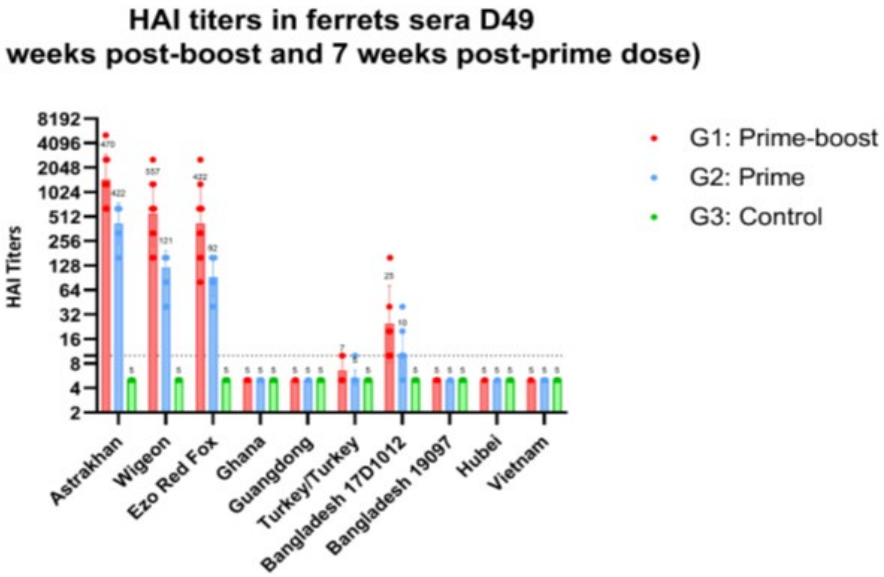
765-N106857	Challenge with wild-type virus homologous and heterologous to the vaccine strain	Ferret	Aflunov (Vietnam) and Aflunov (Turkey)
673-N106850	Challenge with homologous wild-type virus	Ferret	Aflunov (Vietnam)
CBI-PCS-008 & VIV-PCS-001	Challenge with homologous reverse genetics virus	Ferret	Aflunov (Vietnam)

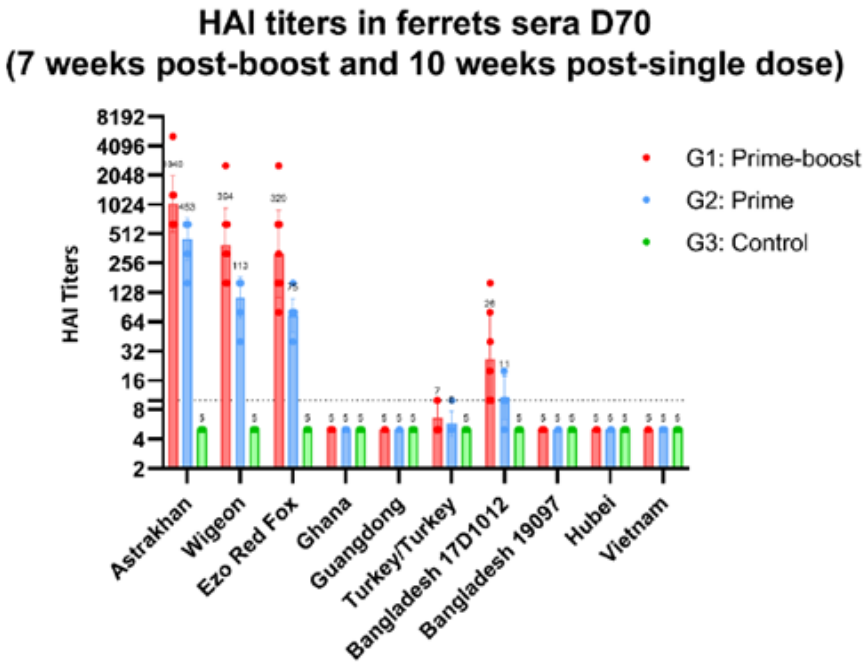
While results from study 765-N106857 are reported in section 5.1 of the SmPC, results from studies 673-N106850 and CBI-PCS-008 & VIV-PCS-001 seem not to be reflected (e.g., in the initial dossier no Indonesia vaccine strain was used). The MAH should revise sub-section "Information from non-clinical studies" in section 5.1 of the SmPC reflecting results from the 2 missing ferret studies, indicating the doses used (7.5 micrograms HA and 7.5 or 15 micrograms in studies 673-N106850 and CBI-PCS-008 & VIV-PCS-001, respectively). In addition, a summary of results from H5N8 Ferret Immunogenicity Study LC-07, #0154-23, should be added in the same sub-section in SmPC section 5.1.

With Step 2 submission, the MAH also provided an addendum of the ferret study *LC-07, #0154-23* named "Homologous and extended heterologous serological testing" in which sera of ferrets vaccinated with 12.5 µg zoonotic influenza vaccine CBER-RG8A A/Astrakhan/3212/2020 in a single or 2-dose vaccination schedule were tested against a set of further pseudoviruses (see below) epidemiologically representative of H5 viruses circulating around the globe such as 2.3.4.4b, 2.3.4.4h, 2.3.2.1a, and 2.3.2.1c.
Serum antibody levels were quantified by HAI assays after vaccination. A similar HAI methodology, as described in the previous report, was followed.

Pseudovirus antigen	Clade
HIV-1-H5N8-M2 Astrakhan UC purified ELN074.Exp038 11172023.	2.3.4.4b
HIV-1 H5N1-M2 IDCDC-RG78 UC purified ELN074.Exp004 04/07/2023	2.3.4.4b
HIV-1 PS A/Ezo red Fox/Hokkaido/1/2022 H5N1-M2 SU132103.ELN074.Exp035.KS11082023	2.3.4.4b
HIV-1 A/chicken/Ghana/AVL-76321VIR7050-39/2021 H5N1-M2 SU132103.ELN074.Exp039	2.3.4.4b
HIV-1 PS A/Guangdong/ 18SF020/2018 H5N6-M2 SU132103.ELN074.Exp037.KS11132023	2.3.4.4h
HIV-1 H5N1 Turkey/Turkey UC purified ELN064.Exp072	2.2.1
HIV-1 PS A/duck/Bangladesh/17D1012/2018 H5N1-M2 SU132103.ELN074.Exp038. KS11172023	2.3.2.1a
HIV-1 PS A/duck/Bangladesh/19097/2013 H5N1-M2 SU132103.ELN074.Exp036. KS11102023	2.3.2.1a
HIV-1 PS A/Hubei/1/2010 H5N1-M2 SU132103. ELN074.Exp037 KS11132023	2.3.2.1a
HIV-1 PS A/duck/Vietnam/NCVD-1584/2012 H5N1-M2 SU132103.ELN074.Exp036 KS11102023	2.3.2.1c

Results

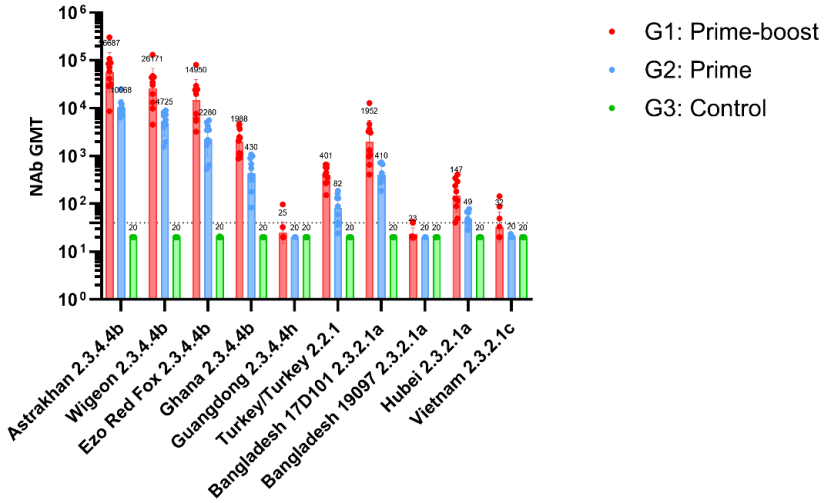




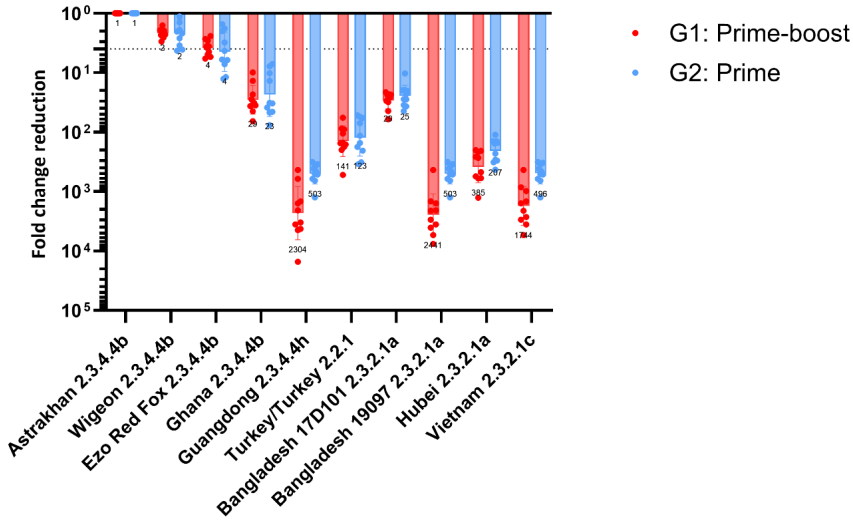
Administration of a booster dose 3 weeks after a prime dose increased the HAI GMT against the homologous antigen by a factor of 3.5 compared to the single-dose group (1:1470 vs 1:422). A similar pattern was observed with the closely related A/American wigeon/South Carolina/22-000345-001/2021 (H5N1) (GMT of 1:557 post-boost vs 1:121 post-single dose) and A/Ezo red Fox/Hokkaido/1/2022 H5N1 (GMT of 1:422 post-boost vs 1:92 post-single dose) strains. Titers were lower for the heterologous strains tested within the 2.3.4.4 subclade and in the case of (A/chicken/Ghana/AVL-763_21VIR7050-39/2021 H5N1), no cross-reactivity was detected (GMT <1:10). Sequence analysis of the HA showed a substitution at position A156T between the Astrakhan and Chicken/Ghana strains. An A156T amino acid change can potentially introduce an N-glycosylation site at position 154, preventing the binding of neutralizing antibodies. No significant titers were observed when the distantly related antigens A/turkey/Turkey/1/2005 (H5N1), A/duck/Bangladesh/19097/2013 (H5N1), A/duck/Vietnam/NCVD-1584/2012 (H5N1), A/Guangdong/18SF020/2018 (H5N6) 2.3.4.4h and A/Hubei/1/2010 (H5N1) 2.3.2.1a were utilized as antigens (GMT <1:10). This lack of reactivity might be due to substantial substitutions in the antigenic and receptor binding sites when compared to the vaccine antigen A/Astrakhan/3212/2020 H5N8. Several amino acid changes were observed for the strains outside clade 2.3.4.4b at different antigenic sites; consequently, no significant cross-reactivity was observed when the post-vaccination ferret sera were tested against PVs within the clade ***2.3.2.1a*** and no cross-reactivity within the clade **2.3.4.4h**, **2.3.2.1c** and **2.2.1.**

NEUTRALIZING ANTIBODY (NAB) TITERS POST-BOOST AND POST-PRIME DOSE

NAb titers 4 weeks post-boost and 7 weeks post-prime dose (D49)

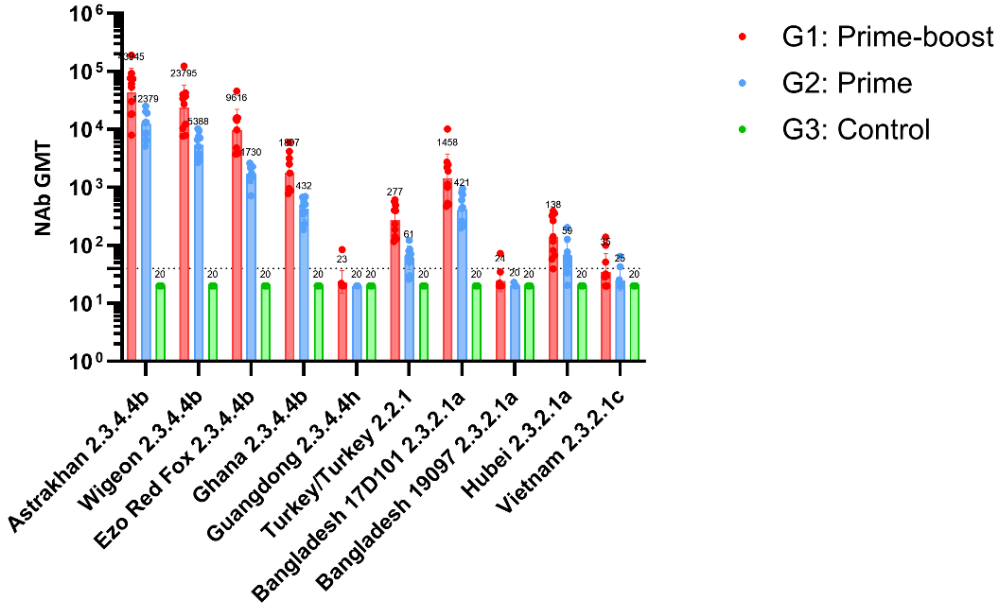


NAb titers fold change reduction over Astrakhan (D49)

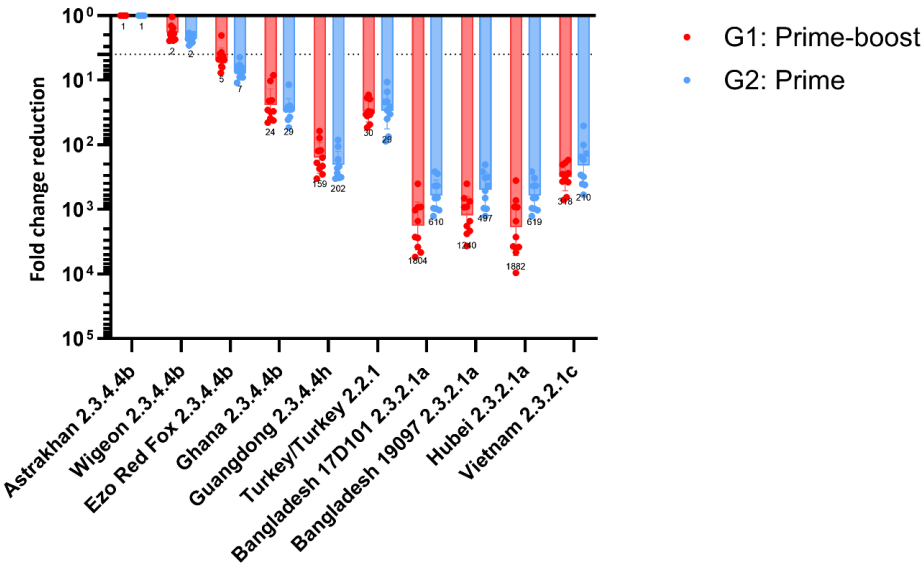


PERSISTENCE OF NAB TITERS POST-PRIME AND BOOST-DOSE

NAb titers 7 weeks post-boost and 10 weeks post-prime dose (D70)



NAb Fold change reduction over Astrakhan (D70)



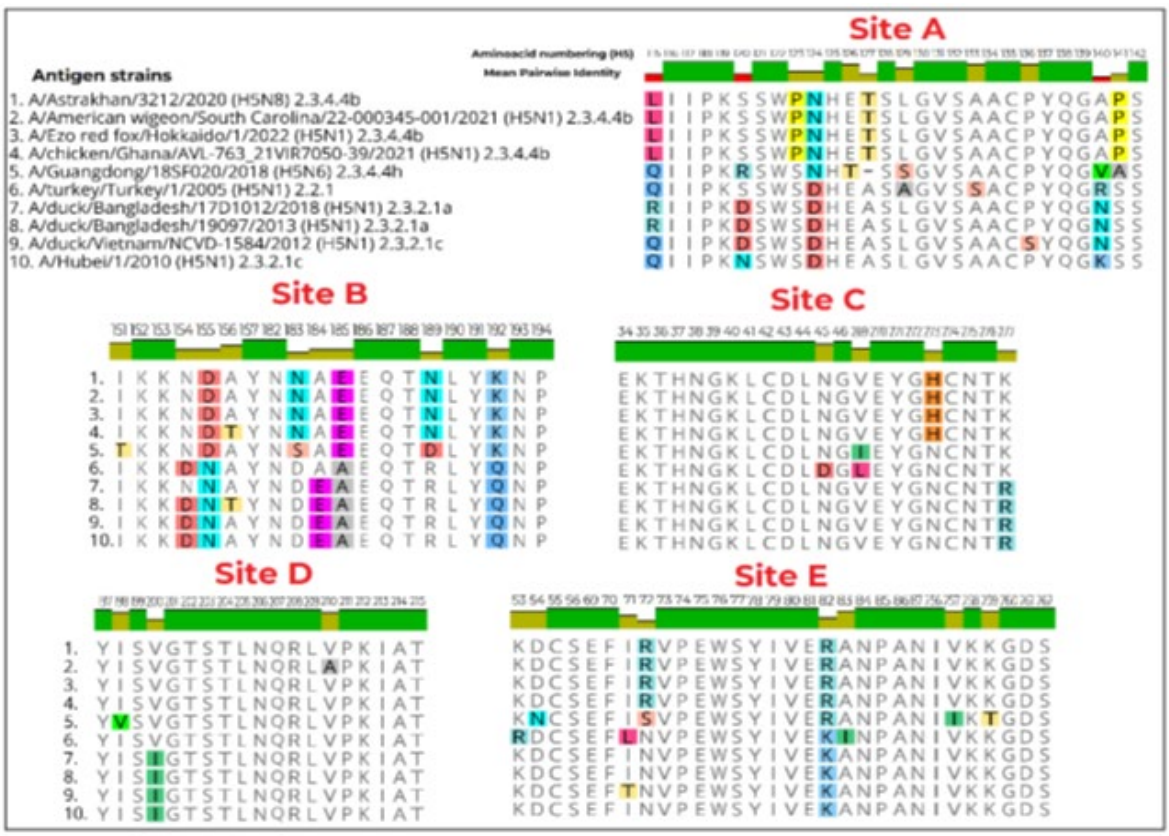
A single-dose vaccination induced significant levels of NAb titers against the homologous (A/Astrakhan/3212/2020 H5N8) and heterologous PVs (A/American wigeon/South Carolina/22-000345-001/2021 H5N1 and A/Ezo red Fox/Hokkaido/1/2022) with GMT of 10068, 4725, and 2280 ten weeks post-single dose and GMT of 56687, 26171, and 14950 three weeks post-boost dose, respectively. Titers were sustained 10 weeks post-prime (12379, 5388, and 1730) and 7 weeks post-boost dose (43945, 23795, and 9616), respectively.

Cross-reactivity was correlated with the amino acid similarity in the HA antigenic sites among the 3 strains within the 2.3.4.4b clade. Interestingly, reduced cross-reactivity of at least 24-fold reduction was observed against the heterologous strain within the same subclade A/chicken/Ghana/AVL-763_21VIR7050-39/20212 (H5N1). This is in line with HAI results for which no cross-reactivity was detected (GMT <1:10). Sequence analysis of the HA showed a substitution at position A156T between the Astrakhan and Chicken/Ghana strains. A156T amino acid change can potentially introduce an N-

glycosylation site at position 154, preventing the binding of neutralizing antibodies raised against the Astrakhan antigen to the Chicken/Ghana HA.
The HAI assay relies on the ability of hemagglutinin-specific antibodies to inhibit the binding between the HA of the virus and the sialic acid receptors on the surface of red blood cells. HAI assays thus quantifies antibodies which can prevent virus-induced agglutination of red blood cells. On the other hand, neutralization assays measure functional antibodies that inhibit viral entry by blocking receptor and non-receptor binding sites and have been described of being more sensitive for the detection of NAb.
Considering NAb titers against additional H5 strains outside the 2.3.4.4b clade, reduced cross-reactivity was observed when the antigens A/turkey/Turkey/1/2005 (H5N1) and A/Hubei/1/2010 (H5N1) were utilized. Cross reactivity testing against A/duck/Bangladesh/19097/2013 (H5N1), A/duck/Vietnam/NCVD-1584/2012 (H5N1), and A/Guangdong/18SF020/2018 (H5N6) was below the limit of detection for most samples.
Reduced or lack of cross reactivity might be due to substantial substitutions in the antigenic and receptor binding sites when compared to the vaccine antigen A/Astrakhan/3212/2020 H5N8.

Comparison of the HA sequence against the Astrakhan strain showed amino acid changes at positions L108M and V214A for Wigeon and A156T for Chicken/Ghana in the HA1 region. The percentage of amino acid similarity of the HAs within the 2.3.4.4b strains ranged from 99.47% to 99.82%. Several amino acid changes were observed when the 2.3.4.4h, 2.3.2.1a, 2.3.2.1c and 2.2.1 strains were compared with the Astrakhan HA sequence, and the percentage of identity ranged from 91.67% to 93.62%. Analyses of the antigenic regions showed substitution of A156T for Chicken/ Ghana at antigenic site B. Several amino acid changes at different antigenic sites, mostly at sites A and B, were observed when the 2.3.4.4h, 2.3.2.1a, 2.3.2.1c and 2.2.1 strains were compared to the Astrakhan HA.

Comparison of the Amino acid changes within the antigenic regions among H5Nx strain used as antigens



HI and MN data demonstrate that the monovalent clade 2.3.4.4b zoonotic influenza vaccine Seqirus CBER-RG8A A/Astrakhan/3212/2020 (H5N8) is immunogenic in ferrets against homologous strain and potentially protects against the majority of heterologous H5 strains within the clade 2.3.4.4b of the vaccine.

No cross-reactivity was observed against H5 strains outside the 2.3.4.4b clade, possibly due to several amino acid changes observed for the strains outside clade 2.3.4.4b at different antigenic sites.

8. Clinical aspects

With regards to the present strain update variation, the MAH is proposing a new wording of indication:

Present	**Proposed**
"Zoonotic Influenza Vaccine Seqirus" based on A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) (clade 2.2.1)	"Zoonotic Influenza Vaccine Seqirus" based on A/Astrakhan/3212/2020 (H5N8)-like strain (CBER-RG8A) (clade 2.3.4.4b)
4.1 Therapeutic indications Active immunisation against **H5N1** subtype of Influenza A virus. This indication is based on immunogenicity data from healthy subjects from the age of 18 years onwards following administration of two doses of the vaccine containing H5N1 subtype strain (see sections 4.4 and 5.1). Zoonotic Influenza Vaccine Seqirus should be used in accordance with official recommendations.	**4.1 Therapeutic indications** Active immunisation of adults against **H5** subtype influenza A viruse**s** (see section 5.1). The vaccine should be used in accordance with official recommendations.

As previously agreed with the EMA/ETF, the MAH did not submit any clinical data within the dossier: supportive clinical data generated by trials using cell-based zoonotic H5N8 vaccines, will be provided post-approval.

In the SmPC the MAH proposes a revised and "broader" version of the indication than that approved for Aflunov/"Zoonotic Influenza Vaccine Seqirus", suggesting a wording that defines influenza A virus based solely on HA and deleting reference to NA. The reasons put forward by the MAH are the following:

- the vaccine is intended to protect against H5 viruses antigenically similar to H5N8/Astrakhan (clade 2.3.4.4b). Stating instead that the vaccine is indicated for active immunisation against H5N8 virus specifically, may unintentionally restrict use, or cause confusion;

- this interpretation seems supported by the H5 HA clade nomenclature as followed by WHO/The World Organisation for Animal Health (OIE)/The Food and Agriculture Organization of the United Nations (FAO) H5 Working Group, which is based on analysis of the H5 sequence data, regardless of the NA subtype;

- the proposed wording of indication would also be similar to the language used for seasonal influenza vaccines, which does not include specific strains as part of the indication;

- since the important change in the vaccine composition is the introduction of a H5 antigen from a clade matching current circulating avian strains independently from the NA component, the reference to N8 is considered not relevant;

- cross reference to section 5.1 in which it is stated that no clinical data exist with Zoonotic Influenza Vaccine Seqirus A/Astrakhan/3212/2020 (H5N8)-like strain (CBER-RG8A) of clade 2.3.4.4b, would clarify that the available clinical immunogenicity data supporting the use of the zoonotic vaccine, were generated by H5N1 vaccine virus strains. Cross-reference to the above sentence in section 5.1 and the specification on the clades in section 2 of the SmPC, are considered sufficient to lead the public health authorities on which of the two co-existing zoonotic vaccines (i.e. H5N8 and Aflunov H5N1), to be used after a careful assessment of the epidemiological situation.

The MAH's proposal was discussed by the EMA/ETF and considered in principle acceptable, however, pending assessment of data at submission of the dossier.

The WHO Global Influenza Surveillance and Response System (GISRS), in collaboration with animal health and veterinary sector colleagues, regularly evaluate CVV and publish development and availability status of A(H5) non–A(H5N1) candidate vaccine viruses (h5-non-h5n1_cvv_20220225.pdf (who.int)). Clade 2.3.4.4b A(H5) CVV based on H5N8 A/Astrakhan/3212/2020 antigenic prototype, have been developed, and the HA of A/Astrakhan/3212/2020 is considered the most closely related to the HPAI A(H5N1) currently circulating strains.

Guideline on Influenza Vaccines - Non-clinical and Clinical Module (EMA/CHMP/VWP/457259/2014) does not exactly advise on the requirements needed for an application of change on strain composition in which HA remains stable (H5) and NA is changed. As discussed during the EMA ITF meeting and with the ETF, provided that the HA subtype does not change from the original registered HA subtype, submission of manufacturing and quality data related to the new strain should be sufficient for the zoonotic strain change. Indeed, no immunogenicity data in humans obtained with A/Astrakhan/3212/2020 (H5N8)-like strain (CBER-RG8A) (clade 2.3.4.4b) vaccine are available and the MAH's intention is not to generate post-approval clinical data with the same egg-based vaccine.

The MAH is proposing a revised and "broader" version of the indication than that approved for Aflunov/"Zoonotic Influenza Vaccine Seqirus", suggesting a wording that defines influenza A virus based solely on HA and deleting reference to NA. In respect to baseline, in the ferret study submitted within this variation, a relevant and durable immune response against the homologous H5N8 strain and a heterologous strain (H5N1) belonging to the same 2.3.4.4b clade is shown, suggesting that the updated vaccine is able to elicit immune response against H5 viruses of clade 2.3.4.4b. However, concerning the MAH's claimed indication specifying use of "Zoonotic influenza vaccine Seqirus" for active immunisation against any H5 influenza A viruses some issues are considered:

- although vaccine NA content is not controlled and NA immunogenicity in vaccinated subjects not measured, it is known that the contribution of NA specific antibodies to vaccine immunity is relevant particularly in protecting against heterologous viruses, since NA specific antibodies bind to epitope domains that are well conserved within a virus subtype (Eichelberger M et al., Cur Opin Imm 2018). Moreover, the impact of different NA on the vaccine safety is currently unknown.

- in the ferret study submitted in the present variation, H5N8 vaccine failed at eliciting a significant immune response against the heterologous H5N1 strain A/turkey/Turkey/1/2005 of 2.2.1 clade. As highlighted by the MAH, lack of reactivity might be due to substantial substitutions in the HA and receptor binding sites of the two strains. This finding is poorly relevant since this subtype is not circulating any longer.

- in a recent study (Neuzil et al., 2023) using MF59-adjuvanted, split A/gyrfalcon/Washington/41088-6/2014 (H5N8) (clade 2.3.4.4c) vaccine, sera of the vaccinees cross-reacted with all the tested subtypes with the clade of concern 2.3.4.4b (i.e., human H5N8 A/Astrakhan, avian H5N8 A/Chicken/Chelabinski, human H5N6 A/Fujian-Sanyuan strains) but the lowest HAI titers were obtained with the latter in which NA was different.

- cross-reactivity clinical data obtained with A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG 23) (clade 2.2.1) vaccine demonstrated a lower heterologous antibody response against the Vietnam strain than against the homologous Turkey strain, and responses to the Anhui/5/2005 CC Ab (clade 2.3.4) strain, the closest strain to clade 2.3.3.4b, were lower than the responses to the Vietnam strain (Seqirus studies V87_25 and V87_26). The same trend has been observed when using the cell-based/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG 23) (clade 2.2.1) vaccine version (Celldemic – studies V89_04 and V89_13). This means that a lower humoral immune response with limited cross-reactivity reaction against different clades of H5 viruses can be found. (Note: these data were shown by the MAH during the ETF meeting in March 2023 to support the need to update the zoonotic strain to match the circulating clade).

In conclusion, in the absence of clinical data, results from ferret study are considered relevant to the definition of clinical efficacy of the "Zoonotic influenza vaccine Seqirus" and support the proposed wording of indication. However, some concerns were raised regarding the potential cross-reactivity against former H5N1 circulating clades and not tested other influenza A(H5) currently circulating clades (e.g., 2.3.2.1).

To guide an appropriate use of the vaccine according to subtype clade, cross reference to SmPC section 4.4 have been added in the indication and the existing sub-section "Cross-reactivity immunity" has been amended as proposed below:

-4.1: Zoonotic Influenza Vaccine Seqirus H5N8 is indicated for active immunisation against H5 subtype influenza A viruses in adults 18 years of age and above (see sections 4.4 and 5.1).

The use of this vaccine should be in accordance with official recommendations.

-4.4: Cross-reactive immunity

There are no clinical cross reactivity data with the Zoonotic Influenza Vaccine Seqirus H5N8. The degree of immune response that may be elicited to influenza A(H5) viruses of subtypes or clades different to that of the vaccine strain Zoonotic Influenza Vaccine Seqirus H5N8, is unknown (see section 5.1 Information from nonclinical studies).

Regarding what anticipated during interactions with the EMA/ETF, the MAH will provide supportive clinical post-approval data:

-Immunogenicity and safety data from the US BARDA funded study (NCT05874713, V205_01) in adults and elderly receiving 2 doses of MF59-adjuvanted H5N8c vaccine as well as heterologous A/Guangdong/18SF020/2018 (clade 2.3.4.4h) H5N6c vaccine. In order to the transferability of the immunogenicity data from cell-based to egg-based vaccines, consideration should be given to whether antigenicity equivalence is confirmed between the working seeds of the 2 vaccines (cell and egg based).

-Immunogenicity and safety data from the extension study NCT05422326, V89_18E1 (also requested as post-approval measure for Celldemic cell-based vaccine). The study investigates whether 2 priming doses of MF59-adjuvanted H5N1 cell culture-derived vaccine (H5N1c) followed by 1 or 2 booster

vaccinations with a cell-based MF59-adjuvanted A/Guangdong/18SF020/2018 H5N6c (clade 2.3.4.4h) vaccine, 3 weeks apart elicit immune responses to the antigens used for priming (H5N1) and boosting (H5N6) after first and second heterologous booster vaccination. In order to the transferability of the immunogenicity data from cell-based to egg-based vaccines, consideration should be given to whether antigenicity equivalence is confirmed between the working seeds of the 2 vaccines (cell and egg based).

9. Changes to the Product Information

As a result of this variation, several sections of the SmPC are being updated. Annex A, Labelling and Package Leaflet (PL) are updated accordingly.

A mix of information coming from H5N1 vaccine (Aflunov: A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) (clade 2.2.1) + A/Vietnam/1194/2004 (H5N1) (clade 1) from which the Zoonotic Influenza Vaccine Seqirus PI stemmed, and from H5N8 vaccine (that are only a minority since no clinical data were generated), is reported. Since the EPARs of Aflunov and Zoonotic Influenza Vaccine Seqirus remain available, a simplification of the Zoonotic Influenza Vaccine Seqirus text has been done focussing on information relevant to the use of the H5N8 vaccine.

Rerer to Attachment 1 for full details of the changes to the Product Information as adopted by the CHMP on 21 March 2024.

9.1.1. Additional monitoring

Although a biological product, Aflunov has never been applied additional monitoring since approved in 2010 before additional monitoring has been introduced (January 2011). At the time of CHMP positive opinion in Sept 2023 for the Aflunov duplicate Zoonotic influenza vaccine Seqirus H5N8, no additional monitoring was applied. However, no relevant EU Aflunov post-marketing experience is available due to the sporadic use of Aflunov since its approval.

In the guideline on Influenza vaccine – Non Clinical and Clinical Module it is reported: "In this guideline the term new vaccine refers to a new medicinal product which requires a stand-alone marketing authorisation. New vaccines include those which are similar to an existing vaccine in terms of the types of antigens and anticipated interaction with the immune system (e.g. quadrivalent inactivated influenza vaccines that are manufactured similarly to trivalent inactivated vaccines). They also include vaccines that include a novel construct or approach (e.g. influenza vaccines based on a single conserved viral protein)."

The current variation regards a strain change for "Zoonotic influenza vaccine Seqirus" within the same marketing authorisation, similar to the flu seasonal vaccines for which no additional monitoring is routinely applied due to the post-marketing experience collected from the previous vaccine campaign. However, "Zoonotic influenza vaccine Seqirus" is a zoonotic vaccine indicated for the active immunisation against HPAI virus with pandemic potential and no clinical information has been generated so far on the specific CVV subtype clade newly introduced, thus an additional monitoring could be recommended. However, since additional monitoring aims to enhance reporting of suspected adverse drug reactions collecting information as early as possible when used in everyday medical practice, as a matter of fact it is not applicable to this zoonotic vaccine which is intended for immunisation during outbreaks. Indeed, among the "other conditions and requirements of the marketing authorisation" in the Annex II several obligations are set for during a pandemic situation which encompass those foreseen by the additional monitoring and include further surveillance activities. Among them, during interactions with the EMA/ETF the MAH committed to apply the Enhanced Passive Safety Surveillance (EPSS) when at least one Member State implements vaccination for a sufficient number of individuals. This will be an adapted seasonal EPSS approach (safety surveillance information and call-in contacts) to ensure an early and rapid

monitoring of the reactogenicity of the "Zoonotic Astrakhan " vaccine. If the EPSS is confirmed by the and agreed by the PRAC in a distinct procedure (not in the scope of the current variation), this measure, together with the submission of frequent simplified PSURs and in case of pandemic situation, is considered a valid alternative to additional monitoring also in view of the fact that the use of this vaccine will be managed by national health systems.

10. Requests for supplementary information

10.1. *Clinical aspects*

During the procedure (non)-Clinical questions raised in requests for supplementary information were resolved.

10.2. Quality Aspects

During the procedure Quality questions raised in requests for supplementary information were resolved.

REFERENCES

Eichelberger M. et al. "Neuraminidase as an influenza vaccine antigen: a low hanging fruit, ready for picking to improve vaccine effectiveness". *Current Opinion in Immunology*, 2018, 53:38–44

Neuzil Kathleen M. et al. "Safety and Immunogenicity of Influenza A/H5N8 Virus Vaccine in Healthy Adults: Durability and Cross-reactivity of Antibody Responses". *Clinical Infectious Diseases* – 2023, Jan 5.

Xie R. et al. "The episodic resurgence of highly pathogenic avian influenza H5 virus". *Nature*, Vol 622, 26 October 2023

Venkatesan P. et al. "Avian influenza spillover into mammals". *Lancet Microbe* - Vol 4 July 2023

11. Recommended conditions for marketing authorisation and product information in case of a positive opinion

11.1. Proposed list of post-authorisation measures

In the context of the obligation of the Marketing authorisation holder (MAH) to take due account of technical and scientific progress, measures related to quality and clinical aspects are recommended for further investigation. Details of these recommendations have been redacted from this version of the assessment report.