Influenza Reagent
Influenza Virus Infectious IVR-241 (A/Puerto Rico/31/2022) (H3N2)
NIBSC code: 23/246
Instructions for use
(Version 1.0, Dated 24/01/2024)

1. INTENDED USE
Reagent 23/246 was prepared from IVR-241 (H3N1), a reassortant of A/Puerto Rico/31/2022 (H3N2) and A/PR/8/34 (H1N1), which was processed in 250μl volumes as liquid stock. The derivation and known passage history of 23/246 are attached.

2. CAUTION
This preparation is not for administration to humans or animals in the human food chain.
The material is not of human or bovine origin. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory’s safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE
No unitage is assigned to this material.

4. CONTENTS
Country of origin of biological material: United Kingdom.
Each ampoule contains 250μl (nominal) of infectious influenza virus as allantoic fluid from SPF embryonated hen’s eggs.

5. STORAGE
Store in the dark at -70°C or below.
Material type: Liquid – will be shipped according to the storage and shipping conditions of the product

6. DIRECTIONS FOR OPENING
Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: If a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

7. USE OF MATERIAL
Ready to use.

8. STABILITY
Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES
N/A.

10. ACKNOWLEDGEMENTS
N/A

11. FURTHER INFORMATION
Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK
Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION
In all publications, including data sheets, in which this material is referenced, it is important that the preparation’s title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET
Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance:</td>
</tr>
<tr>
<td>Clear liquid</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
</tr>
<tr>
<td>Flammable: No</td>
</tr>
<tr>
<td>Other (specify): Live influenza virus</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation:</td>
</tr>
<tr>
<td>Effects of ingestion:</td>
</tr>
<tr>
<td>Effects of skin absorption:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggested First Aid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation:</td>
</tr>
<tr>
<td>Ingestion:</td>
</tr>
<tr>
<td>Contact with eyes:</td>
</tr>
<tr>
<td>Contact with skin:</td>
</tr>
</tbody>
</table>
Action on Spillage and Method of Disposal

Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate virucidal agent. Rinse area with an appropriate virucidal agent followed by water. Absorbent materials used to treat spillage should be treated as biological waste.

15. LIABILITY AND LOSS
In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents. Unless expressly stated otherwise by NIBSC, NIBSC’s Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) (“Conditions”) apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient’s attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*</th>
<th>United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net weight</td>
<td>0.25g per vial</td>
</tr>
<tr>
<td>Toxicity Statement</td>
<td>Non-toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable</td>
<td></td>
</tr>
<tr>
<td>Attached</td>
<td>No</td>
</tr>
</tbody>
</table>

Passage history of IVR-241 (H3N2)

<table>
<thead>
<tr>
<th>Cumulative number of passages</th>
<th>Passage numbers at each stage</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3</td>
<td>E3</td>
<td>3030726546</td>
<td>CDC, USA</td>
</tr>
<tr>
<td>E9</td>
<td>E3/E6</td>
<td>Lot 630B</td>
<td>Seqirus, Australia</td>
</tr>
<tr>
<td>E10</td>
<td>E3/E6/E1</td>
<td>47960’</td>
<td>MHRA, UK</td>
</tr>
</tbody>
</table>

* The HA titre of this virus using 0.7% Guinea pig red blood cells is 512. The infectious titre is unknown.

Sterility: No visible contamination was detected in a variety of media (tryptone soya broth, thioglycolate broth, Sabouraud’s broth and blood agar plates) after 14 days incubation.

The HA and NA sequence of this virus are available at GISAID with the accession number EPI_ISL_18516475.
Derivation of IVR-241  
*A/Puerto Rico/31/2022 – like High Growth Reassortant*

A/Puerto Rico/31/2022 (IVR-241) is a H3N2 high growth reassortant influenza virus.

**PREPARATION**
The preparation of A/Puerto Rico/31/2022 (IVR-241) high growth reassortant influenza virus was conducted in Vaccine and PNS Development at CSL Seqirus.

The high yielding parent strain used was A/Puerto Rico/8/34.

**MATERIALS**
The following materials of biological origin were used during the preparation of high growth reassortant IVR-241:

**Virus Isolate:**
The virus isolate was obtained from CDC, Atlanta via Seqirus Holly Springs.

Supply details are:
A/Puerto Rico/31/2022  
CDC ID number: 3030726546  
Passages prior to receipt at Seqirus: 3

**Eggs:**
Specific Pathogen Free (SPF) eggs were used for all passages at CSL Seqirus.

**Antiserum:**
Trypsin-periodate treated sheep hyperimmune antiserum Lot# AS367, Sub-lot #5053 raised against influenza virus A/Puerto Rico/8/34.

The antiserum was derived from sheep born and raised in Australia.

*Note on Transmissible Spongiform Encephalopathies (TSEs):*
Australia and New Zealand have been declared TSE free in accordance with OIE guidelines. Detailed information on Australia’s animal health status can be obtained from the following Animal Health Australia website link: [https://animal.health.australia.com.au](https://animal.health.australia.com.au)

The trypsin used is 10x solution of gamma irradiated porcine pancreatic trypsin;  
Gibco Cat #15090046, Manufacturers Lot No. 2317956.
PASSAGE HISTORY:

Mixed infection passage (E3/D1) A/Puerto Rico/31/2022 wild type virus @ $10^4 \times A/Puerto Rico/8/34$ (H1N1) @ $10^7$

↓

1<sup>st</sup> Antiserum Passage (E3/D2) Inoculum @ $10^{-3}$ with antisera to A/Puerto Rico/8/34 (H1N1)

HA titre = 520

↓

2<sup>nd</sup> Antiserum Passage (E3/D3) Inoculum @ $10^{-3}$ with antisera to A/Puerto Rico/8/34 (H1N1)

HA titre $\geq$ 1576

↓

3<sup>rd</sup> Antiserum/1<sup>st</sup> Limit Dilution Passage (E3/D4) Inoculum @ $10^{-7}$

HA titre $\geq$ 1689

↓

2<sup>nd</sup> Limit Dilution Passage (E3/D5) Inoculum @ $10^{-9}$

HA titre $\geq$ 1810

↓

3<sup>rd</sup> Limit Dilution Passage Lot 630B (E3/D6) Inoculum @ $10^{-5}$

Mean HA titre $\geq$ 1689

IVR-241

** Virus sample diluted to $10^{-3}$, dilution was mixed with antisera to A/Puerto Rico/8/34 (H1N1) and incubated for 1 hour at room temperature. Incubated virus/antisera sample was serially diluted and inoculated into eggs.

Total number of passages post mixed infection = 5
Total number of passages since this virus was received from an approved laboratory = 6

HA titres were determined using guinea pig red blood cells at room temperature.
### TESTING OF A/PUERTO RICO/31/2022 INFLUENZA VIRUS (IVR-241)

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (by real-time RT-PCR)</td>
<td>2:6 (A/Puerto Rico/8/34 : A/Puerto Rico/31/2022) Reassortant A/Puerto Rico/31/2022 (wild type virus) genes H3, N2 genes were detected. A/Puerto Rico/8/34 genes PA and M were detected. NP, NS, PB1 and PB2 genes from A/Puerto Rico/8/34 were not detected, indicating that the reassortant NP, NS, PB1 and PB2 genes are from A/Puerto Rico/31/2022 (wild type virus).</td>
</tr>
<tr>
<td>Gene</td>
<td>A/Puerto Rico/8/34</td>
</tr>
<tr>
<td>------</td>
<td>--------------------</td>
</tr>
<tr>
<td>H3</td>
<td>✓</td>
</tr>
<tr>
<td>N2</td>
<td>✓</td>
</tr>
<tr>
<td>H1</td>
<td>X</td>
</tr>
<tr>
<td>N1</td>
<td>X</td>
</tr>
<tr>
<td>PB1</td>
<td>X</td>
</tr>
<tr>
<td>PB2</td>
<td>X</td>
</tr>
<tr>
<td>PA</td>
<td>✓</td>
</tr>
<tr>
<td>NP</td>
<td>X</td>
</tr>
<tr>
<td>M</td>
<td>✓</td>
</tr>
<tr>
<td>NS</td>
<td>X</td>
</tr>
</tbody>
</table>

✓ - positive by PCR  
X - negative by PCR  
NT - Not Tested

### Disclaimer:
The material i.e. high growth reassortant virus IVR-241 and the information provided in this derivation report are provided on an "as is" basis and as such without any warranty or representation of any kind (expressed or implied) including, without limitation, of satisfactory quality or fitness for a particular purpose.
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