Influenza Reagent
Influenza Virus Infectious NYMC X-393A
(A/West Virginia/30/2022) (H1N1)
NIBSC code: 23/154
Instructions for use
(Version 1.0, Dated 21/07/2023)

1. INTENDED USE
Reagent 23/154 was prepared from NYMC X-393A (H1N1), a reassortant of A/West Virginia/30/2022 (H1N1) and X-157 (H3N2), which was processed in 250µl volumes as liquid stock. The derivation and known passage history of 23/154 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 chicken eggs.

5. STORAGE
Store in the dark at -70°C or below.

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 should be stored on receipt as indicated on the label.
NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES
NA

10. ACKNOWLEDGEMENTS
NA

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: Clear liquid</td>
</tr>
<tr>
<td>Corrosive: No</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: Likelihood of influenza virus infection</td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggested First Aid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation: Seek medical advice</td>
</tr>
<tr>
<td>Ingestion: Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Contact with skin: Wash thoroughly with water</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Action on Spillage and Method of Disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spillage of 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 biologically hazardous waste</td>
</tr>
</tbody>
</table>

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*: United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.</td>
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</table>

<table>
<thead>
<tr>
<th>Net weight: 0.25g per ampoule</th>
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<tbody>
<tr>
<td>Toxicity Statement: Non-toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable.</td>
</tr>
<tr>
<td>Attached: No</td>
</tr>
</tbody>
</table>

attached:

Passage history of NYMC X-393A (H1N1)

<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>3030726621</td>
<td>CDC, USA</td>
</tr>
<tr>
<td>E12</td>
<td>E3/E9</td>
<td>E#6552</td>
<td>NYMC, USA</td>
</tr>
<tr>
<td>E13</td>
<td>E3/E9/E1</td>
<td>47650*</td>
<td>MHRA, UK</td>
</tr>
</tbody>
</table>

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

Sterility: No visible contamination was detected in a variety of media (tryptose 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_17980707.
Derivation of NYMC X-393A
A/West Virginia/30/2022 (H1N1pdm) with NYMC X-157 CL-3
High Yield A H1N1pdm Reassortant (6:2)
with A/PR/8/34 M, PB1, PB2, PA, NS and NP genes and
A/West Virginia/30/2022 HA and NA genes

Exper. # 4896 I
A/West Virginia/30/2022, H1N1pdm
CDC 3030726621
Spf 3
HA: 256

Passages at New York Medical College

Passage No.   1

10^{-1}
HA — 1:128

A/West Virginia/30/2022 (H1N1pdm) x NYMC X-157 CL-3

2

10^{-4} + 10^{-2}
HA — 1:64

3

10^{-1}
HA — 0
+ X-157 HANA antisera (as)
X-157 HANA antibodies (ab)

4

10^{-1}
HA-1:1024
+ X-157 HANA antisera (as)
X-157 HANA antibodies (ab)

5

10^{-3}
HA — 512
+ X-157 HANA antisera (as)
X-157 HANA antibodies (ab)

6

10^{-4}
HA — 1:256

7

10^{-8}
HA — 1:1024
HA Yield by UPLC Analysis (µg HA/ml allantoic fluid)

<table>
<thead>
<tr>
<th></th>
<th>wt (wild type)</th>
<th>X-393A</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.1</td>
<td>5.6</td>
<td>1.8</td>
</tr>
</tbody>
</table>

HA and NA genes were identified as A/West Virginia/30/2022 by RT-PCR/RFLP gene analysis. The M, PB1, PB2, PA, NS and NP genes were identified as A/PR/8/34 by RT-PCR/RFLP analysis.

SPF eggs were used for all reassortant passages.

Virus seed was shown to be sterile. Sterility testing was performed by streaking the sample on blood agar plates and incubating for 48 hours at 37 °C.

All titers performed with chicken red blood cells.