Influenza Reagent
Influenza virus infectious NYMC X-341+1P (H3N2)
NIBSC code: 20/264
Instructions for use
(Version 4.0, Dated 22/03/2021)

1. INTENDED USE
Reagent 20/264 is prepared from X-341 + 1P (H3N2) which was processed in 250μl volumes as liquid stock. The derivation and known passage history of X-341 + 1P (H3N2) is 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 vial 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

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/

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>Flammable: No</td>
</tr>
<tr>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
</tr>
<tr>
<td>Irritant: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Toxicological properties</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>

Suggested First Aid

Inhalation: Seek medical advice

Ingestion: Seek medical advice

Contact with eyes: Wash with copious amounts of water. Seek medical advice

Contact with skin: Wash thoroughly with water.

Action on Spillage and Method of Disposal

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.

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.

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Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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
16. INFORMATION FOR CUSTOMS USE ONLY

<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>E1-E7</td>
<td>E7</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>E8-E17</td>
<td>E7/E10</td>
<td>E#6441</td>
<td>NYMC, USA</td>
</tr>
<tr>
<td>E18</td>
<td>E7/E10/E1</td>
<td>45780</td>
<td>NIBSC, UK</td>
</tr>
</tbody>
</table>

Passage history of X-341+1P(H3N2)

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 is available at GISAID with the accession number EPI_ISL_882956.
Derivation of NYMC X-341 +1P
A/Hong Kong/2671/2019 (H3N2) with A/PR/8/34
High Yield A H3N2 Reassortant (2:6)
with A/PR/8/34 M and NP genes and
A/Hong Kong/2671/2019 HA,NA,PB1,PB2,PA and NS genes

Exper. # 4850
A/Hong Kong/2671/2019
H3N2
#193956 E7 (Am3Al 4)
GP32(10^{-5}) 8/10/2019

Passages at New York Medical College

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>HA titer</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>10^{-2}</td>
</tr>
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</table>

A/Hong Kong/2671/2019 (H3N2) x A/PR/8/34

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>HA titer</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>10^{-2} + 10^{-3}</td>
</tr>
<tr>
<td>3</td>
<td>10^{-1}</td>
</tr>
</tbody>
</table>

+ A/PR/8/34 antisera (as)
A/PR/8/34 HANA antibodies (ab)

<table>
<thead>
<tr>
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<th>HA titer</th>
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<tbody>
<tr>
<td>4</td>
<td>10^{-1}</td>
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</tbody>
</table>

+ A/PR/8/34 antisera (as)
A/PR/8/34 HANA antibodies (ab)

<table>
<thead>
<tr>
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<th>HA titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10^{-3}</td>
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</table>

+ A/PR/8/34 antisera (as)
A/PR/8/34 HANA antibodies (ab)

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>HA titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>10^{-4}</td>
</tr>
</tbody>
</table>
The HA yield of X-341 was shown to be 4.2 ug/ml by UPLC analysis. The HA yield for A/Hong Kong/2671/2019 was 4.2 ug/ml by UPLC analysis.

HA, NA, PB1, PB2, PA, and NS genes were identified as A/Hong Kong/26712019 by RT-PCR/RFLP gene analysis. M 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.