Influenza Reagent
Influenza virus infectious X-181
NIBSC code: 19/206

Instructions for use
(Version 3.0, Dated 06/05/2020)

1. INTENDED USE
Reagent 19/206 is prepared from X-181 (H1N1pdm) which was processed for freeze drying in 250 µl volumes as described by Campbell, P.J., Journal of Biological Standardisation, 1974, 2, 249-267. The known passage history of X-181 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.

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 embryonated SPF hen’s eggs.

5. STORAGE
Store in the dark at -20°C or below

Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING
DIN ampoules have an ‘easy-open’ coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL
Reconstitute the contents of one ampoule of reagents with 250µl of sterile distilled water. Leave for a minimum of 5 minutes before use to allow for complete solution of freeze dried material. A range of dilutions (e.g. 10^{-3} to 10^{-4}) should be made in a suitable medium for initial cultivation.

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/jctlm/

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

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

### Physical and Chemical properties

<table>
<thead>
<tr>
<th>Physical appearance:</th>
<th>Corrosive:</th>
</tr>
</thead>
<tbody>
<tr>
<td>White powder</td>
<td>No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stable:</th>
<th>Corrosive:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hygroscopic:</th>
<th>Irritant:</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flammable:</th>
<th>Handling:</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>See caution, Section 2</td>
</tr>
</tbody>
</table>

Other (specify): Live influenza virus

### Toxicological properties

<table>
<thead>
<tr>
<th>Effects of inhalation:</th>
<th>Likelihood of influenza virus infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not established, avoid ingestion</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effects of ingestion:</th>
<th>Not established, avoid contact with skin</th>
</tr>
</thead>
</table>

Suggested First Aid

<table>
<thead>
<tr>
<th>Inhalation:</th>
<th>Seek medical advice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ingestion:</th>
<th>Seek medical advice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contact with eyes:</th>
<th>Wash with copious amounts of water. Seek medical advice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contact with skin:</th>
<th>Wash thoroughly with water.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Action on Spillage and Method of Disposal

Spillage of contents should be taken up with absorbent material wetted with a 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.

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><strong>Country of origin for customs purposes</strong>*</th>
<th>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>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Net weight</strong></th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toxicity Statement</strong></td>
<td>Non-toxic</td>
</tr>
<tr>
<td><strong>Veterinary certificate or other statement if applicable.</strong></td>
<td>Attached: No</td>
</tr>
</tbody>
</table>
Passage history of X-181

<table>
<thead>
<tr>
<th>Passage level</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-E5</td>
<td></td>
<td>NYMC, New York, USA</td>
</tr>
<tr>
<td>E6</td>
<td>E#5857</td>
<td>NYMC, New York, USA</td>
</tr>
<tr>
<td>E7</td>
<td>31710</td>
<td>NIBSC, Hertfordshire, UK</td>
</tr>
<tr>
<td>E8</td>
<td>45030</td>
<td>NIBSC, Hertfordshire, UK</td>
</tr>
</tbody>
</table>

E = SPF eggs

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_435701.

Attached derivation (page 3 and 4) as received from NYMC (derivation dated 13 August 2009)
Derivation of NYMC X-181 (5:3) hyH1N1sw hy A/California/07/2009

RE-reassortment of NYMC X-179A with NYMC X-157
HA, NA and PB1 genes donated by X-179A
(by A/California/07/2009 origin)
PB2, PA, M, NP and NS genes donated by X-157 (these five genes are of A/PR/8/34 origin)

Exper. # 4641 July 14, 2009

Post-reassortant NYMC X-179A X NYMC X-157

Passage No.____

1

$10^3$ + $10^3$

HA—128

10$^1$ + NYMC X-157 HANA As

HA—512

2

10$^1$ + X-157 HANA Abs

HA—1024

3

$10^7 (E^{**4})$ + X-157 HANA Abs

HA—2048

4

$10^9 (E^{**4})$

HA—2048

5

$10^5 (E^{**4})$

X-181 #5857 NYMC archive

Shipped to CDC 7/29/09

6

$10^5$

HA—4096

X-181 #5857 NYMC archive

Shipped to FDA, mfgers, WHO labs, 8/13-14/09

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory
HA and NA identified as A/CA/07/09 serologically by HI and NI tests.
HA, NA and PB1 genes were identified as A/CA/07/09 (donated by X-179A) by RT-PCR/RFLP analysis. The identity of the remaining genes confirmed by RT-PCR/RFLP analysis.
SPAFAES eggs were used exclusively for all passages. HA titers were tested using chicken red blood cells at room temp.
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.