Influenza Reagent
Influenza Infectious NYMC X-299
NIBSC code: 17/162
Instructions for use
(Version 2.0, Dated 23/11/2017)

1. INTENDED USE
Reagent 17/162 is prepared from NYMC X-299 (A/Montana/50/2016 x X-157B) H1N1pdm09 which was processed for freeze drying in 250μl volumes as described by Campbell, PJ, Journal of Biological Standardisation. 1974, 2, 249-267. The derivation and known passage history of NYMC X-299 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 ampoule contains 250μl (nominal) of infectious influenza virus as allantoic fluid from SPF embryonated 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 manufacturers instructions provided with the ampoule breaker.

7. USE OF MATERIAL
Reconstitute the contents of one ampoule of reagent 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 10⁻⁵) should be made in a suitable medium for initial cultivation.

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>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: white powder</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
</tbody>
</table>

Other (specify): Live influenza virus

<table>
<thead>
<tr>
<th>Toxicological properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation: Likelihood of influenza virus infection</td>
<td></td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
<td></td>
</tr>
<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
<td></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...
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</td>
</tr>
<tr>
<td>sufficiently processed to be classed as originating from the country of</td>
</tr>
<tr>
<td>supply, for example a change of state such as freeze-drying.</td>
</tr>
<tr>
<td>Net weight: 0.25g per ampoule</td>
</tr>
<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>

**Passage history of NYMC X-299**

<table>
<thead>
<tr>
<th>Passage</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-E8</td>
<td></td>
<td>New York Medical College, USA</td>
</tr>
<tr>
<td>E9</td>
<td>E#6294</td>
<td>New York Medical College, USA</td>
</tr>
<tr>
<td>E10</td>
<td>42920</td>
<td>NIBSC, Hertfordshire, UK</td>
</tr>
</tbody>
</table>

Total number of passages post mixed infection: 9

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 on GISAID with the accession number EPI_ISL_281222.
Derivation of NYMC X-299
A/Montana/50/2016 (H1N1pdm09) genetic grp 6B.1 with NYMC X-157B
High Yield A H1N1pdm09 Reassortant (6:2)
with A/PR/8/34 M, PB1, PB2, PA, NS, and NP genes and
A/Montana/50/2016 HA, and NA genes

Exper. # 4796
A/Montana/50/2016
#3025627640
E3 (1/23/17)
HA: 128

Passages at New York Medical College

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>HA (Fold)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$10^{-1}$</td>
<td>HA—128</td>
</tr>
<tr>
<td>2</td>
<td>$10^{-3}$ + $10^{-3}$</td>
<td>HA—1024</td>
</tr>
<tr>
<td>3</td>
<td>$10^{-1}$</td>
<td>+ X-157 HANA antisera (as) X-157 HANA antibodies (ab)</td>
</tr>
<tr>
<td>4</td>
<td>$10^{-1}$</td>
<td>+ X-157 HANA antisera (as) X-157 HANA antibodies (ab)</td>
</tr>
<tr>
<td>5</td>
<td>$10^{-1}$</td>
<td>+ X-157 HANA antisera (as) X-157 HANA antibodies (ab)</td>
</tr>
<tr>
<td>6</td>
<td>$10^{-4}$</td>
<td>HA—2048</td>
</tr>
</tbody>
</table>
HA and NA genes were identified as A/Montana/50/2016 by RT-PCR/RFLP gene analysis. PB1, PB2, PA, M, NS, and NP genes were identified as A/PR/8/34 by RT-PCR/RFLP analysis.

SPF eggs were used for all reassortant passages.

All HA titers were tested using chicken red blood cells (cRBC) at room temperature.

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.
Dear Dr. Bucher,

We appreciate your submission of influenza reassortant(s) to CDC for analysis. Data from your laboratory and other collaborating laboratories worldwide contribute significantly towards the influenza vaccine recommendations made each year by WHO.

Your reassortant was antigenically characterized by a “two way” hemagglutination-inhibition (HI) test using a panel of post-infection ferret antisera.

![Table]

Your reassortant had HI reactivity patterns that were consistent with the corresponding wild type virus, and it is antigenically similar to A/Michigan/45/2015. Ferret antiserum raised against the A/MONTANA/50/2016 X-299 virus inhibit the majority of recently circulating viruses in the HI assay. Therefore, it passed the two way test.

The HA and NA gene of your reassortant were sequenced and compared to that of their wild type parental virus A/MONTANA/50/2016. The reassortant virus differed from the parental virus (E2 passage) at amino acid residue 187 in the HA. The parental virus possessed a change of D187A compared to the HA sequence of the original clinical specimen, while the reassortant had a change of D187V. There is no amino acid difference between the reassortant and its parental virus in the NA.

If you have any questions, please contact us.

Sincerely,

Dr. Xiyan Xu
Deputy Director
WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza
Influenza Division, CDC

Dr. Jacqueline Katz
Director
WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza
Influenza Division, CDC

07/24/2017
**HEMAGGLUTINATION INHIBITION REACTIONS OF INFLUENZA A(H1N1)pdm09 VIRUSES**

**DATE TESTED:** 7/13/17

<table>
<thead>
<tr>
<th>STRAIN DESIGNATION</th>
<th>MB/45</th>
<th>MB/45</th>
<th>MT/50</th>
<th>MT/50</th>
<th>MT/80</th>
<th>PASSAGE</th>
<th>DATE COLL.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 AMICHIGAN 45/2015</td>
<td>2460</td>
<td>2460</td>
<td>5120</td>
<td>2560</td>
<td>2560</td>
<td>E3(12/7/15)</td>
<td>9/7/2015</td>
</tr>
<tr>
<td>2 AMICHIGAN 45/2015</td>
<td>1280</td>
<td>2660</td>
<td>2560</td>
<td>2560</td>
<td>1280</td>
<td>M1/C3(5/13/16)</td>
<td>9/7/2015</td>
</tr>
<tr>
<td>3 AMONTANA 50/2016</td>
<td>640</td>
<td>1280</td>
<td>1280</td>
<td>1280</td>
<td>1280</td>
<td>E4(1/27/17)</td>
<td>10/10/2016</td>
</tr>
<tr>
<td>4 AMONTANA 50/2016</td>
<td>1280</td>
<td>2560</td>
<td>2560</td>
<td>2560</td>
<td>2560</td>
<td>C2(1/24/17)</td>
<td>10/10/2016</td>
</tr>
<tr>
<td>5 AMONTANA 50/2016 X-299</td>
<td>1280</td>
<td>1280</td>
<td>2560</td>
<td>2560</td>
<td>1280</td>
<td>E3E9</td>
<td></td>
</tr>
</tbody>
</table>

*A virus is considered consistent with the wild type if it reacts with ferret antisera raised to the reference strain giving an HI titer equal to or within two-fold of the HI titer of the wild type reference strain.*