Influenza Reagent
Influenza Virus Infectious NYMC X-223
NIBSC code: 13/264
Instructions for use
(Version 1.0, Dated 01/04/2014)

1. INTENDED USE
Reagent 13/264 is prepared from NYMC X-223 which was processed for freeze
drying in 250μl volumes as described by Campbell, PJ, Journal of
Biological Standardisation, 1974, 2,249-267. The known passage history
of NYMC X-223 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 manufactures 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
10⁻⁶) 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>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: white powder</td>
</tr>
<tr>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
</tr>
<tr>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
</tr>
<tr>
<td>Handling: See caution, Section 2</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>
</tr>
</tbody>
</table>

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

Passage history of NYMC X-223 (Post mixed infection)

<table>
<thead>
<tr>
<th>Passage</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E5 (prior to receipt at NYMC)</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>E5/E1 – E5/E7</td>
<td>NYMC, New York, USA</td>
<td></td>
</tr>
<tr>
<td>E5/E8</td>
<td>E#6033</td>
<td>NYMC, New York, USA</td>
</tr>
<tr>
<td>E9</td>
<td>35390</td>
<td>NIBSC, Hertfordshire, UK</td>
</tr>
</tbody>
</table>
Derivation of NYMC X-223 High Yield H3N2 Reassortant (6:2)  
With A/PR/8/34 PA, PB2, PB1, NP, NS and M genes  
and A/Texas/50/2012 HA and NA genes

Experiment #4711 (11/2/12)  
A/Texas/50/2012 (H3N2) CDC #: 2012704893 E5 (10/18/12) HA: 256

**Passage No.**  
1 to 5  
Passages prior to receipt at NYMC (E5)

**Reassortment passage at NYMC:**  
A/Texas/50/2012 x A/PR/8/34

<table>
<thead>
<tr>
<th>Passage</th>
<th>Dilution</th>
<th>Result HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>10⁻¹ + 10⁻⁴</td>
<td>1.2048</td>
</tr>
<tr>
<td>7</td>
<td>10⁻¹</td>
<td>1.1024</td>
</tr>
<tr>
<td>8</td>
<td>10⁻³</td>
<td>1.256</td>
</tr>
<tr>
<td>9</td>
<td>10⁻³</td>
<td>1.512</td>
</tr>
<tr>
<td>10</td>
<td>10⁻⁴</td>
<td>1.1024</td>
</tr>
<tr>
<td>11</td>
<td>10⁻⁹</td>
<td>1.256</td>
</tr>
<tr>
<td>12</td>
<td>10⁻⁷</td>
<td>1.512</td>
</tr>
<tr>
<td>13</td>
<td>10⁻⁵</td>
<td>1.2048</td>
</tr>
</tbody>
</table>

**NYMC X-223 E5E8**  
E# 6033 NYMC archive

HA and NA were identified as A/Texas/50/2012 serologically by HI and NI tests and confirmed by RT-PCR/RFLP analysis. Internal genes PA, PB2, PB1 NP, NS and M were identified as A/PR/8/34 and HA, NA as A/Texas/50/2012 by RT-PCR/RFLP. SPFAS eggs were used for all reassortant passages. All HA titers were tested using guinea pig red blood cells at room temp. Virus seeds were shown to be sterile by streaking samples on sheep blood agar plates and incubating for 48 hours at 37 degrees C.
Dear Dr. Bucher,

We appreciate your submitting influenza reassortants 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.

The results we obtained with your reassortants are listed and interpreted below.

Your reassortant was characterized by a "one-way" hemagglutination-inhibition test using post-infection ferret antisera and guinea pig red blood cells (GP).

<table>
<thead>
<tr>
<th>CDC ID#</th>
<th>Specimen ID#</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013706003</td>
<td>A/HAWAII/22/2012 X-225</td>
<td>CONSISTENT WITH A/HAWAII/22/2012-LIKE (H3N2) PASS</td>
</tr>
<tr>
<td>2013706004</td>
<td>A/HAWAII/22/2012 X-225A</td>
<td>CONSISTENT WITH A/HAWAII/22/2012-LIKE (H3N2) PASS</td>
</tr>
<tr>
<td>2013706001</td>
<td>A/TEXAS/50/2012 X-223</td>
<td>CONSISTENT WITH A/TEXAS/50/2012-LIKE (H3N2) PASS</td>
</tr>
<tr>
<td>2013706002</td>
<td>A/TEXAS/50/2012 X-223A</td>
<td>CONSISTENT WITH A/TEXAS/50/2012-LIKE (H3N2) PASS</td>
</tr>
</tbody>
</table>

Your reassortants have HI reactivity patterns that are consistent with their corresponding wild type viruses.

If you have any questions, please contact us.

Sincerely,

Dr. Xiyan Xu
Team Leader
Virus Reference Team
Virus Surveillance and Diagnosis Branch
Influenza Division, CDC

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

National Institute for Biological Standards and Control
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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory
Doris Bucher, Ph.D.
Department of Microbiology and Immunology
New York Medical College
Basic Science Building
Valhalla, NY 10595

Dear Dr. Bucher,

We appreciate your submitting influenza reassortants 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.

The results we obtained with your reassortants are listed and interpreted below.

Your reassortants were characterized by a “two-way” hemagglutination-inhibition test using post-infection ferret antisera and guinea pig red blood cells.

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<tr>
<th>CDC ID#</th>
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<tr>
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<td>CONSISTENT WITH A/HAWAII/22/2012 (H3N2) PASS</td>
</tr>
<tr>
<td>2013706001</td>
<td>A/TEXAS/50/2012 X-223</td>
<td>CONSISTENT WITH A/TEXAS/50/2012 (H3N2) PASS</td>
</tr>
</tbody>
</table>

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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
Dear Dr. Bucher,

We appreciate your submitting influenza reassortants to CDC for analysis.

The HA and NA genes of your reassortants were sequenced and compared to that of their wild type parental virus A/Texas/50/2012. The results we obtained with your reassortants are listed and interpreted below.

<table>
<thead>
<tr>
<th>CDC ID#</th>
<th>Specimen ID#</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013706001</td>
<td>A/Texas/50/2012 X223</td>
<td>HA: Ile-226-Asn and Lys-387-Glu</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA: No change detected</td>
</tr>
<tr>
<td>2013706002</td>
<td>A/Texas/50/2012 X223A</td>
<td>HA: Ile-226-Asn</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA: No change detected</td>
</tr>
</tbody>
</table>

A number of amino acid changes were detected in the HA genes of the reassortants. Further analysis is warranted to better understand the significance of the changes.

If you have any questions, please contact us.

Sincerely,

[Signatures]

Dr. Alexander Klimov
Deputy Director
WHO Collaborating Centre for Surveillance, Epidemiology and Control of Influenza
Influenza Division, CDC

Dr. Xiyen Xu
Team Leader
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