Influenza Reagent
Influenza virus infectious NYMC BX-39
NIBSC code: 12/202
Instructions for use (Version 1.0, Dated 01/11/2012)

1. INTENDED USE
Reagent 12/202 is prepared from NYMC BX-39 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 BX-39 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 10⁻⁸) 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>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:</td>
</tr>
<tr>
<td>Likelihood of influenza virus infection</td>
</tr>
<tr>
<td>Effects of ingestion:</td>
</tr>
<tr>
<td>Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption:</td>
</tr>
<tr>
<td>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>
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</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*</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>
<tr>
<td>Net weight:</td>
<td>NA</td>
</tr>
<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 BX-39 (Post mixed infection)

<table>
<thead>
<tr>
<th>Passage</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 – E6</td>
<td></td>
<td>NYMC, New York, USA</td>
</tr>
<tr>
<td>E7</td>
<td>E#5916</td>
<td>NYMC, New York, USA</td>
</tr>
<tr>
<td>E8</td>
<td>33430</td>
<td>NIBSC, Hertfordshire, UK</td>
</tr>
</tbody>
</table>

Derivation of NYMC BX-39
B/Hubei-Wujiagang/158/2009 (Yamagata lineage) – like High Yield Reassortant (2:2:4) with B/Panama/45/90 PA, NS, B/Lee/40 PB2 and NP genes

Exper. # 4658 5/19/10
B/Hubei-Wujiagang/158/2009  CDC#2010728941  E2/E2 (2/16/10) HA:32
BX-38-1P-1: B/Lee/40-like high yield reassortant with B/Panama/45/90 PA and NS genes

Post-reassortment
Passage No.  B/Hubei-Wujiagang/158/2009   X   BX-38-1P-1 (E1)

1

\[
10^3 + \quad 10^3 \\
\text{HA—1:1024}
\]

1

\[
10^3 + \quad \text{B/Lee/40 NA as (as) B/Lee/40 HANA ab}
\]

\[
\text{HA—1:128}
\]

2

\[
10^3 + \quad \text{B/Lee/40 NA as B/Lee/40 HANA ab}
\]

\[
\text{HA—1:128}
\]

3

\[
10^3 + \quad \text{B/Lee/40 NA as B/Lee/40 HANA ab}
\]

\[
\text{HA—1:64}
\]

4

\[
10^3 + \quad \text{B/Lee/40 NA as B/Lee/40 HANA ab}
\]

\[
\text{HA—1:256}
\]

5

\[
10^4 + \quad \text{B/Lee/40 NA as B/Lee/40 HANA ab}
\]

\[
\text{HA—1:128}
\]

6

\[
10^8 + \quad \text{B/Lee/40 NA as B/Lee/40 HANA ab}
\]

\[
\text{HA—1:64}
\]

7

\[
10^5 + \quad \text{B/Lee/40 NA as B/Lee/40 HANA ab}
\]

\[
\text{HA—1:128}
\]
HA—1:512
NYMC BX-39
E#5916 NYMC archive
Shipped to CDC—Dec. 7, 2010
CDC ID# 2011700329

HA and NA identified as B/Hubei-Wujiagang/158/2009 by RT-PCR/RFLP analysis of HA and NA genes. RT-PCR/RFLP analysis also identified PB1 and M genes as B/Hubei-Wujiagang/158/2009, PA and NS genes as B/Panama/45/90, and PB2 and NP genes as B/Lee/40.

SPAFAS eggs used for all reassortant passages. HA titer was tested using chicken red blood cells at room temp.
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.

<table>
<thead>
<tr>
<th>CDC ID#</th>
<th>Specimen ID#</th>
<th>Results</th>
</tr>
</thead>
</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. Alexander Klimov
Deputy Director
WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza