Influenza Reagent
Influenza virus infectious BX–85C
NIBSC code: 19/256
Instructions for use
(Version 3.0, Dated 12/08/2021)

1. INTENDED USE
Reagent 19/256 is prepared from BX–85C (B-Victoria) 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 BX–85C 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 allantoinic 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
Vials have a ‘flip-up’ circular cap. Either on the cap or the collar of the vial, there is an indication of the point at which to lever off the cap. This exposes an area of the stopper through which reconstitution and withdrawal of the preparation can be made using a hypodermic needle and syringe. If use of a pipette is preferred, then fully remove the metal collar using, for example, forceps, taking care to avoid cuts by wearing appropriate gloves. Remove the stopper for access. Care should be taken to prevent loss of the contents.

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^-1 to 10^-5) 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/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:</td>
</tr>
<tr>
<td>White powder</td>
</tr>
<tr>
<td>Stable:</td>
</tr>
<tr>
<td>Hygroscopic:</td>
</tr>
<tr>
<td>Flammable:</td>
</tr>
<tr>
<td>Other (specify):</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation:</td>
</tr>
<tr>
<td>Effects of ingestion:</td>
</tr>
<tr>
<td>Effects of skin absorption:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggested First Aid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation:</td>
</tr>
<tr>
<td>Ingestion:</td>
</tr>
<tr>
<td>Contact with eyes:</td>
</tr>
<tr>
<td>Contact with skin:</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.
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>
<tr>
<td><strong>Net weight</strong></td>
<td>NA</td>
</tr>
<tr>
<td><strong>Toxicity Statement</strong></td>
<td>Non-toxic</td>
</tr>
<tr>
<td><strong>Veterinary certificate or other statement if applicable, Attached</strong></td>
<td>No</td>
</tr>
</tbody>
</table>
# Passage history of BX-8SC

<table>
<thead>
<tr>
<th>Passage level</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-E3</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>E4-E11</td>
<td>NYMC, New York, USA</td>
<td></td>
</tr>
<tr>
<td>E12</td>
<td>E#6415</td>
<td>NYMC, New York, USA</td>
</tr>
<tr>
<td>E13</td>
<td>45230</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_419002.
Derivation of NYMC BX-85C

B/Washington/02/2019 (Victoria lineage) - like Reassortant (1:7)
B/Lee: B/Washington with B/Lee/40 NP gene; B/Washington/02/2019 PA, PB1, PB2, HA, NA, M and NS genes

Exper. # 4847 08/13/19
B/Washington/02/2019(Victoria lineage) CDC ID# 3026019537
Passage*: E3 (Date of collection: 1/19/2019) (Date of Harvest: 4/16/2019) HA titer: 256

NYMC BX-42: Hybrid strain with B/Panama/45/1990 PB1, PB2, PA, NS, HA, NA and B/Lee/40 NP and M genes

**Passage No.**

<table>
<thead>
<tr>
<th>Passage at NYMC</th>
<th>Passages prior to receipt at NYMC (E3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pre-reassortment passage</td>
<td></td>
</tr>
</tbody>
</table>

**B/Washington/02/2019 X NYMC BX-42**

2

\[ 10^3 + 10^3 \]

\[ \text{HA—1:256} \]

3

\[ 10^3 \]

\[ \text{HA—1:512} \]

+ B/Panama/45 HANA antibodies (ab)

4

\[ 10^3 \]

\[ \text{HA—1:1024} \]

+ B/Panama/45 HANA ab

5

\[ 10^3 \]

\[ \text{HA—1:1024} \]

+ B/Panama/45 HANA ab

6

\[ 10^3 \]

\[ \text{HA—1:256} \]

7

\[ 10^3 \]

\[ \text{HA—1:512} \]

8

\[ 10^7 \]

\[ \text{HA—1:512} \]
BX-85C: HA, NA, PA, PB1, PB2, M and NS genes identified as B/Washington/02/2019 by RT-PCR/RFLP analysis. NP gene is from B/Lee/40.

SPAFAS eggs were used for all passages.
HA titers were performed using chicken 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 ºC.

UPLC HA yield:
BX-85C = 20 ug/ml
B/Washington/02 = 17.7 ug/ml

BX-85C HA yield vs WT: 1.13 (fold)