Influenza Reagent
Influenza virus infectious BX-57
NIBSC code: 20/134
Instructions for use
(Version 3.0, Dated 18/02/2021)

1. INTENDED USE
Reagent 20/134 is prepared from BX-57 (B/Hong Kong/3417/2014
(Yamagata lineage) x B/Lee/40) which was processed for freeze drying in
250 μl volumes as described by Campbell, PJ, Journal of Biological
Standardisation, 1974, 2, 249-267. The known passage history of BX-57
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
freeze dried 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 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^{-2} to
10^{-3}) 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

Physical and Chemical properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance:</td>
<td>White powder</td>
</tr>
<tr>
<td>Stable:</td>
<td>Yes</td>
</tr>
<tr>
<td>Hygroscopic:</td>
<td>No</td>
</tr>
<tr>
<td>Flammable:</td>
<td>No</td>
</tr>
<tr>
<td>Corrosive:</td>
<td>No</td>
</tr>
<tr>
<td>Oxidising:</td>
<td>No</td>
</tr>
</tbody>
</table>

Toxicological properties

<table>
<thead>
<tr>
<th>Effect</th>
<th>Likelihood of influenza virus infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation:</td>
<td>Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of ingestion:</td>
<td>Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption:</td>
<td>Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

Suggested First Aid

<table>
<thead>
<tr>
<th>Situation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation:</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Ingestion:</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes:</td>
<td>Wash with copious amounts of water. Seek</td>
</tr>
<tr>
<td></td>
<td>medical advice</td>
</tr>
<tr>
<td>Contact with skin:</td>
<td>Wash thoroughly with water.</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

| Country of origin for customs purposes*: United Kingdom |
| * 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. |
| Net weight: NA |
| Toxicity Statement: Non-toxic |
| Veterinary certificate or other statement if applicable. Attached: No |

**Passage history of BX-57**

<table>
<thead>
<tr>
<th>Cumulative number of passages</th>
<th>Passage numbers at each stage</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-E4</td>
<td>E4</td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>E5-E14</td>
<td>E10</td>
<td>6160</td>
<td>NYMC</td>
</tr>
<tr>
<td>E15</td>
<td>E1</td>
<td>45430</td>
<td>NIBSC, Hertfordshire, UK</td>
</tr>
</tbody>
</table>

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_574587
Derivation of NYMC BX-57
B/Hong Kong/3417/2014 (Yamagata lineage) - like High Yield Reassortant (1:3:4)
B/Lee:B/Panama:B/HK
With B/Lee/40 NP gene; B/Panama/45/90 PB1, PB2 and NS genes;
B/Hong Kong/3417/2014 PA, HA, NA and M genes

Exper. # 4764  4/14/15
B/Hong Kong/3417/2014 (Yamagata lineage) from WHO Collaborating Centre for Influenza
National Institute for Medical Research, London, UK
NYMC BX-46: Hybrid strain with B/Panama/45/90 PB1, PB2, PA, NS and B/Lee/40 HA, NP, NA and
M genes

Passage No. Passages prior to receipt at NYMC (E4)
1 to 4

Passage at NYMC

1 pre-reassortment passage

B/Hong Kong/3417/2014 X NYMC BX-46

<table>
<thead>
<tr>
<th>Passage</th>
<th>Initial Dilution</th>
<th>Final Dilution</th>
<th>HA titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>$10^{-1}$</td>
<td>$10^{-3}$</td>
<td>1:128</td>
</tr>
<tr>
<td>3</td>
<td>$10^{-3}$</td>
<td></td>
<td>1:256</td>
</tr>
<tr>
<td></td>
<td>+ B/Lee/40 HANA antibodies (ab)</td>
<td>B/Lee/40 NA antibodies (ab)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ B/Lee/40 HANA ab</td>
<td>B/Lee/40 NA ab</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>$10^{-3}$</td>
<td></td>
<td>1:128</td>
</tr>
<tr>
<td>5</td>
<td>$10^{-3}$</td>
<td></td>
<td>1:128</td>
</tr>
<tr>
<td></td>
<td>+ B/Lee/40 HANA</td>
<td>B/Lee/40 NA ab</td>
<td></td>
</tr>
</tbody>
</table>

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, NA, PA and M genes were identified as B/Hong Kong/3417/2014, NP gene as B/Lee/40, PB1, PB2 and NS genes as B/Panama/45/90 by RT-PCR/RFLP analysis. 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. The sterility test is not performed according to a method of the USP <71> / Ph. Eur. 2.6.1 / 21 CFR 610.12.