1. INTENDED USE
Reagent 21/350 is prepared from BX-59A (B-Yamagata Lineage) which was processed in 250µl volumes as liquid stock. The known passage history of BX-59A is attached.

2. CAUTION
The material is not of human or bovine origin. This preparation is not for administration to humans or animals.

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 vial contains 250µl (nominal) of infectious influenza virus as allantoic fluid from SPF embryonated hen’s eggs.

5. STORAGE
Store in the dark at -70ºC or below.

Material type: Liquid – will be shipped according to the storage and shipping conditions of the product.

6. DIRECTIONS FOR OPENING
Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: If a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

7. USE OF MATERIAL
Ready to use.

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
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>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Clear liquid</td>
<td>Effects of inhalation: Likelihood of influenza virus infection.</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Suggested First Aid</td>
</tr>
<tr>
<td>Other: Live influenza virus</td>
<td>Inhalation: Seek medical advice</td>
</tr>
<tr>
<td>Handling: See caution, Section 2</td>
<td>Ingestion: Seek medical advice</td>
</tr>
<tr>
<td>(specify):</td>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td></td>
<td>Contact with skin: Wash thoroughly with water.</td>
</tr>
</tbody>
</table>

Action on Spillage and Method of Disposal
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological 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: 0.25g per vial.
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. Attached: No

Passage history of BX-59A (Post-mixed infection)

<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>unknown</td>
<td></td>
</tr>
<tr>
<td>E5-E12</td>
<td>E4/E8</td>
<td>NYMC, USA</td>
<td></td>
</tr>
<tr>
<td>E13</td>
<td>E4/E9</td>
<td>#6208</td>
<td>NYMC, USA</td>
</tr>
<tr>
<td>E14</td>
<td>E4/E9/E1</td>
<td>41800</td>
<td>NIBSC, 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_217972.
Derivation of NYMC BX-59A
B/California/12/2015 (Yamagata lineage) - like High Yield Reassortant (1:1:6)
B/Lee:B/Panama:B/California
With B/Lee/40 NP gene; B/Panama/45/90 PB2 gene; B/California/2015 PB1, PA, HA, NA, M and NS genes

Exper. #4774  9/8/15
B/California/12/2015 (Yamagata lineage)  CDC ID# 3000094524  E4 (6/1/2015)  HA:16
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.  
1 to 4  

Passages prior to receipt at NYMC (E4)

Passage at NYMC

pre-reassortment passage

B/California/12/2015 X NYMC BX-46

2

10^1 + 10^3

HA—1:256

3

10^3 + B/Lee/40 HANA antibodies (ab)  
  B/Lee/40 NA antibodies (ab)

HA—1:64

4

10^3 + B/Lee/40 HANA ab  
  B/Lee/40 NA ab

HA—1:64
HA, NA, PB1, PA, M and NS genes were identified as B/California/12/2015, NP gene as B/Lee/40, PB2 gene 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 °C. The sterility test is not performed according to a method of the USP <71> / Ph. Eur. 2.6.1 / 21 CFR 610.12.