Influenza Reagent
Influenza Virus Infectious NIB-103
NIBSC code: 17/184
Instructions for use
(Version 2.0, Dated 23/11/2017)

1. INTENDED USE
Reagent 17/184 is prepared from NIB-103 (A/Noes/FI 103/16 x IVR-145) by the process of freeze drying. It is issued to its reference code as a white powder. The reference should be regarded as potentially hazardous to health.  It should be used 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.

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 of infectious influenza virus as allantoic fluid from SPF embryonated hen's eggs. NIBSC follows the policy of WHO with respect to its reference materials.

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 breacker 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 breacker.

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 the 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/ctlm/
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: white powder</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Live influenza virus</td>
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</tr>
</tbody>
</table>

Suggested First Aid
Inhalation: Seek medical advice
Ingestion: Seek medical advice
Contact with eyes: Wash with copious amounts of water. Seek medical advice
Contact with skin: Wash thoroughly with water.

Action on Spillage and Method of Disposal
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.

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
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 ampoule |
| Toxicty Statement: | Non-toxic |
| Veterinary certificate or other statement if applicable. | Attached: No |

Passage history of NIB-103 (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>NIBSC, Hertfordshire, UK</td>
</tr>
<tr>
<td>E7</td>
<td>42610</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 on GISAID with the accession number EPI_ISL_281392.

Derivation of NIB-103
Strain: A/Norway/3806/2016 (H3N2)
Received from CRICK 162860 1s2c25, E7
Passage undertaken at NIBSC #42480, E8

Mixed Infection:  A/Norway/3806/2016 (10^{-2}) x IVR-145 (10^{-3})
HA Titre: 2048

1st Antiserum passage: Inoculum 10^{-5} with IVR-145 antiserum
HA Titre: 512

2nd Antiserum passage: Inoculum 10^{-4} with IVR-145 antiserum
HA Titre: 512

3rd Antiserum passage: Inoculum 10^{-3} with IVR-145 antiserum
HA Titre: 512

4th Antiserum passage: Inoculum 10^{-2} with IVR-145 antiserum
HA Titre: 1024

5th passage: Inoculum 10^{-9}
HA Titre: 512

6th passage: Inoculum 10^{-7}
HA Titre: 256

7th passage: Inoculum 10^{-5}
HA Titre: 512

Total number of passages since mixed infection= E7

Lot: 42610
SPF eggs were used for all passages.

RT-PCR/RFLP analysis indicates that NIB-103 has HA, NA, NP, NS, PB2, PA and PB1 genes from A/Norway/3806/2016 and the MX gene from A/PR/8/34, making it a 1:7 reassortant.
The Crick Worldwide Influenza Centre,  
The Francis Crick Institute,  
1 Midland Road, London NW1 1AT, UK  
info@crick.ac.uk, www.crick.ac.uk

Monday, 04 September 2017

NIB-103 (A/Norway/3806/2016)

Your high yield/growth reassortant virus NIB-103 derived from A/Norway/3806/2016 has now been analysed in a 2-way test and its HA and NA gene sequences determined.

In HI assays using guinea pig red blood cells in the presence of oseltamivir the post infection antiserum raised against egg-propagated cultivar of A/Norway/3806/2016 (F45/16) recognised NIB-103 with a titre equal to the titre of the antiserum for the homologous virus. The post infection antiserum raised against egg-propagated cultivar of A/Hong Kong/4801/2014 (F12/15) recognised NIB-103 with a titre at a titre 2-fold lower than the titre of the antiserum for the homologous virus. Antiserum raised against NIB-103 (NIB F13/17) recognised against the egg-propagated cultivar of A/Norway/3806/0216 at the same titre as it recognised the reassortant virus, and the antiserum raised against NIB-103 also recognised against the egg-propagated cultivar of A/Hong Kong/4801/2016 at a titre equal to the titre of the antiserum for the reassortant virus. The results of the test are shown in the annex below.

The gene sequence of the HA and NA genes of NIB-103 was determined and compared with the cell culture-propagated and egg-propagated cultivars of A/Norway/3806/2016. Alignments are attached. You will see that the HA gene sequence of NIB-103 differed from the HA gene sequence of the egg-propagated cultivar of A/Norway/3806 (Is2 c25) at residue 203 of HA1 (T203I). The HA gene sequences of both the egg-propagated cultivar of A/Norway/3806/2016 and the reassortant NIB-103 also differed from the cell culture-propagated cultivar of A/Norway/3806/2016 at two positions: those associated with egg-adaptation T160K in HA1 (resulting in the loss of the glycosylation site at residue 158 of HA1, and L194P in HA1, the second common site of egg-adaptation in 3C.2a viruses. There were no changes in seen the NA sequences.

These results indicate that NIB-103 can be considered as antigenically like its parent egg-propagated A/Norway/48012/2016 and also antigenically like the prototype virus A/Hong Kong/4801/2014.

I hope that you find the results useful.

With best wishes,
Yours sincerely,

John McCauley
Director, WHO Collaborating Centre for Reference and Research on Influenza
The Francis Crick Institute.
Annex 1. HI analysis of NIB-103.

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2017-08-11

<table>
<thead>
<tr>
<th>WIC number</th>
<th>Viruses</th>
<th>Other</th>
<th>Collection</th>
<th>Passage</th>
<th>A/HK</th>
<th>A/Florida</th>
<th>NIB-103</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Reference viruses</th>
<th>Virus</th>
<th>Passage history</th>
<th>Ferret number</th>
<th>Genetic group</th>
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<tbody>
<tr>
<td>A/Hong Kong/4881/2014</td>
<td>Isolate 1</td>
<td>3C.2a</td>
<td>2014-02-20</td>
<td>E5/E2</td>
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<tr>
<td>A/Florida/309/2003</td>
<td>Isolate 1 clone 26</td>
<td>3C.2a</td>
<td>2016-06-26</td>
<td>E8</td>
</tr>
<tr>
<td>NIB-103 (A/Florida/2003)</td>
<td>Isolate 1 clone 26</td>
<td>3C.2a</td>
<td>2016-06-26</td>
<td>E8</td>
</tr>
</tbody>
</table>

Assay: HI (Guinea Pig RBC with 20nM Oseltamivir)

Vaccine: S4H

RBC: Guinea Pig

Virus: Influenza A(H3N2)

Date: 2017-08-11