Influenza Reagent
Influenza Virus Infectious NIB-92
NIBSC code: 15/148
Instructions for use
(Version 2.0, Dated 09/11/2015)

1. INTENDED USE
Reagent 15/148 is prepared from NIB-92 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 NIB-92 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 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 10⁻⁵) 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:
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: white powder</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
</tr>
<tr>
<td>Flammable: No</td>
</tr>
<tr>
<td>Irritant: No</td>
</tr>
<tr>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Oxidising: No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation: Likelihood of influenza virus infection</td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption: 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>
</tr>
</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

| **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 NIB-92 (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>40560</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.
Derivation of NIB-92
A/Victoria/673/2014 (H3N2)-like High Growth Reassortant

Strain: A/Victoria/673/2014 (H3N2)
Received from V/IDRL #1409666-1, E4
Passage undertaken at NIBSC #40230, E5

Mixed Infection: A/Victoria/673/2014 (10^3) × IVR-145 (10^4)

1st Antiserum passage: Inoculum 10^3 with IVR-145 antiserum
HA Titre: 512

2nd Antiserum passage: Inoculum 10^3 with IVR-145 antiserum
HA Titre: 256

3rd Antiserum passage: Inoculum 10^2 with IVR-145 antiserum
HA Titre: 1024

4th Antiserum passage: Inoculum 10^2 with IVR-145 antiserum
HA Titre: 128

5th passage: Inoculum 10^5
HA Titre: 1024

6th passage: Inoculum 10^5
HA Titre: 512

7th passage: Inoculum 10^5
HA Titre: 320
Lot: 40560
Total number of passages since mixed infection = E7
SPF eggs were used for all passages.
RT-PCR/RFLP analysis indicates that NIB-92 has HA and NA genes from A/Victoria/673/2014 and NP, NS, PB1, PB2, PA and MX genes from JVR-145 making it a 6:2 reassortant.

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.
Tuesday, 14 July 2015

We have the 2-way results for the HI results for NIB-92 (based on A/Victoria/673/2014).

The antiserum you raised against A/Victoria/673/2014 recognised NIB-92 in an HI test using guinea pig red blood cells done in the presence of 20nM Oseltamivir at a titre equal to the titre of the antiserum for the homologous virus A/Victoria/673/2014; the antiserum you raised against NIB-92 recognised A/Victoria/673/2014 in the HI test at a two-fold higher than the titre of the antiserum for NIB-92. Therefore NIB-92 is antigenically indistinguishable from its parent A/Victoria/673/2014.

The results are below.

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2015-07-14

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Collection Date</th>
<th>Passage History</th>
<th>A/Vic 673/14 (A/Vic/673/2014)</th>
<th>NIB-92</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Victoria/673/2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIB-92 (A/Victoria/673/2014)</td>
<td>3C.2a</td>
<td>E5</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>3C.2a</td>
<td>E7</td>
<td>640</td>
<td>640</td>
</tr>
</tbody>
</table>

I hope that you find the results useful.

With best wishes,

Digitally signed by John William McCauley  
Date: 2015.07.14 16:00:24 +01'00'

John McCauley BSc PhD  
Director, Crick Worldwide Influenza Centre.
We have more HI results for the HI results for NIB-92 (based on A/Victoria/673/2014). These results are to confirm that NIB-92 is antigenically like A/Hong Kong/4801/2014 as well as like A/Victoria/673/2014, as we described in the letter of 14th July 2015.

An antiserum raised against egg-propagated A/Hong Kong/4801/2014 recognised NIB-92 in an HI test using guinea pig red blood cells done in the presence of 20nM Oseltamivir at a titre equal to the titre of the antiserum for the homologous virus A/Hong Kong/4801/2014; the antiserum you raised against NIB-92 recognised A/Hong Kong/4801/2014 in the HI test at a titre two-fold higher than its titre for NIB-92. This antiserum also recognised its parent A/Victoria/673/2014, at a titre two-fold higher than it recognised NIB-92. The antiserum raised against A/Victoria/673/2014 recognised NIB-92 at a titre equal to the titre of the antiserum for the homologous virus.

Therefore, NIB-92 is antigenically indistinguishable from its parent A/Victoria/673/2014, and it is also antigenically like A/Hong Kong/4801/2014.

The results are below.

<table>
<thead>
<tr>
<th>Antigenic analyses of influenza (H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2015-10-23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>A/Hong Kong/4801/2014</td>
</tr>
<tr>
<td>A/Victoria/673/2014</td>
</tr>
<tr>
<td>NIB-92 (A/Victoria/673/2014)</td>
</tr>
</tbody>
</table>

Reference viruses

NIB 92

1. < = <80 ND = NOT DONE

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National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. Tel 44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory
I hope that you find the results useful.

With best wishes,
Digitally signed by John William McCauley
Date: 2015.10.26 17:02:47 Z
John McCauley BSc PhD
Director, Crick Worldwide Influenza Centre.