Influenza Reagent
Influenza Virus Infectious NIB-104
NIBSC code: 17/194
Instructions for use
(Version 2.0, Dated 23/11/2017)

1. INTENDED USE
Reagent 17/194 is prepared from NIB-104 (A/Singapore/INFIMH-16-0019/2016 x IVR-145) (H3N2) which was processed for freeze drying in 250μl volumes as described by Campbell, P.J. Journal of Biological Standardisation, 1974, 2249-267. The derivation and known passage history of NIB-104 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 manufacturers 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. 10⁻³ 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:
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>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>White powder</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable:</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic:</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable:</td>
<td>Handling: See caution, Section 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation:</td>
<td>Likelihood of influenza virus infection</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              | |
|----------------------------------| |
| 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
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>Country of origin for customs purposes*:</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>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Net weight:</th>
<th>0.25g per ampoule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity Statement:</td>
<td>Non-toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable.</td>
<td>Attached: No</td>
</tr>
</tbody>
</table>

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**Passage history of NIB-104 (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>42770</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_282213.
Derivation of NIB-104
A/Singapore/INFIMH-16-0019/2016 x IVR-145 (H3N2)-like High Growth Reassortant

Strain: A/Singapore/INFIMH-16-0019/2016 (H3N2)
Received from VIDRL, E5
Passage undertaken at NIBSC #42520, E6

Mixed Infection: A/Singapore/INFIMH-16-0019/2016 (10^3) x IVR-145 (10^6)

1\(^{st}\) Antiserum passage: Inoculum 10\(^{-3}\) with IVR-145 antiserum
HA Titre: 128

2\(^{nd}\) Antiserum passage: Inoculum 10\(^{-3}\) with IVR-145 antiserum
HA Titre: 256

3\(^{rd}\) Antiserum passage: Inoculum 10\(^{2}\) with IVR-145 antiserum
HA Titre: 512

4\(^{th}\) Antiserum passage: Inoculum 10\(^{2}\) with IVR-145 antiserum
HA Titre: 512

5\(^{th}\) passage: Inoculum 10\(^{8}\)
HA Titre: 256

6\(^{th}\) passage: Inoculum 10\(^{9}\)
HA Titre: 512

7\(^{th}\) passage: Inoculum 10\(^{5}\)
HA Titre: 128
Lot: 42770

Total number of passages since mixed infection = E7
SPF eggs were used for all passages.

RT-PCR/RFLP analysis indicates that NIB-104 has HA, NA and NS genes from A/Singapore/INFIMH-16-0019/2016 and all remaining genes from IVR-145 (A/PR/8/34), making it a 5:3 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.
**Influenza Virus Seed Lot**

Identity Test Report for: National Institute for Biological Standards and Control

<table>
<thead>
<tr>
<th>Sample ID No.</th>
<th>NIB-104</th>
<th>Test Code</th>
<th>NA</th>
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</thead>
<tbody>
<tr>
<td>Seed Lot No.</td>
<td>NA</td>
<td>Date submitted</td>
<td>15/09/2017</td>
</tr>
<tr>
<td>Sample name</td>
<td>A/Singapore/INFMH-16-0019/2016</td>
<td>WHO ID No.</td>
<td>1709635</td>
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</tbody>
</table>

**Test applied**

- Haemagglutination Inhibition Assay

**Assay Date:** 26 Sep 2017

**Assay performed by:** Tasoula Zakis

### HI titre with reference antisera

<table>
<thead>
<tr>
<th>Reference antigen</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
<th>A8</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/SWITZERLAND/9715293/2013 A(H3N2)</td>
<td>640</td>
<td>80</td>
<td>160</td>
<td>&lt;80</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>A/NEW CALEDONIA/71/2014 A(H3N2)</td>
<td>40</td>
<td>2560</td>
<td>640</td>
<td>&lt;80</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>640</td>
<td>320</td>
</tr>
<tr>
<td>A/HONG KONG/4801/2014 A(H3N2)</td>
<td>40</td>
<td>2560</td>
<td>640</td>
<td>&lt;80</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>640</td>
<td>320</td>
</tr>
<tr>
<td>A/MICHIGAN/45/2015 A(H1N1)pdm</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>1280</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>B/BRISBANE/33/2008 (B VIC)</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>320</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>B/PHUKET/3073/2013 (B YAM)</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>640</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>A/SINGAPORE/INFMH-16-0019/2016 (WT) A(H3N2)</td>
<td>40</td>
<td>1280</td>
<td>320</td>
<td>&lt;80</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>640</td>
<td>320</td>
</tr>
</tbody>
</table>

### Test antigen

- NIB-104

**Actual antisera used were raised to:**

- A1 A/SWITZERLAND/9715293/2013 A(H3N2)
- A2 A/NEW CALEDONIA/71/2014 A(H3N2)
- A3 A/HONG KONG/4801/2014 A(H3N2)
- A4 A/MICHIGAN/45/2015 A(H1N1)pdm
- A5 B/BRISBANE/33/2008 (B VIC)
- A6 B/PHUKET/3073/2013 (B YAM)
- A7 A/SINGAPORE/INFMH-16-0019/16 (WT) A(H3N2)
- A8 NIB-104(A/SINGAPORE/INFMH-16-0019/2016) NIBSC F12/17 Reassortant A(H3N2)

**Conclusion:** NIB-104 has a HI reactivity pattern that is consistent with the wild-type egg propagated virus A/SINGAPORE/INFMH-16-0019/16 and therefore passes the One-Way HI test. NIB-104 also passes the Two-Way HI test based on results obtained with antisera produced against the reassortant virus NIB-104 (A8; supplied by NIBSC).

Pass [ ] Fail [ ] Warn [ ]

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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