Influenza Reagent
Influenza virus infectious IVR-165
NIBSC code: 11/220
Instructions for use
(Version 1.0, Dated 12/03/2012)

This material is not for in vitro diagnostic use.

1. INTENDED USE

2. CAUTION
This preparation is not for administration to humans.

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 handled only in appropriate containment facilities by fully trained competent staff. It should be used and disposed of in accordance with national safety guidelines and your laboratory’s safety procedures.

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 allantotic 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. Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker is provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar. Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF 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/products/biological_reference_materials/frequently_ asked_questions/how_are_international_units.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering_information/frequently_asked_questions.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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: White powder</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>Handling: See caution, Section 2</td>
<td>Inhalation: Seek medical advice</td>
</tr>
<tr>
<td>Other (specify): Live influenza virus</td>
<td>Ingestion: Seek medical advice</td>
</tr>
<tr>
<td></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 and waste disposal procedures should follow those outlined in your facility standard laboratory operating procedures. Appropriate disinfectants would include Chlorine based chemicals, 70% Ethanol and phenolic compounds when used according to manufacturer’s specified recommendations.
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 IVR-165

<table>
<thead>
<tr>
<th>Passage level</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-E3</td>
<td></td>
<td>VIDRL, Melbourne, Australia</td>
</tr>
<tr>
<td>D1 - D6</td>
<td>VI-1555</td>
<td>CSL, Melbourne, Australia</td>
</tr>
<tr>
<td>E6</td>
<td>34520</td>
<td>NIBSC, Hertfordshire, UK</td>
</tr>
</tbody>
</table>

E = SPF eggs

Attached derivation as received from CSL
HI data as received from VIDRL, Melbourne.
Derivation of IVR-165
A/Victoria/361/2011 – like High Growth Reassortant

PREPARATION
Preparation of IVR-165, lot VI-1555, an A/Victoria/361/2011 (H3N2) high growth reassortant influenza virus was conducted in the Influenza Development department, R&D, CSL Limited.

MATERIALS
The following materials of biological origin were used during preparation of high growth reassortant IVR-165:

**Virus Isolate:** The virus isolate was obtained from the WHO Collaborating Centre for Reference & Research on Influenza, Melbourne (WHO-CC).
Supply details are:
A/Victoria/361/2011 (Type A, Subtype H3N2)
WHO-CC Laboratory number: SL/1110498
Passages prior to receipt at WHO-CC: Nil
Passages undertaken in WHO-CC: E3, HA=128(guinea pig red cells)

**Eggs:** SPF Premium Plus eggs were used for all passages.

**Antiserum:** Trypsin-periodate treated sheep hyperimmune antiserum Lot# AS367, sub-lot # 4720, raised against influenza virus A/Puerto Rico/8/34.
The sheep antiserum was derived from sheep born and raised in Australia.

*Note on Transmissible Spongiform Encephalopathies (TSEs):*
Australia and New Zealand have been declared TSE free in accordance with OIE guidelines. Detailed information on Australia’s animal health status can be obtained from the following Animal Health Australia website link:
The trypsin used is 10x solution of gamma irradiated porcine pancreatic trypsin; Invitrogen / Gibco Cat # 15090, Lot No. 798572

**PASSAGE HISTORY:**
*Mixed infection passage:* A/Victoria/361/2011 (H3N2) wild type virus @10⁵ x A/Puerto Rico/8/34 (H1N1) (@10³) HA titre 1040 (with CRBC)
1st Antiserum Passage Inoculum @ 10⁻³ with antiserum to A/Puerto Rico/8/34 (H1N1) HA titre ND (with CRBC)

2nd Antiserum Passage Inoculum @ 10⁻³ with antiserum to A/Puerto Rico/8/34 (H1N1) HA titre 502 (with CRBC)

HA titre ≥1154 (with GPRBC)

1st Limit dilution passage Inoculum @ 10⁻⁸

HA titre 710 (with CRBC)

2nd Limit dilution passage Inoculum @ 10⁻⁹

HA titre 1114 (with CRBC)

Preparation of IVR-165 Lot VI-1555
Inoculum @ 10⁻⁵

mean HA titre 454 (with CRBC)

Total number of passages post mixed infection = 5
Total number of passages since this virus was received from an approved laboratory = 6
HA titres were determined using chicken red blood cells at room temperature.
Guinea Pig red cells were also used at 2nd Antiserum passage.

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**TESTING OF INFLUENZA VIRUS SPF LOT VI-1555:**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sterility</strong></td>
<td>Pending</td>
</tr>
<tr>
<td><strong>Antigenicity</strong></td>
<td>Seed lot VI-1555 (IVR-165) has a HI reactivity pattern that is consistent with the wild type A/Victoria/361/2011 virus. See WHO report on one-way HI testing attached</td>
</tr>
<tr>
<td><strong>Genotype</strong>&lt;br&gt;(by real time RT-PCR)</td>
<td>6i2&lt;br&gt;H1, N1 from A/Victoria/361/2011&lt;br&gt;Remaining 6 internal genes from PR-S&lt;br&gt;&lt;br&gt;A/Victoria/361/2011</td>
</tr>
</tbody>
</table>
Disclaimer:
The material i.e. high growth reassortant virus IVR-165 and the information provided in this derivation report are provided on an “as is” basis and as such without any warranty or representation of any kind (express or implied) including, without limitation, of satisfactory quality or fitness for a particular purpose.
Prepared by:
Leonora Pancho
Manager
Influenza Development, R&D, CSL Limited
Wednesday, 15th February 2012
# Influenza Virus Seed Lot Identity Test Report for: CSL Limited

## Reference Antigens

<table>
<thead>
<tr>
<th>Reference antigen</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A(H1N1) pdm</th>
<th>B VIC</th>
<th>B YAM</th>
<th>H1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/PERTH/16/2009 A(H3)</td>
<td>320</td>
<td>&lt;20</td>
<td>320</td>
<td>20</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>A/BRISBANE/10/2007 A(H3)</td>
<td>20</td>
<td>320</td>
<td>80</td>
<td>20</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>A/PERTH/10/2010 A(H3)</td>
<td>80</td>
<td>&lt;20</td>
<td>640</td>
<td>40</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>A/BRISBANE/59/2007 A(H1)</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>A/CALIFORNIA/07/2009 A(H1N1) pdm</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>&lt;20</td>
<td>320</td>
</tr>
<tr>
<td>B/BRISBANE/33/2008 B VIC</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;40</td>
<td>&lt;40</td>
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<td>&lt;20</td>
</tr>
<tr>
<td>B/WISCONSIN/1/2010 B YAM</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>A/VICTORIA/361/2011 A(H3) (WT)</td>
<td>40</td>
<td>80</td>
<td>640</td>
<td>40</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

## Test Antigen

| VI-1555 | 160 | 160 | 640 | 640 | <40 | <40 | <20 | <20 |

## Actual Antiseras Used

- A2: A/BRISBANE/10/2007
- A3: A/PERTH/10/2010
- A4: A/VICTORIA/361/2011
- A(H1N1) pdm: A/CALIFORNIA/07/2009
- B VIC: B/BRISBANE/33/2008
- B YAM: B/WISCONSIN/1/2010

## Conclusion

Seed lot VI-1555 (IVR-165) has a HI reactivity pattern that is consistent with the wild type A/Victoria/361/2011 virus.

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Pass □ Fail □ Warn □

Ian Barr  
Deputy Director  
15.02.2012