Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza virus infectious IVR-158
NIBSC code: 10/226
Instructions for use
(Version 1.0, Dated 03/12/2010)

1. INTENDED USE

The influenza reference virus IVR-158 is a reassortant prepared by CSL Ltd using classical reassortant methodology from A/Brisbane/10/2010 H1N1pdm virus and IVR-6 virus. Reagent 10/226 is prepared from IVR-158 and 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 IVR-158 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 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 allantoic fluid from embryonated SPF hen's eggs.

5. STORAGE

Store in the dark at -20^{0}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

A range of dilutions (e.g. 10^{-3} to 10^{-5}) should be made in a suitable medium for initial cutivation.

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

Physical and Chemical properties			
Physical appearance: White powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	Live influenza virus with surface proteins derived from H1N1pdm virus.		

Toxicological properties	
Effects of inhalation:	Likelihood of influenza virus infection
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin

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 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

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

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-158

Passage level	Lot	Laboratory
E1-E4		CSL, Melbourne, Australia
E5	VI-1541	CSL, Melbourne, Australia
E6	32840	NIBSC, Hertfordshire, UK

E = SPF eggs

Attached derivation as received from CSL
HI data as received from VIDRL, Melbourne.

Derivation of IVR-158

A/Brisbane/10/2010 (H1N1)pdm – like High Growth Reassortant

PREPARATION

Preparation of IVR-158, lot VI-1541, an A/Brisbane/10/2010 (H1N1pdm)-like 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-158:

Virus Isolate:

The virus isolate was obtained from the WHO Collaborating Centre for Reference & Research on Influenza, Melbourne (WHO-CC).

Supply details are:
A/Brisbane/10/2010 (Type A, Subtype H1N1 pdm)
WHO-CC Laboratory number: SL/1007014-1
Passages prior to receipt at WHO-CC: Nil
Passages undertaken in WHO-CC: E2, HA=1024 (turkey red cells)

Eggs

: SPF Premium Plus eggs were used for all passages.

Antiserum:

Trypsin-periodate treated sheep hyperimmune antiserum Lot# AS348, sub-lot # 4685, raised against influenza virus IVR-6 a reassortant of A/Texas/1/77 H3N2 x A/Puerto Rico/8/34.
The sheep antiserum was derived from sheep born and raised in Australia.

The trypsin used is 10x solution of gamma irradiated porcine pancreatic trypsin;
SAFC Catalog No. 59427C

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 obtaind from the following Animal Health Australia website link: www.animalhealthaustralia.com.au/aahc/status/ahia.cfm

SAFC Catalog No. 59427C trypsin is obtained from the United States or other countries deemed free of Bovine Spongiform Encephalopathy (BSE).

PASSAGE HISTORY:

Mixed infection passage: A/Brisbane/10/2010 (H1N1pdm) @10-5 x IVR-6* (H3N2) @10-3
HA titre ≥ 1325

↓

1st Antiserum Passage Inoculum @ 10-3 with antiserum to IVR-6 HA titre 453

↓

2nd Antiserum Passage Inoculum @ 10-3 with antiserum to IVR-6 HA titre 520

↓

1st Limit dilution passage Inoculum @ 10-8 HA titre ≥ 1576

↓

2nd Limit dilution passage Inoculum @ 10-9 HA titre ≥ 1576

↓

3rd Limit dilution passage Inoculum @ 10-9 HA titre ≥ 1576

↓

Preparation of IVR-158, Lot VI-1541
Inoculum @ 10-5 mean HA titre ≥ 884

*IVR-6 is an A/Texas/1/77 (H3N2)-like High Growth Reassortant derived from A/Texas/1/77 (H3N2) x A/Puerto Rico/8/34 (H1N1). IVR-6 is a 5:3 reassortant containing HA, NA and PB1 from A/Texas/1/77. The remaining internal genes are derived from PR8.

Total number of passages post mixed infection = 6
Total number of passages since this virus was received from an approved laboratory = 7
HA titres were determined using chicken red blood cells at room temperature.

<table>
<tr><th>TESTING OF INFLUENZA VIRUS SPF LOT VI-1525: Test</th><th colspan="4">Result</th></tr>
<tr><td>Sterility</td><td colspan="4">Sterile (after 7days on test)</td></tr>
<tr><td>Antigenicity</td><td colspan="4">Seed lot VI-1541 has a HI reactivity pattern that is consistent with an A/California/07/2009-like virus.
See WHO report on HI testing attached</td></tr>
<tr><td rowspan="12">Genotype
(by real time RT-PCR)</td><td colspan="4">5:2:1
H1, N1 from A/Brisbane/210/2010
6 internal genes from IVR-6 i.e.
PB1 from A/Texas/1/77 (H3N2)
Remaining 5 internal genes from PR-8</td></tr>
<tr><td>Gene Segment</td><td>A/Brisbane/10/2010</td><td>PR8</td><td>A/Texas/1/77</td></tr>
<tr><td>PB2</td><td>-</td><td>🕘</td><td>N/A</td></tr>
<tr><td>PB1</td><td>-</td><td>N/A</td><td>🕘</td></tr>
<tr><td>PA</td><td>-</td><td>🕘</td><td>N/A</td></tr>
<tr><td>HA – H1</td><td>🕘</td><td>N/A</td><td>N/A</td></tr>
<tr><td>HA - H3</td><td>N/A</td><td>N/A</td><td>🕘</td></tr>
<tr><td>NP</td><td>-</td><td>🕘</td><td>N/A</td></tr>
<tr><td>NA – N1</td><td>🕘</td><td>N/A</td><td>N/A</td></tr>
<tr><td>NA-N2</td><td>N/A</td><td>N/A</td><td>🕘</td></tr>
<tr><td>M</td><td>-</td><td>🕘</td><td>N/A</td></tr>
<tr><td>NS</td><td>-</td><td>🕘</td><td>N/A</td></tr>
<tr><td>Infectivity
EID50 (log_{10}/0.2mL)</td><td colspan="4">7.75</td></tr>
<tr><td>Appearance
(Electron Microscopy)</td><td colspan="4">The following morphologies were reported (in order of abundance):
Small spheres, small kidneys, medium spheres, small tadpoles, short filaments.</td></tr>
</table>

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Influenza Virus Seed Lot

Identity Test Report for: CSL Limited Sample ID No.	1043743	Test Code	CSL: QA 0050
Seed Lot No.	VI-1541	Date submitted	09.09.2010
Sample name	IVR-158(A/Brisbane/10/2010)	WHO ID No.	1009312

Test applied	**Haemagglutination Inhibition Assay**	Assay Date	10 September 2010
Assay performed by		T. Mastorakos	

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HI titre with reference antisera							
Reference antigen	A1	A2	A3	H1	H3	B VIC	B YAM
A/CALIFORNIA/07/2009 A(H1N1)pdm	**1280**	320	1280	<20	<20	<20	<20
A/AUCKLAND/1/2009 A(H1N1)pdm	1280	**320**	1280	<20	<20	<20	<20
A/BRISBANE/2013/2009 A(H1N1)pdm	1280	160	**1280**	<20	<20	<20	<20
A/BRISBANE/59/2007 A(H1)	<20	<20	<20	**320**	<20	<20	<20
A/PERTH/16/2009 A(H3)	<20	<20	<20	<20	**320**	<20	<20
B/BRISBANE/33/2008	<20	<20	<20	<20	<20	**1280**	<20
B/FLORIDA/4/2006	<20	<20	<20	<20	<20	<20	**640**
A/BRISBANE/10/2010 (WT)	640	160	1280	<20	<20	<20	<20
Test antigen							
VI-1541	640	80	640	<20	<20	<20	<20

Actual antisera used were raised to:	
A1	A/CALIFORNIA/07/2009
A2	A/AUCKLAND/1/2009
A3	A/BRISBANE/2013/2009
H1	A/BRISBANE/59/2007
H3	A/PERTH/16/2009
B VIC	B/BRISBANE/33/2008
B YAM	B/FLORIDA/4/2006

Conclusion: Seed lot VI-1541 has a HI reactivity pattern that is consistent with an A/California/07/2009-like virus.

Ian Barr Deputy Director 10.09.2010