Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious IVR-153
NIBSC code: 09/236
Instructions for use
(Version 1.0, Dated 19/11/2009)

1. INTENDED USE

The influenza reference virus IVR-153 is a reassortant prepared by CSL Ltd using classical reassortant methodology from A/California/7/2009 (H1N1)v virus and IVR-6 virus, with the HA and NA genes donated from A/California/7/2009 (H1N1)v and the six internal genes donated from IVR-6. Reagent 09/236 is prepared from IVR-153 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-153 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.
IVR-153 has been tested in ferrets and found to be attenuated relative to wild type A/California/7/2009 virus.

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 H1N1v 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.

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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-153

Passage level	Lot	Laboratory
E1-E4		CSL, Melbourne, Australia
E5	VI-1525	CSL, Melbourne, Australia
E6	31270	NIBSC, Hertfordshire, UK

E = SPF eggs

Attached derivation (page 3 -6) as received from CSL 25th June 2009

FINAL REPORT ON THE PREPARATION AND TESTING OF:

Influenza Virus Reassortant Nº IVR-153, A/California/7/2009 (H1N1)v-like,

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

SPF LOT Nº VI-1525

PREPARATION

Preparation of A/California/7/2009 (H1N1)v-like influenza virus IVR-153, lot VI-1525 was carried out following procedures set out in Standard Operating Procedure RDS0030 and is documented on Batch Process Sheets: RDB0917 Lot 231 and RBD0936 Lot VI-1525.

This work was conducted in the Influenza Development department, R&D, CSL Limited.

VIRUS ISOLATE

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

A/California/7/2009 (H1N1) (Type A, Subtype H1N1)

Passages prior to receipt at WHO-CC:	E2,	HA=64
CDC ID #:		2009712112
WHO-CC Laboratory number:		0905025
Passages undertaken in WHO-CC:		nil

Derivation of A/California/7/2009 (H1N1)v-like influenza virus IVR-153, SPF lot VI-1525:

Mixed infection passage:	A/California/7/2009 (H1N1) Wild Type Virus @10^{-3} IVR-6* (H3N2) @10^{-3}	X HA titre ≥ 1810
	↓	
1st Antiserum Passage	Inoculum @ 10^{-3} with antiserum to IVR-6	HA titre 269
	↓	
2nd Antiserum Passage	Inoculum @ 10^{-3} with antiserum to IVR-6	HA titre 1076
	↓	
1st Limit dilution passage	Inoculum @ 10^{-8}	HA titre 663
	↓	
2nd Limit dilution passage	Inoculum @ 10^{-10}	HA titre 520
	↓	
Preparation of SPF Lot VI-1525	Inoculum @ 10^{-5}	mean HA titre ≥ 628

*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 = 5
Total number of passages since this virus was received from an approved laboratory = 6

SPF eggs supplied by SPAFAS were used for all passages.
HA titre was tested using chicken red blood cells at room temperature.

TESTING OF INFLUENZA VIRUS SPF LOT VI-1525:

<table>
<tr><th>Test</th><th colspan="3">Result</th></tr>
<tr><td>Sterility</td><td colspan="3">Pass</td></tr>
<tr><td>HA sequence</td><td colspan="3">A/California/7/2009 (H1N1)v-like
1 amino acid change Q240R</td></tr>
<tr><td>Antigenicity</td><td colspan="3">See WHO report on HI testing attached</td></tr>
<tr><td rowspan="3">Genotype
(by real time RT-PCR)</td><td colspan="3">6:2
i.e. 6 internal genes from IVR-6, which gives</td></tr>
<tr><td>A/California/7/2009</td><td>PR8</td><td>A/Texas/1/77</td></tr>
<tr><td>H1
N1</td><td>M
PA
PB2
NP
NS</td><td>PB1</td></tr>
<tr><td>Infectivity
EID50 (log_{10}/0.2mL)</td><td colspan="3">8.4</td></tr>
<tr><td>Appearance
(Electron Microscopy)</td><td colspan="3">The following morphologies were reported (in order of abundance): Small spheres, small ellipsoids, short filaments, medium filaments, medium spheres.</td></tr>
<tr><td>Pathogenicity
(Ferret pathogenicity test)</td><td colspan="3">Pass.
The IVR-153 reassortant virus is attenuated in ferrets relative to wt CA/07/09 (H1N1v) virus.</td></tr>
</table>

Disclaimer:

The material i.e. reassortant high growth virus IVR-153 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.

Biocontainment requirements for handling the candidate reassortant vaccine virus

The candidate reassortant vaccine virus contains infectious materials and should be handled only in appropriate containment facilities.

Attenuation of IVR-153 has been demonstrated by ferret pathogenicity testing. Therefore virus processing and /or vaccine production using fully trained and competent staff in accordance with national safety guidelines.
Recipient laboratories must accept full responsibility for the use and disposal of all materials.

Please note that by opening the enclosed vials containing the reassortant candidate vaccine viruses you agree to handle this virus following the containment guidance contained in WHO Technical Report Series No. 941.

Prepared by:
Peter Schoofs
Manager
Influenza Development, R&D
CSL Limited

Thursday, 25 June 2009

WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA
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Influenza Virus Seed Lot

Identity Test Report for: CSL Limited

Sample ID No.	951291	Test Code	CSL: QA 0050
Seed Lot No.	VI-1525	Date submitted	29.05.2009
Sample name	A/California/07/2009 (IVR - 153)	WHO ID No.	0905159

Test applied	**Haemagglutination Inhibition Assay**	Assay Date	29.05.2009
Assay performed by	T. Mastorakos & Chantal Baas		

		HI titre with reference antisera						
Reference antigen		A1	A2	A3	A4	B VIC	B YAM	H3
A/BRISBANE/59/2007	A(H1)	**80**	160	<20	<20	<20	<20	<40
IVR – 150 (A/GUAM/1/2007)	A(H1)	160	**1280**	<20	<20	<20	<20	<40
A/CALIFORNIA/04/2009	A(H1)	<20	<20	**160**	80	<20	<20	<40
A/CALIFORNIA/07/2009	A(H1)	<20	<20	640	**1280**	<20	<20	<40
B/VICTORIA/304/2006		<20	<20	<20	<20	**320**	<20	<40
B/FLORIDA/4/2006		<20	<20	<20	<20	20	**320**	<40
A/BRISBANE/10/2007	A(H3)	<20	<20	<20	<20	<20	<20	**640**
Test antigen								
VI-1525		<20	<20	1280	1280	<20	<20	<40

Actual antisera used were raised to:	A1	A/BRISBANE/59/2007
	A2	IVR – 150 (A/GUAM/1/2007)
	A3	A/CALIFORNIA/04/2009
	A4	A/CALIFORNIA/07/2009
	B VIC	B/VICTORIA/304/2006
	B YAM	B/FLORIDA/4/2006
	H3	A/BRISBANE/10/2007

Conclusion: Seed lot VI-1525 (IVR-153) has a HI reactivity pattern that is consistent with an A/California/07/2009-like virus.

Pass	Fail	Warn
■	☐	☐

Ian Barr
Deputy Director
29.05.2009

C:\Documents and Settings\wilsond\Local Settings\Temporary Internet Files\OLK149F\VI-1525.doc