



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

**1. INTENDED USE**

The influenza reference virus IVR-165 is a reassortant prepared by CSL Ltd using classical reassortant methodology from A/Victoria/361/2011 virus and A/Puerto Rico/8/34 virus. Reagent 11/220 is prepared from IVR-165 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-165 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°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 (labeled) end. Ensure that the disposable ampoule safety breaker 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<sup>-3</sup> to 10<sup>-5</sup>) 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](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](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	
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](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**

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> NA
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> No

**Passage history of IVR-165**

Passage level	Lot	Laboratory
E1-E3		VIDRL, Melbourne, Australia
D1 - D6	VI-1555	CSL, Melbourne, Australia
E6	34520	NIBSC, Hertfordshire, UK

E = SPF eggs

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



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

### 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:

[www.animalhealthaustralia.com.au/aahc/status/ahia.cfm](http://www.animalhealthaustralia.com.au/aahc/status/ahia.cfm)

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 @10<sup>-5</sup> x A/Puerto Rico/8/34 (H1N1)  
(@10<sup>-3</sup> HA titre 1040 (with CRBC

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1<sup>st</sup> Antiserum Passage Inoculum @ 10<sup>-3</sup> with antiserum to A/Puerto Rico/8/34 (H1N1) HA titre ND (with CRBC)



2<sup>nd</sup> Antiserum Passage Inoculum @ 10<sup>-3</sup> with antiserum to A/Puerto Rico/8/34 (H1N1) HA titre 502 (with CRBC)  
HA titre ≥1154 (with GPRBC)



1<sup>st</sup> Limit dilution passage Inoculum @ 10<sup>-8</sup> HA titre 710 (with CRBC)



2<sup>nd</sup> Limit dilution passage Inoculum @ 10<sup>-9</sup> HA titre 1114 (with CRBC)



Preparation of IVR-165 Lot VI-1555  
Inoculum @ 10<sup>-5</sup> 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 2<sup>nd</sup> Antiserum passage.

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

Test	Result	
Sterility	Pending	
Antigenicity	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	
Genotype (by real time RT-PCR)	6:2 H1, N1 from A/Victoria/361/2011 Remaining 6 internal genes from PR-8	
	A/Victoria/361/2011	PR8
	H3	
	N2	



**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:

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Wednesday, 15<sup>th</sup> February 2012



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INFLUENZA** MELBOURNE AUSTRALIA 10 Wreckyn St, North Melbourne, Victoria, 3051, Australia Phone:  
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## Influenza Virus Seed Lot Identity Test Report for: CSL Limited

Sample ID No.	1144782	Test Code	CSL: QA 0050
Seed Lot No.	VI-1555	Date submitted	14/02/2011
Sample name	IVR-165 (A/Victoria/361/2011)	WHO ID No.	1202012

<b>Test applied</b>	<b>Haemagglutination Inhibition Assay</b>	Assay Date	15 February 2012
Assay performed by	T. Mastorakos		

Reference antigen	HI titre with reference antisera									
	A1	A2	A3	A4	A(H1N1) pdm	B VIC	B YAM	H1		
A/PERTH/16/2009 A(H3)	<b>320</b>	<20	320	20	<40	<40	<20	<20		
A/BRISBANE/10/2007 A(H3)	20	<b>320</b>	80	20	<40	<40	<20	<20		
A/PERTH/10/2010 A(H3)	80	<20	<b>640</b>	40	<40	<40	<20	<20		
A/BRISBANE/59/2007 A(H1)	<20	<20	<20	<20	<40	<40	<20	<b>320</b>		
A/CALIFORNIA/07/2009 A(H1N1) pdm	<20	<20	<20	<20	<b>1280</b>	<40	<20	<20		
B/BRISBANE/33/2008 B VIC	<20	<20	<20	<20	<40	<b>1280</b>	<20	<20		
B/WISCONSIN/1/2010 B YAM	<20	<20	<20	<20	<40	<40	<b>160</b>	<20		
A/VICTORIA/361/2011 A(H3) (WT)	40	80	160	<b>640</b>	<40	<40	<20	<20		
<b>Test antigen</b>										
VI-1555	160	160	640	640	<40	<40	<20	<20		
Actual antisera used were raised to:	A1	A/PERTH/16/2009								
	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								
	H1	A/BRISBANE/59/2007								

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

Pass <input checked="" type="checkbox"/>	Fail <input type="checkbox"/>	Warn <input type="checkbox"/>
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**Ian Barr**  
Deputy Director  
15.02.2012