Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious IVR-178
NIBSC code: 14/308
Instructions for use
(Version 2.0, Dated 10/11/2015)

1. INTENDED USE

Reagent 14/ 308 is prepared from IVR-178 which was 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-178 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^{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

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

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 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

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

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-178 (Post mixed infection)

Passage	Lot	Laboratory
E1-E5		BioCSL, Australia
E6	VI-1602	BioCSL, Australia
E7	40200	NIBSC, Hertfordshire. UK

Derivation of IVR-178

A/New Caledonia/71/2014 – like High Growth Reassortant

A/New Caledonia/71/2014 (IVR-178, Lot VI-1602) is a H3N2 (3C.2a) high growth reassortant influenza virus.

PREPARATION

The preparation of A/New Caledonia/71/2014 (IVR-178, Lot VI-1602) high growth reassortant influenza virus was conducted in R&D Influenza Operations Department at bioCSL.

The high yielding parent strain used was A/Puerto Rico/8/34.

MATERIALS

The following materials of biological origin were used during the preparation of high growth reassortant IVR-178:

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

Supply details are:

A/New Caledonia/71/2014

WHO-CC Laboratory number:	1409151-1
Passages prior to receipt at WHO-CC:	None
Passages undertaken in WHO-CC:	5

Eggs: Specific Pathogen Free (SPF) Premium Plus eggs were used for all passages at bioCSL.

Antiserum: Trypsin-periodate treated sheep hyperimmune antiserum Lot# AS367, Sub-lot # 4830, raised against influenza virus A/Puerto Rico/8/34 .

The 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 obtaind from the following Animal Health Australia website link: http://www.animalhealthaustralia.com.au/programs/biosecurity

The trypsin used is 10x solution of gamma irradiated porcine pancreatic trypsin;

Invitrogen / Gibco Cat # 15090046, Lot No. 1142809

PASSAGE HISTORY:

Mixed infection passage:	A/New Caledonia/71/2014 wild type virus @10^{-5} x A/Puerto Rico/8/34 (H1N1) @10^{-3}	HA titre =788
	↓	
1st Antiserum Passage	Inoculum @ 10^{-3} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = ND
	↓	
2nd Antiserum Passage	Inoculum @ 10^{-3} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = 34
	↓	
1st Limit Dilution Passage	Inoculum @ 10^{-7}	HA titre = 160
	↓	
2nd Limit Dilution Passage	Inoculum @ 10^{-8}	HA titre = 92
	↓	
3rd Limit Dilution Passage	Inoculum @ 10^{-8}	HA titre = 65
	↓	
Preparation of IVR-178	*Lot VI-1602* Inoculum @ 10^{-5}	Mean HA titre = 102

*ND = Not detected

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.

TESTING OF A/NEW CALEDONIA/71/2014 INFLUENZA VIRUS SEED LOT (IVR-178, LOT VI-1602)

Test	Result		
Sterility (in accordance with EP/BP/USP)	Pass		
Antigenicity	Seed lot VI-1602 (IVR-178) has a HI reactivity pattern that is consistent with the wild type A/New Caledonia/71/2014 virus. Refer to WHO-CC Influenza Virus Seedlot Identity Test Report (attached)		
Genotype (by real time RT-PCR)	**5 : 3 (A/Puerto Rico/8/34 : A/New Caledonia/71/2014) Reassortant** A/Puerto Rico/8/34 PB1, PB2, PA, NP and Matrix genes were detected. NS gene not detected. A/New Caledonia/71/2014 (wild type virus) H3 and N2 genes were detected. NS gene from A/Puerto Rico/8/34 was not detected, indicating that the reassortant NS gene is from A/New Caledonia/71/2014 (wild type virus).		
	Gene	**A/Puerto Rico/8/34**	**A/New Caledonia/71/2014**
	H3		√
	N2		√
	H1	X	
	N1	X	
	PB1	√	NT
	PB2	√	NT
	PA	√	NT
	NP	√	NT
	M	√	NT
	NS	X	NT
Infectivity EID50 (log_{10}/0.2mL)	8.36		
Appearance (Electron Microscopy)	The following morphologies were reported (in order of abundance): Small spheres, small kidneys, short filaments, flattened virions.		

√ - positive by PCR X - negative by PCR NT – Not Tested

Disclaimer:

The material i.e. high growth reassortant virus IVR-178 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:

Lynda Allan
Scientist
R&D Operations-Influenza, bioCSL

Date: 06 / 03 / 15

Authorised by:

C N. Wadey

Christine Wadey
Associate Director
R&D Operations, bioCSL

Date: 06 / 03 / 15

WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA
MELBOURNE AUSTRALIA

792 Elizabeth St, Melbourne, Victoria, 3000, Australia
Phone: +61 3 9342 9300 Fax: +61 3 9342 9329
www.influenzacentre.org

Influenza Virus Seed Lot

Identity Test Report for: CSL Limited

Sample ID No.	1394753	Test Code	CSL: QA 0050
Seed Lot No.	VI-1602	Date submitted	13/02/2015
Sample name	IVR-178(A/New Caledonia/71/2014)	WHO ID No.	1502064

Test applied	**Haemagglutination Inhibition Assay**	Assay Date	13 February 2015
Performed by	Tasoula Mastorakos		

	HI titre with reference antisera					
Reference antigen	A1	A2	A3	A4	A5	A6
A/SWITZERLAND/9715293/2013	**640**	40	80	<80	<20	<20
A/NEW CALEDONIA/71/2014	40	**40**	320	<80	<20	<20
A/CANBERRA/82/2014	80	80	**320**	<80	<20	<20
A/CALIFORNIA/07/2009 A(H1N1)pdm	<20	<20	<20	**10240**	<20	<20
B/BRISBANE/33/2008 (B VIC)	<20	<20	<20	<80	**2560**	<20
B/PHUKET/3073/2013 (B YAM)	<20	<20	<20	<80	<20	**1280**
Test antigen						
VI-1602	40	80	320	<80	<20	<20

Actual antiserum used were raised to:	A1	A/SWITZERLAND/9715293/2013
	A2	A/NEW CALEDONIA/71/2014
	A3	A/CANBERRA/82/2014
	A4	A/CALIFORNIA/07/2009 A(H1N1)pdm
	A5	B/BRISBANE/33/2008 (B VIC)
	A6	B/PHUKET/3073/2013 (B YAM)

Conclusion: Seed lot VI-1602 has a HI reactivity pattern that is consistent with the wild-type virus A/New Caledonia/71/2014.

Pass ■	Fail □	Warn □

Ian Barr
Deputy Director
13.02.2015

S:\WHOFLU\Group\QC testing\WHO ID Reports\VI-1602.doc

WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA
MELBOURNE AUSTRALIA

792 Elizabeth St, Melbourne, Victoria, 3000, Australia
Phone: +61 3 9342 9300 Fax: +61 3 9342 9329
www.influenzacentre.org

Influenza Virus Seed Lot

Two way HI test for: CSL Limited

Sample ID No.	1394753	Test Code	N/A
Seed Lot No.	VI-1602	Date submitted	13/02/15
Sample name	IVR-178(A/New Caledonia/71/2014)	WHO ID No.	1502064

Test applied	**Haemagglutination Inhibition Assay**	Assay Date	5 March 2015
Performed by	Tasoula Mastorakos		

	HI titre with reference antisera					
Reference antigen	A1	A2	A3	A4	A5	
A/SOUTH AUSTRALIA/55/2014 H3N2	**320**	160	80	80	20	
A/SWITZERLAND/9715293/2013 H3N2	160	**640**	80	80	20	
A/NEW CALEDONIA/71/2014 H3N2	40	40	**640**	320	1280	
A/CANBERRA/82/2014 H3N2	40	40	640	**320**	1280	
Test antigen						
VI-1602 (IVR-178)	40	80	1280	320	**2560**	
Actual antiserum used were raised to:	A1	A/SOUTH AUSTRALIA/55/2014 (H3N2)				
	A2	A/SWITZERLAND/9715293/2013 (H3N2)				
	A3	A/NEW CALEDONIA/71/2014 (H3N2)				
	A4	A/CANBERRA/82/2014 (H3N2)				
	A5	IVR-178(A/NEW CALEDONIA/71/2014) (H3N2)				

Conclusion: Ferret antisera (A5) raised to seed lot VI-1602 (IVR-178) had HI titres that were similar (<4-fold) with its reactivity with A/New Caledonia/71/2014 wild type and with the homologous virus, hence VI-1602 passes the 2-way HI test.

Pass	Fail	Warn
■	□	□

Ian Barr
Deputy Director
11.3.2015

The Francis Crick Institute, Mill Hill Laboratory,
The Ridgeway, Mill Hill, London NW7 1AA. UK
info@crick.ac.uk www.crick.ac.uk

THE FRANCIS CRICK INSTITUTE

Thursday, 05 November 2015

Dr Ian Barr
WHO Collaborating Centre for Reference and Research on Influenza
VIRDL
Melbourne
Australia.

Dear Ian,

Here is a set of 2-way results for the HI results for IVR-178 (based on A/New Caledonia/71/2014).

Two antisera raised against A/Hong Kong/4801/2014 recognised IVR-178 in an HI test using guinea pig red blood cells done in the presence of 20nM Oseltamivir at titres 2-fold reduced over the titre of the antiserum for the homologous virus A/Hong Kong/4801/2014. An antiserum raised against IVR-178 recognised A/Hong Kong/4801/2014 in the HI test at a titre 2-fold higher than its titre for IVR-178; this antiserum also recognised A/New Caledonia/71/2014, at the same titre as it recognised A/Hong Kong/4801/2014. An antiserum raised against A/New Caledonia/71/2014 recognised IVR-178 at a titre within 2-fold of the titre of the homologous titre of the antiserum.

Therefore, IVR-178 is antigenically like its parent A/New Caledonia/71/2014, and it is also antigenically like A/Hong Kong/4801/2014.

The results are below.

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2015-11-03

	Viruses	Collection Date	Passage History	Post Infection Ferret antisera A/HK 4801/14 Egg F12/15	A/HK 4801/14 F42/15	A/New Cal 71/14 NIBSC F11/15	IVR-178 (A/NC/71/14) F44/15
	REFERENCE VIRUSES						
142089	A/Hong Kong/4801/2014	2014-02-26	E6/E2 Isolate 1	640	1280	2560	5120
150355	A/New Caledonia/71/2014	2014-08-13	E5/E2	640	1280	2560	5120
	IVR-178 (A/New Caledonia/71/14)		E5/D7/E1	320	640	1280	2560

1. < = <40 ND = NOT DONE

I hope that you find the results useful.

With best wishes,

Digitally signed by John William McCauley
Date: 2015.11.05 17:28:21 Z

John McCauley BSc PhD
Director, Crick Worldwide Influenza Centre.