Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious IVR-186
NIBSC code: 17/210
Instructions for use
(Version 2.0, Dated 04/04/2018)

1. INTENDED USE

Reagent 17/210 is prepared from IVR-186 (A/Singapore/INFIMH-16-0019/2016 x A/PR/8/34) (H3N2) which was processed for freeze drying in 250µl volumes as described by Campbell, PJ, Journal of Biological Standardisation, 1974, 2,249-267. The derivation and known passage history of IVR-186 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

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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: 0.25g per ampoule
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached:** No

Passage history of IVR-186

Passage	Lot	Laboratory
E1-E6		Seqirus, Australia
E7	VI-1620	Seqirus, Australia
E8	43310	NIBSC, Hertfordshire, UK

Sterility: no visible contamination was detected in a variety of media (tryptose soya broth, thioglycolate broth, Sabouraud's broth and blood agar plates) after 14 days incubation.

The HA and NA sequence of this virus is available from GISAID with the accession number EPI_ISL_285605.

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REPORT

Derivation of IVR-186
A/Singapore/INFIMH-16-0019/2016 – like High Growth Reassortant

A/Singapore/INFIMH-16-0019/2016 (IVR-186, Lot VI-1620) is a H3N2 high growth reassortant influenza virus.

PREPARATION

The preparation of A/Singapore/INFIMH-16-0019/2016 (IVR-186, Lot VI-1620) high growth reassortant influenza virus was conducted in R&D Influenza Operations Department at Seqirus.

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-186:

Virus Isolate:

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

Supply details are:
A/Singapore/INFIMH-16-0019/2016

WHO-CC Laboratory number:	1607127-1
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 Seqirus.

Antiserum:

Trypsin-periodate treated sheep hyperimmune antiserum Lot# AS367, Sub-lot # 4886 and 4929, 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 obtained 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. 1567868 and 1738602.

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PASSAGE HISTORY:

Mixed infection passage:	A/Singapore/INFIMH-16-0019/2016 wild type virus @10^{-3} x A/Puerto Rico/8/34 (H1N1)@10^{-3}	HA titre = 1114
	↓	
1st Antiserum Passage	Inoculum @ 10^{-3} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = 343
	↓	
2nd Antiserum Passage	Inoculum @ 10^{-3} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = 557
	↓	
*3rd Antiserum Passage / 1st Limit Dilution Passage **	Inoculum @ 10^{-7} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = 144
	↓	
*4th Antiserum Passage / 2nd Limit Dilution Passage **	Inoculum @ 10^{-8} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = 640
	↓	
3rd Limit Dilution Passage	Inoculum @ 10^{-6}	HA titre ≥ 1154
	↓	
Preparation of IVR-186	*Lot VI-1620* Inoculum @ 10^{-5}	Mean HA titre ≥ 493

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.

* Virus sample diluted to 10^{-3} dilution was mixed with antiserum to A/Puerto Rico/8/34 (H1N1) and incubated for 1 hour at room temperature. Incubated virus antiserum sample was serially diluted and inoculated into eggs.

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TESTING OF A/SINGAPORE/INFIMH-16-0019/2016 INFLUENZA VIRUS SEED LOT (IVR-186, LOT VI-1620)

Test	Result		
Sterility (in accordance with EP/BP/USP)	Pass		
Antigenicity	Pass		
Genotype (by real time RT-PCR)	**6 : 2 (A/Puerto Rico/8/34 : A/Singapore/INFIMH-16-0019/2016) Reassortant** A/Puerto Rico/8/34 PB1, PB2, PA, NP, Matrix and NS genes were detected. A/Singapore/INFIMH-16-0019/2016 (wild type virus) H3 and N2 genes were detected.		
	Gene	**A/Puerto Rico/8/34**	**A/Singapore/GP2646/2016**
	H3		√
	N2		√
	H1	X	
	N1	X	
	PB1	√	NT
	PB2	√	NT
	PA	√	NT
	NP	√	NT
	M	√	NT
	NS	√	NT
Infectivity EID50 (log_{10}/0.2mL)	7.69		
Appearance (Electron Microscopy)	The following morphologies were reported (in order of abundance): Whole virions (small spheres, medium spheres, a few filaments), lipid.		

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

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Disclaimer:

The material i.e. high growth reassortant virus IVR-186 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 (expressed or implied) including, without limitation, of satisfactory quality or fitness for a particular purpose.

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Date: 14 / Nov / 2017

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Influenza Virus Seed Lot

Identity Test Report for: Seqirus

Sample ID No.	1729459	Test Code	QE0050
Seed Lot No.	VI-1620	Date submitted	19/09/2017
Sample name	IVR-186(A/Singapore/INFIMH-16-0019/2016)	WHO ID No.	1710595

Test applied	**Haemagglutination Inhibition Assay**	Assay Date:	13 Nov 2017
Assay performed by:	Tasoula Zakis		

	HI titre with reference antisera							
Reference antigen	A1	A2	A3	A4	A5	A6	A7	A8
A/SWITZERLAND/9715293/2013 (AH3)	**640**	160	320	<80	<20	<20	160	40
A/NEW CALEDONIA/71/2014 (AH3)	40	**1280**	640	<80	<20	<20	640	640
A/HONG KONG/4801/2014 (AH3)	40	2560	**640**	<80	<20	<20	1280	1280
A/MICHIGAN/45/2015 A(H1N1)pdm	<20	<20	<20	**2560**	<20	<20	<20	<20
B/BRISBANE/33/2008 (B VIC)	<20	<20	<20	<20	**640**	<20	<20	<20
B/PHUKET/3073/2013 (B YAM)	<20	<20	<20	<20	<20	**1280**	<20	<20
A/SINGAPORE/INFIMH-16-0019/2016 (WT) (AH3)	40	2560	320	<80	<20	<20	**640**	640
Test antigen								
VI-1620	40	1280	320	<80	<20	<20	640	640

Actual antisera used were raised to:	A1	A/SWITZERLAND/9715293/2013	A(H3N2)
	A2	A/NEW CALEDONIA/71/2014	A(H3N2)
	A3	A/HONG KONG/4801/2014	A(H3N2)
	A4	A/MICHIGAN/45/2015	A(H1N1)pdm
	A5	B/BRISBANE/33/2008	(BVIC)
	A6	B/PHUKET/3073/2013	(B YAM)
	A7	A/SINGAPORE/INFIMH-16-0019/2016	(WT)
	A8	IVR-186(A/SINGAPORE/INFIMH-16-0019/2016)	

Conclusion: IVR-186 has a HI reactivity pattern that is consistent with the wild-type egg propagated virus A/Singapore/INFIMH-16-0019/2016 and therefore passes the One-Way HI test. IVR-186 also passes the Two-Way-HI test based on results obtained with antisera produced against the reassortant virus IVR-186 (A8).

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13.11.2017