Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious IVR-195
NIBSC code: 19/106
Instructions for use
(Version 3.0, Dated 21/05/2019)

1. INTENDED USE

Reagent 19/106 is prepared from IVR-195 (A/Kansas/14/2017 x A/PR/8/34) 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-195 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

Vials have a 'flip-up' circular cap. Either on the cap or the collar of the vial, there is an indication of the point at which to lever off the cap. This exposes an area of the stopper through which reconstitution and withdrawal of the preparation can be made using a hypodermic needle and syringe. If use of a pipette is preferred, then fully remove the metal collar using, for example, forceps, taking care to avoid cuts by wearing appropriate gloves. Remove the stopper for access. Care should be taken to prevent loss of the contents.

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

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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

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REPORT

Derivation of IVR-195
A/Kansas/14/2017 – like High Growth Reassortant

A/Kansas/14/2017 (IVR-195, Lot VI-1640) is a H3N2 high growth reassortant influenza virus.

PREPARATION

The preparation of A/Kansas/14/2017 (IVR-195, Lot VI-1640) 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/8/1934.

MATERIALS

The following materials of biological origin were used during the preparation of high growth reassortant IVR-195:

Virus Isolate:

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

Supply details are:
A/Kansas/14/2017:

WHO-CC Storage Lot number:	10006626
Passages prior to receipt at WHO-CC:	7
Passages undertaken in WHO-CC:	8

Eggs:

Specific Pathogen Free (SPF) eggs were used for all passages at Seqirus.

Antiserum:

Trypsin-periodate treated sheep hyperimmune antiserum Lot# AS367, subLot #4968, raised against influenza virus A/Puerto Rico/8/1934.

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. 1609000237.

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PASSAGE HISTORY:

Mixed infection passage:	A/Kansas/14/2017 wild type virus @10^{-3} x A/Puerto Rico/8/34 (H1N1)@10^{-5}	HA titre = 408*
	↓	
1st Antiserum Passage	Inoculum @ 10^{-3} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = 710
	↓	
*2nd Antiserum/1st Limit Dilution Passage***	Inoculum @ 10^{-4} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = 299
	↓	
*3rd Antiserum/2nd Limit Dilution Passage***	Inoculum @ 10^{-4} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = >1325
	↓	
3rd Limit Dilution Passage	Inoculum @ 10^{-6}	HA titre = >1576
	↓	
Preparation of IVR-195	*Lot VI-1640* Inoculum @ 10^{-5}	Mean HA titre =1114

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 guinea pig red blood cells (or fowl red blood cells as indicated by *) 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 at indicated dilution.

TESTING OF A/KANSAS/14/2017 INFLUENZA VIRUS SEED LOT (IVR-195, VI-1640)

Test	Result
Sterility (in accordance with EP/BP/USP)	Results pending
Antigenicity	Passed

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REPORT

Genotype (by real time RT-PCR)	**5 : 3 (A/Puerto Rico/8/34 : A/Kansas/14/2017) Reassortant** A/Puerto Rico/8/34 PB1, PB2, NP, Matrix and NS genes were detected. A/Kansas/14/2017 (wild type virus) H3 and N2 genes were detected.		
	Gene	**A/Puerto Rico/8/34**	**A/Kansas/14/2017**
	H3		√
	N2		√
	H1	X	
	N1	X	
	PB1	√	NT
	PB2	√	NT
	PA	X	NT
	NP	√	NT
	M	√	NT
	NS	√	NT
Infectivity EID50 (log_{10}/0.2mL)	Results pending		
Appearance (Electron Microscopy)	The following morphologies were reported (in order of abundance): Whole virions (spheres, short filaments, irregular particles, medium filaments).		

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

Disclaimer:

The material i.e. high growth reassortant virus IVR-195 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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Passage history of IVR-195 (post mixed infection)

Passage	Lot	Laboratory
E2-E7	unknown	Seqirus, Australia
E8	VI-1640	Seqirus, Australia
E9	44300	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 at GISAID with the accession number EPI_ISL_356702.