Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious IVR-228 (H3N2)
NIBSC code: 21/246
Instructions for use
(Version 2.0, Dated 24/09/2021)

1. INTENDED USE

Reagent 21/246 is prepared from IVR-228 which was processed in 250µl volumes as liquid stock. The derivation and known passage history of IVR-228 (H3N2) 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 vial contains 250µl (nominal) of infectious influenza virus as allantoic fluid from SPF embryonated hen's eggs.

5. STORAGE

Store in the dark at -70^{0}C or below

6. DIRECTIONS FOR OPENING

Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: If a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

7. USE OF MATERIAL

Ready to use

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: Clear liquid		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 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.

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: 0.25g per vial

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

Passage history of IVR-228 (H3N2)

Cumulative number of passages	Passage numbers at each stage	Lot	Laboratory
E1-E4	E4	Unknown	Unknown
E5-E12	E4/E8	Lot 539	Seqirus, Australia
E13	E4/E8/E1	46560	NIBSC, 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_4458423.

Seqirus A CSL Company

REPORT

PASSAGE HISTORY:

Mixed infection passage: (E4/D1)	A/Darwin/9/2021 wild type virus @10^{-3} x A/Puerto Rico/8/34 (H1N1) @10^{-5}	HA titre ≥ 1325
	↓	
1st Antiserum Passage (E4/D2)	Inoculum @ 10^{-3} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre ≥ 1280
	↓	
2nd Antiserum Passage (E4/D3)	Inoculum @ 10^{-3} with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = 1154
	↓	
*3rd Antiserum/1st Limit Dilution Passage*** (E4/D4)	Inoculum @ 10^{-7}	HA titre = 905
	↓	
*4th Antiserum/2nd Limit Dilution Passage*** (E4/D5)	Inoculum @ 10^{-7}	HA titre ≥1810
	↓	
3rd Limit Dilution Passage (E4/D6)	Inoculum @ 10^{-10}	HA titre ≥1236
	↓	
4th Limit Dilution Passage (E4/D7)	Inoculum @ 10^{-10}	HA titre ≥1810
	↓	
5th Limit Dilution Passage Lot 539 (E4/D8) IVR-228	Inoculum @ 10^{-5}	Mean HA titre ≥1684

** 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.

Total number of passages post mixed infection = 7
Total number of passages since this virus was received from an approved laboratory = 8

HA titres were determined using guinea pig red blood cells at room temperature.

TESTING OF A/DARWIN/9/2021 INFLUENZA VIRUS (IVR-228)

Test	Result		
Genotype (by real time RT-PCR)	**6 : 2 (A/Puerto Rico/8/34 : A/Darwin/9/2021) Reassortant** A/Darwin/9/2021 (wild type virus) genes H3 and N2 were detected. A/Puerto Rico/8/34 genes PB1, PB2, PA, NP, Matrix and NS were detected.		
	Gene	**A/Puerto Rico/8/34**	**A/Darwin/9/2021**
	H3		✓
	N2		✓
	H1	X	
	N1	X	
	PB1	✓	NT
	PB2	✓	NT
	PA	✓	NT
	NP	✓	NT
	M	✓	NT
	NS	✓	NT

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

Disclaimer:

The material i.e. high growth reassortant virus IVR-228 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.