



**Influenza Reagent**  
**Influenza Virus Infectious IVR-221 (H3N2)**  
**NIBSC code: 20/318**  
**Instructions for use**  
**(Version 8.0, Dated 24/07/2024)**

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**1. INTENDED USE**

Reagent 20/318 is prepared from IVR-221 (H3N2), a reassortant of A/Tasmania/503/2020 (H3N2) and A/PR/8/34 (H1N1), which was processed in 250µl volumes as liquid stock. The derivation and known passage history of 20/318 are 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°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](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

<b>Physical and Chemical properties</b>	
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	
<b>Toxicological properties</b>	
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
<b>Suggested First Aid</b>	
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.
<b>Action on Spillage and Method of Disposal</b>	
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](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> 0.25g per vial
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> No

Passage history of IVR-221 (H3N2)

Cumulative number of passages	Passage numbers at each stage	Lot	Laboratory
E5	E5	SL10039709	VIDRL, Australia
E14	E5/E9	Lot 496B	Seqirus, Australia
E15	E5/E9/E1	46010*	NIBSC, UK

\*The HA titre of this virus in 0.7% guinea pig red blood cells is 128. The infectious titre is unknown.

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

The HA and NA sequence of this virus are available at GISAID with the accession number EPI\_ISL\_1391371.



## REPORT

### Derivation of IVR-221 A/Tasmania/503/2020 – like High Growth Reassortant

A/Tasmania/503/2020 (IVR-221) is a H3N2 high growth reassortant influenza virus.

#### PREPARATION

The preparation of A/Tasmania/503/2020 (IVR-221) high growth reassortant influenza virus was conducted in R&D Influenza Operations Technical Development 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-221:

##### Virus Isolate:

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

Supply details are:

A/Tasmania/503/2020

WHO-CC Storage Lot number: SL10039709

Passages prior to receipt at WHO-CC: 0

Passages undertaken in WHO-CC: 5

##### Eggs:

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

##### Antiserum:

Trypsin-periodate treated sheep hyperimmune antiserum Lot# AS367, Sub-lot # 5014, 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: <https://www.animalhealthaustralia.com.au/what-we-do/disease-surveillance/tse-freedom-assurance-program/>

The trypsin used is 10x solution of gamma irradiated porcine pancreatic trypsin; Invitrogen / Gibco Cat # 15090046, Lot No. 1808000092.



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### PASSAGE HISTORY:

<i>Mixed infection passage:</i> (E5/D1)	A/Tasmania/503/2020 wild type virus @10 <sup>-3</sup> x A/Puerto Rico/8/34 (H1N1) @10 <sup>-3</sup>	HA titre ≥1810
	↓	
<i>1<sup>st</sup> Antiserum Passage</i> (E5/D2)	Inoculum @ 10 <sup>-3</sup> with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre = ND
	↓	
<i>2<sup>nd</sup> Antiserum Passage</i> (E5/D3)	Inoculum @ 10 <sup>-3</sup> with antiserum to A/Puerto Rico/8/34 (H1N1)	HA titre ≥1576
	↓	
<i>1<sup>st</sup> Limit Dilution Passage</i> (E5/D4)	Inoculum @ 10 <sup>-6</sup>	HA titre ≥1280
	↓	
<i>2<sup>nd</sup> Limit Dilution Passage</i> (E5/D5)	Inoculum @ 10 <sup>-7</sup>	HA titre ≥ 1810
	↓	
<i>3<sup>rd</sup> Limit Dilution Passage</i> (E5/D6)	Inoculum @ 10 <sup>-9</sup>	HA titre = 905
	↓	
<i>4<sup>th</sup> Limit Dilution Passage</i> (E5/D7)	Inoculum @ 10 <sup>-10</sup>	HA titre ≥1280
	↓	
<i>5<sup>th</sup> Limit Dilution Passage</i> (E5/D8)	Inoculum @ 10 <sup>-10</sup>	HA titre =640
	↓	
<i>6<sup>th</sup> Limit Dilution Passage</i> Lot 496B (E5/D9) IVR-221	Inoculum @ 10 <sup>-5</sup>	Mean HA titre ≥ 874



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Total number of passages post mixed infection = 8

Total number of passages since this virus was received from an approved laboratory = 9

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

ND =Not detected.

### TESTING OF A/TASMANIA/503/2020 INFLUENZA VIRUS (IVR-221)

Test	Result		
Genotype (by real time RT-PCR)	<b>5 : 3 (A/Puerto Rico/8/34 : A/Tasmania/503/2020) Reassortant</b>		
	A/Tasmania/503/2020 (wild type virus) genes H3 and N2 were detected. A/Puerto Rico/8/34 genes PB2, PA, NP, Matrix and NS were detected.		
	PB1 genes from A/Puerto Rico/8/34 were not detected, indicating that the reassortant PB1 genes are from A/Tasmania/503/2020 (wild type virus).		
	Gene	A/Puerto Rico/8/34	A/Tasmania/503/2020
	H3		✓
	N2		✓
	H1	X	
	N1	X	
	PB1	X	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-221 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



## REPORT

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(expressed or implied) including, without limitation, of satisfactory quality or fitness for a particular purpose.

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