Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious SAN-010
(A/Darwin/9/2021) (H3N2)
NIBSC code: 23/114
Instructions for use
(Version 1.0, Dated 12/05/2023)

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1. INTENDED USE

Reagent 23/114 is prepared from SAN-010 (H3N2), a reassortant of A/Darwin/9/2021 (H3N2) and A/Puerto Rico/8/34 (H1N1), which was processed in 250µl volumes as liquid stock. The derivation and known passage history of 23/114 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 ampoule 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
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.

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

Passage history of SAN-010 (H3N2)

Cumulative number of passages	Passage numbers at each stage	Lot	Laboratory
E3	E3	SL10042638	VIDRL, Australia
E10	E3/E7	SP-2021-010	Sanofi, USA
E11	E3/E7/E1	46700*	MHRA, UK

* The HA titre of this virus using 0.7% Guinea Pig Red blood cells is 512. The infectious titre is unknown.

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 are available at GISAID with the accession number EPI_ISL_17352397.

Derivation of SAN-010

A/Darwin/9/2021 High Growth Reassortant

A/Darwin/9/2021 (SAN-010) is an H3N2 high growth reassortant influenza virus.

A/Darwin/9/2021 (SAN-010) is an H3N2 high growth reassortant influenza virus developed in Sanofi Flu Reassortant Lab, department Bacterial and Viral Technology at Sanofi, US.

Sanofi Lot #: SP-2021-010

Wildtype Virus:

A/Darwin/9/2021 (the virus isolate was obtained from the VIDRL)

VIDRL Lot #: SL10042638

Passages prior to receipt at Sanofi: 3

Donor Virus:

A/Puerto Rico/8/1934 (SP Lot # 2239)

Eggs:

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

Antiserum:

Rabbit antisera raised against influenza virus A/Puerto Rico/8/1934 was used in the process.

Passage History

	Co-infection passage	A/Darwin/9/2021 (H3N2) wild type virus @ 10^{-2} x A/Puerto Rico/8/1934 (H1N1) @ 10^{-3}	HA titer GP:5120
		↓	
1	1st antiserum passage	Inoculum with A/Puerto Rico/8/1934 HANA antibodies @ 1:40 dilution	HA titer GP:2560
		↓	
2	2nd antiserum passage	Inoculum with A/Puerto Rico/8/1934 HANA antibodies @ 1:40 dilution	HA titer GP:5120
		↓	
3	3rd antiserum passage	Inoculum with A/Puerto Rico/8/1934 HANA antibodies @ 1:40 dilution	HA titer GP:1280
		↓	
4	1st Limit dilution passage	Inoculum @ 10^{-6} dilution	HA titer GP:10240
		↓	
5	2nd Limit dilution passage	Inoculum @ 10^{-7}	HA titer GP:5120
		↓	
6	Final amplification	Inoculum @ 10^{-5}	HA titer GP: 10240

Passages prior to receipt at Sanofi = 3

Total number of passages post co-infection = 6

Testing of A/Darwin/9/2021 SAN-010

Test	Results		
Sterility	No growth on Thioglycolate Medium and Trypticase Soy Broth after 10 days.		
Infectivity	$10^{8.7}$ EID_{50}/ mL		
Gene Ratio Determined by qPCR and confirmed by NGS.	5:3 reassortant HA, NA, & PB1 genes from A/Darwin/9/2021. Internal genes PB2, PA, NP, M, & NS from A/Puerto Rico/8/1934.		
	Gene	**A/Puerto Rico/8/1934**	**A/Darwin/9/2021**
	HA		+
	NA		+
	PB2	+	
	PB1		+
	PA	+	
	NP	+	
	M	+	
	NS	+	
Final HA titer for A/Darwin/9/2021 SAN-010 = 10240			
HA titers were determined using 1.0% guinea pig red blood cells at room temperature.			
HA-HPLC showed 3.8x increase compared to the original wildtype virus			

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