Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious CBER-48A
(A/Sydney/5/2021) (H1N1)
NIBSC code: 22/214
Instructions for use
(Version 3.0, Dated 22/04/2024)

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1. INTENDED USE

Reagent 22/214 is prepared from CBER-48A (H1N1), a ressortant of A/Sydney/5/2021 (H1N1) and A/Beijing/32/92 X-117 (H3N2), which was processed in 250µl volumes as liquid stock. The derivation and known passage history of 22/214 are attached.

2. CAUTION

The material is not of human or bovine origin. This preparation is not for administration to humans or animals.

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 -70ºC or below.
Material type: Liquid – will be shipped according to the storage and shipping conditions of the product

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 are held at NIBSC within assured, temperature-controlled storage facilities. 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 biological waste.

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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.

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 CBER-48A (H1N1)

Cumulative number of passages	Passage numbers at each stage	Lot	Laboratory
E3	E3	Unknown	VIDRL, Australia
E13	E3/E10	Unknown	CBER, USA
E14	E3/E10/E1	47240 *	MHRA (NIBSC), UK

*The HA titre of this virus using 0.7% turkey red blood cells is 512. 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 sequences of this virus are available at GISAID with the accession number EPI_ISL_ 16022232.

Report 31: Preparation and Testing of A/Sydney/5/2021 (H1N1) CBER-48A

Date reassortment initiated: 7 February 2022
Reassortment performed by: Laura Couzens

Date of report: 25 August 2022
Date of revision: 03 August 2023
Report reviewed by: Dr. Muhammad Shahabuddin

1. Summary

A/Sydney/5/2021 (H1N1), a representative H1N1 6B.1A.5a.2 group virus, was supplied to CBER by WHO Collaborating Centre for Reference and Research on Influenza, Melbourne Australia. The first passage at CBER yielded a stock with 128 HAU. To produce a high yield (HY) virus with potential to be recommended as a candidate vaccine virus, the wt virus was reassorted with a donor virus, A/Beijing/32/92 X-117 (H3N2) which has six internal genes from A/PR/8/34. A/Sydney/5/2021 reassortants were selected in the presence of rabbit antiserum raised against the HA/NA of A/BJ/32/92, and cloned by limiting dilution. All passages were performed in embryonated SPF chicken eggs (Charles River). While several reassortant viruses were characterized for antigenic relatedness to the parent wild type virus, CBER-48A is the only virus described in this report.

2. Source of virus isolate

Virus received from:	WHO-CC, Melbourne Australia, VIDRL
Strain Designation:	A/Sydney/5/2021 (H1N1)
Passages prior to receipt at CBER:	E3

3. Preparation of A/Sydney/5/2021 (H1N1) CBER-48A

The reassortant virus was prepared in embryonated SPF chicken eggs throughout. No other animal-derived material was used.

Passage 1 @10^{-3} (initial amplification at CBER, E1) HAU: 128
The amplified virus is labeled as CBER Lot 208, prepared on 27 January 2022

Passage 2 @10^{-2} (reassortment, E2)
A/BJ/32/92 (H3N2) Lot 109 was used as the donor virus at 10^{-3} dilution

Passage 3 (selection 1, E3) HAU: >2048
Rabbit anti- A/BJ/32/92 HA/NA (1:10) was mixed with virus (1:1 ratio) before inoculating eggs

Passage 4 (selection 2, E4) HAU: >2048
Rabbit anti- A/BJ/32/92 HA/NA (1:10) was mixed with virus (1:1 ratio) before inoculating eggs

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Passage 5	(selection 3, E5)	HAU: >2048

Rabbit anti- A/BJ/32/92 HA/NA (1:10) was mixed with virus (1:1 ratio) before inoculating eggs

Passage 6	(selection 4, E6)	HAU: 512

Rabbit anti- A/BJ/32/92 HA/NA (1:10) was mixed with virus (1:1 ratio) before inoculating eggs

Passage 7 @10^{-5}	(limiting dilution 1, E7)	HAU: 1024
Passage 8 @10^{-6}	(limiting dilution 2, E8)	HAU: 1024
Passage 9 @10^{-6}	(limiting dilution 3, E9)	HAU: ≥2048
Passage 10 @10^{-5}	(final amplification, E10)	HAU: 1024

The amplified A/Sydney/5/2021 CBER-48A virus (E3/E10) was prepared on 21 March 2022 and was the source material for one and two-way antigenic analysis, gene constellation determination, and measurement of HA concentration. This virus was used to infect ferrets to generate antiserum for two-way antigenic analysis.

4. Testing performed on A/Sydney/5/2021 (H1N1) CBER-48A

Test	Test description	Acceptance criteria	Result	Conclusion
Gene Constellation	TaqMan® real-time reverse-transcription PCR	HA and NA genes must be exclusively from wild type virus	HA: wild type NA: wild type M: PR8 NS1: PR8 NP: PR8 PA: PR8 PB1: PR8 PB2: PR8	PR8: wt 6:2
One-way antigenic test	HI titer of ferret anti-wt[a] against wt[b] and test virus	HI titers of test virus and wt parent equal or within 2-fold	Titer vs wt: 2560 Titer vs CBER-48A: 1280	Pass
Two-way antigenic test	HI titers of ferret anti-wt[a] and anti-test virus[c] against wt and test virus	HI titers of test virus and wt parent equal or within 2-fold	See antigenic analysis results, attached	Pass
Sterility	Inoculation of blood agar plates[d]	No growth	No growth	Pass
HA units	Titration on turkey red blood cells	HAU of test virus greater or equal to wt	wt[e]: 128 HAU CBER-48A: 1024 HAU	Pass

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HA concentration	Isotope dilution mass spectrometry	HA yield of test virus greater than wt (Results shown as HA/total protein %)	wt[e]: 9.8% CBER-48A: 15.9% comparator: ND	Pass
HA and NA sequence	Performed by WHO-CC, Melbourne	List HA and NA sequence changes between reassortant and parental viruses	HA: D187E NA: no changes	HA and NA sequence

[a]Ferret anti-wild type A/Sydney/5/2021 (H1N1) serum was generated at WHO-CC Melbourne by infecting a ferret with wild type A/Sydney/5/2021
[b]Wild type A/Sydney/5/2021
[c]Ferret anti- A/Sydney/5/2021 CBER-48A sera were generated at CBER by infecting two ferrets with the final amplified virus (E3/E10)
[d]Tryptic™ Soy Agar with 5% Sheep Blood
[e]Wild type A/Sydney/5/2021 (E3/E1), Lot 208

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