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 (H3N2), which was processed in 250µl volumes as liquid stock. The known passage history of 22/214 is attached.

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

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.

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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Clear liquid</td>
<td>Effects of inhalation: Likelihood of influenza virus infection.</td>
</tr>
<tr>
<td>Corrosive: No</td>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
<tr>
<td>Oxidising: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Suggested First Aid</td>
</tr>
<tr>
<td>Irritant: No</td>
<td>Inhalation: Seek medical advice</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Ingestion: Seek medical advice</td>
</tr>
<tr>
<td>Handling: See caution, Section 2</td>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Other (specify): Live influenza virus</td>
<td>Contact with skin: Wash thoroughly with water.</td>
</tr>
</tbody>
</table>

Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological 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.

16. INFORMATION FOR CUSTOMS USE ONLY

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*</th>
<th>United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>* 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.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Net weight:</th>
<th>0.25g per vial.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity Statement:</td>
<td>Non-toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable.</td>
<td>Attached: No</td>
</tr>
</tbody>
</table>

**Passage history of CBER-48A (H1N1)**

<table>
<thead>
<tr>
<th>Cumulative number of passages</th>
<th>Passage numbers at each stage</th>
<th>Lot</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3</td>
<td>E3</td>
<td>Unknown</td>
<td>VIDRL, Australia</td>
</tr>
<tr>
<td>E13</td>
<td>E3/E10</td>
<td>Unknown</td>
<td>CBER, USA</td>
</tr>
<tr>
<td>E14</td>
<td>E3/E10/E1</td>
<td>47240 *</td>
<td>MHRA (NIBSC), UK</td>
</tr>
</tbody>
</table>

*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 (tryptose 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_1602232.
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  Report reviewed by: Dr. Muhammad Shahabuddin

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 (H3N2). 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.

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

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

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 @10⁶ (limiting dilution 2, E8)  HAU: 1024  
Passage 9 @10⁶ (limiting dilution 3, E9)  HAU: ≥2048  
Passage 10 @10⁵ (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.

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Test description</th>
<th>Acceptance criteria</th>
<th>Result</th>
<th>Conclusion</th>
</tr>
</thead>
</table>
| Gene Constellation           | TaqMan® realtime reversetranscription 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-wt⁴ against wt⁵ and test virus                           | HI titer 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 titer of ferret anti-wt⁴ and antitest virus against wt and test virus          | HI titer of test virus and wt parent equal or within 2-fold                         | See antigenic analysis results, attached                              | Pass       |
| Sterility                    | Inoculation of blood agar plates⁶                                                | No growth                                                                           | No growth                                                             | Pass       |
| HA units                     | Titration on turkey red blood cells                                              | HAU of test virus greater or equal to wt                                            | wt³: 128 HAU  
CBER-48A: 1024 HAU                                                   | Pass       |
| HA concentration            | Isotope dilution mass spectrometry                                              | HA yield of test virus greater than wt (Results shown as HA/total protein %)       | wt³: CBER-48A: comparator:                                           | Not performed |
| 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 |
aFerret 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
bWild type A/Sydney/5/2021 Ferret anti- A/Sydney/5/2021 CBER-48A sera were generated at CBER by infecting two ferrets with the final amplified virus (E3/E10)
cTryptic™ Soy Agar with 5% Sheep Blood
dWild type A/Sydney/5/2021 (E3/E1), Lot 208