Influenza Reagent
Influenza Virus Infectious CNIC-2206
(A/Guizhou-Liuzhite/326/2022) (H3N2)
NIBSC code: 22/180
Instructions for use
(Version 1.0, Dated 14/10/2022)

1. INTENDED USE
Reagent 22/180 is prepared from CNIC-2206 (H3N2), a ressortant of A/Guizhou-Liuzhite/326/2022 and A/PR/8/34, which was processed in 250µl volumes as liquid stock. The known passage history of CNIC-2206 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 -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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Clear liquid</td>
</tr>
<tr>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
</tr>
<tr>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
</tr>
<tr>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Live influenza virus</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
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</thead>
<tbody>
<tr>
<td>Effects of inhalation: Likelihood of influenza virus infection.</td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggested First Aid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation: Seek medical advice</td>
</tr>
<tr>
<td>Ingestion: Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Contact with skin: Wash thoroughly with water.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Action on Spillage and Method of Disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
</tr>
</tbody>
</table>
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 CNIC-2206 (H3N2)

<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>E9</td>
<td>E9</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>E16</td>
<td>E9/E7</td>
<td>20220815A</td>
<td>CNIC, China</td>
</tr>
<tr>
<td>E17</td>
<td>E9/E7/E1</td>
<td>47180</td>
<td>NIBSC, UK</td>
</tr>
</tbody>
</table>

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_15157782.
**CNIC-2206 HIGH GROWTH REASSORTANT REPORT**

**High Growth Reassortant**  
CNIC-2206 (Lot:20220815A)  

**Parental Wild Type virus**  
A/Guizhou-Lijizhe/326/2022  

**Conclusion**  
TWO-WAY PASS

- **Two-way HI test**
  The high growth reassortant (HGR) virus was antigenically characterized by a “two-way” Hemagglutination Inhibition (HI) test. Ferret antisera raised against the wild type parental virus A/Guizhou-Lijizhe/326/2022 well inhibited the HGR CNIC-2206 (within two-fold reduction of HI titers compared with the homologous virus). Similarly, ferret antisera raised against the HGR CNIC-2206 well inhibited the parental virus. Therefore, CNIC-2206 is antigenically similar to A/Guizhou-Lijizhe/326/2022 and passes the two-way test.

  **HEMAGGLUTINATION INHIBITION ASSAY OF INFLUENZA A VIRUSES**
  TESTED 09/28/2022

<table>
<thead>
<tr>
<th>REFERENCE FERRET ANTISERA</th>
<th>A/Guizhou-Lijizhe/326/2022</th>
<th>CNIC-2206</th>
<th>PASSAGE HISTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Guizhou-Lijizhe/326/2022</td>
<td>1280</td>
<td>320</td>
<td>E9</td>
</tr>
<tr>
<td>CNIC-2206</td>
<td>640</td>
<td>640</td>
<td>E9/E7</td>
</tr>
</tbody>
</table>

- **GENETIC CHARACTERIZATION**
  Whole genome sequencing of HGR CNIC-2206 was performed. Comparing with wild type parental virus A/Guizhou-Lijizhe/326/2022 (E9), the HA gene of CNIC-2206 acquired amino acids change at positions D225G and P227S. There was no amino acid change in the NA.

  For the internal genes, CNIC-2206 has M, NP, NS, PA, PB1 and PB2 genes from A/PR/8/34, making it a 6:2 reassortant.

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WHO Collaborating Center for Reference and Research on Influenza  
National Institute for Viral Disease Control and Prevention, China CDC
Derivation of CNIC-2206 High Growth Reassortant

Strain: A/Guizhou-Luohite/326/2022
Passage undertaken at CNIC

1st Mixed Infection: A/Guizhou-Luohite/326/2022 (10^{-3}) \times A/PR/8/34 (10^{-9})
HA Titer: 2048

2nd Antiserum passage: Inoculum 10^{-3} with A/PR/8/34 & NA antiserum
HA Titer: 64

3rd Antiserum passage: Inoculum 10^{-1} with A/PR/8/34 & NA antiserum
HA Titer: 64

4th Antiserum passage: Inoculum 10^{-2} with A/PR/8/34 & NA antiserum
HA Titer: 8

5th passage: Inoculum 10^{-7}
HA Titer: 512

6th passage: Inoculum 10^{-4}
HA Titer: 8

7th passage: Inoculum 10^{-5}
HA Titer: 32

Total number of passages since mixed infection=7
SPF eggs were used for all passages.