Influenza Reagent
Influenza Virus Infectious BVR-25 (B-Victoria Lineage)
NIBSC code: 21/216

Instructions for use
(Version 2.0, Dated 24/09/2021)

1. INTENDED USE
Reagent 21/216 is prepared from BVR-25 which was processed in 250µl volumes as liquid stock. The derivation and known passage history of BVR-25 (B-Victoria Lineage) is 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/

NIBSC follows the policy of WHO with respect to its reference materials.

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>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>
</tbody>
</table>

| 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.

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*: 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>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Net weight: 0.25g per vial</th>
</tr>
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</table>

Toxicity Statement: Non-toxic

Veterinary certificate or other statement if applicable.

Attached: No

Passage history of BVR-25 (B-Victoria Lineage)

<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>E1-E4</td>
<td>E4</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>E5-E13</td>
<td>E4/E9</td>
<td>LOT 497C</td>
<td>Seqirus, Australia</td>
</tr>
<tr>
<td>E14</td>
<td>E4/E9/E1</td>
<td>46500</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_4458445.
PASSAGE HISTORY:

Mixed infection passage
(E4/D1)
B/Victoria/2110/2019 wild type virus
@10³ x B/Brisbane/46/2015 @10⁻⁸
↓
1st Antiserum Passage
(E4/D2)
Inoculum @ 10³ with Mab to
B/Brisbane/60/2008
↓
2nd Antiserum/1st Limit
Dilution Passage
(E4/D3)
Inoculum @ 10⁶ with Mab to
B/Brisbane/60/2008
↓
3rd Limit Dilution Passage
(E4/D4)
Inoculum @ 10⁹
↓
4th Limit Dilution Passage
(E4/D5)
Inoculum @ 10⁶
↓
5th Limit Dilution Passage
(E4/D6)
Inoculum @ 10³
↓
6th Limit Dilution Passage
(E4/D7)
Inoculum @ 10⁴
↓
7th Limit Dilution Passage
(E4/D8)
Inoculum @ 10⁻⁸
↓
8th Limit Dilution Passage
Lot 497C (E4/D9)
BVR-25

HA titre = 39
HA titre ≥ 1689
HA titre = 844
HA titre = 1040
HA titre ≥ 1256
HA titre ≥ 1749
HA titre ≥ 1154
HA titre ≥ 1810
Mean HA titre = 403
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 fowl red blood cells at room temperature.

** Virus sample diluted to 10⁻¹ dilution was mixed with Mab to B/Brisbane/60/2008 and incubated for 1 hour at room temperature. Incubated virus/MAb sample was serially diluted and inoculated into eggs at indicated dilution.

**TESTING OF B/VICTORIA/2110/2019 (BVR-25) INFLUENZA VIRUS**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (by DNA sequencing)</td>
<td>1:7 (B/Brisbane/46/2015: B/Victoria/2110/2019) Reassortant B/Victoria/2110/2019 (wild type virus) HA, NA, PB1, PB2, PA, NP and NS genes were detected B/Brisbane/46/2015 (HGP) Matrix (M) gene was detected.</td>
</tr>
<tr>
<td>Gene</td>
<td>B/Victoria/2110/2019</td>
</tr>
<tr>
<td>HA</td>
<td>√</td>
</tr>
<tr>
<td>NA</td>
<td>√</td>
</tr>
<tr>
<td>PB1</td>
<td>√</td>
</tr>
<tr>
<td>PB2</td>
<td>√</td>
</tr>
<tr>
<td>PA</td>
<td>√</td>
</tr>
<tr>
<td>NP</td>
<td>√</td>
</tr>
<tr>
<td>M</td>
<td>X</td>
</tr>
<tr>
<td>NS</td>
<td>√</td>
</tr>
</tbody>
</table>

√ - positive by Sequencing  
X - negative by Sequencing

Disclaimer:
The material i.e. high growth reassortant virus BVR-25 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 (expressed or implied) including, without limitation, of satisfactory quality or fitness for a particular purpose.