Influenza Reagent
Influenza Virus Infectious
B/Austria/1359417/2021
(B-Victoria lineage) BVR-26
NIBSC code: 22/204
Instructions for use
(Version 1.0, Dated 13/10/2022)

1. INTENDED USE
Reagent 22/204 is prepared from BVR-26 (B-Victoria lineage), a reassortant of B/Austria/1359417/2021 and B/Brisbane/46/2015, which was processed in 250µl volumes as liquid stock. The known passage history of BVR-26 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>
<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>Stable: Yes</td>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Live influenza virus</td>
<td></td>
</tr>
</tbody>
</table>

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

**Passage history of BVR-26 (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>E3</td>
<td>E3</td>
<td>VW10042525</td>
<td>VIDRL, Australia</td>
</tr>
<tr>
<td>E12</td>
<td>E3/E9</td>
<td>533B</td>
<td>Seqirus, Australia</td>
</tr>
<tr>
<td>E13</td>
<td>E3/E9/E1</td>
<td>46570</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_15158311.
Derivation of BVR-26
B/Austria/1359417/2021 – High Growth Reassortant

B/Austria/1359417/2021 (BVR-26) is a B high growth reassortant influenza virus.

PREPARATION
The preparation of B/Austria/1359417/2021 (BVR-26) high growth reassortant influenza virus was conducted in R&D Influenza Operations Department at Seqirus.

The high yielding parent strain used was B/Brisbane/46/2015.

MATERIALS
The following materials of biological origin were used during the preparation of high growth reassortant BVR-26:

Virus Isolate:
The virus isolate was obtained from the WHO Collaborating Centre for Reference & Research on Influenza, Melbourne.

Supply details are:
B/Austria/1359417/2021
Laboratory number: VW10042525
Passages prior to receipt at Seqirus: 3

Eggs:
Specific Pathogen Free (SPF) eggs were used for all passages at Seqirus.

Antiserum:
Monoclonal Antibody BBR3.7E8.1B9 (15/03/19B) raised against influenza virus B/Brisbane/60/2008.

The MAb was prepared from murine hybridoma cells derived from mice immunized with inactivated B/Brisbane/60/2008 influenza virus.

Note on Transmissible Spongiform Encephalopathies (TSEs):
Australia and New Zealand have been declared TSE free in accordance with OIE guidelines. Detailed information on Australia’s animal health status can be obtained from the following Animal Health Australia website link: https://animalhealthaustralia.com.au

The MAb was gamma irradiated at 35SieGrays prior to use.
### PASSAGE HISTORY:

<table>
<thead>
<tr>
<th>Passage Type</th>
<th>Inoculum</th>
<th>HA titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed injection passage:</td>
<td>B/Austria/1359417/2021 wild type virus @10&lt;sup&gt;-3&lt;/sup&gt; x B/Br/66/2015 @10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Antiserum Passage</td>
<td>Inoculum @ 10&lt;sup&gt;-3&lt;/sup&gt; with Mab to B/Br/66/2008</td>
<td>10</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Antiserum Passage</td>
<td>Inoculum @ 10&lt;sup&gt;-3&lt;/sup&gt; with Mab to B/Br/66/2008</td>
<td>520</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; Antiserum/1st Limit Dilution Passage**</td>
<td>Inoculum @ 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>57</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; Antiserum/2nd Limit Dilution Passage**</td>
<td>Inoculum @ 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>453</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt; Limit Dilution Passage</td>
<td>Inoculum @ 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>970</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt; Limit Dilution Passage</td>
<td>Inoculum @ 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>251</td>
</tr>
<tr>
<td>7&lt;sup&gt;th&lt;/sup&gt; Limit Dilution Passage</td>
<td>Inoculum @ 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>299</td>
</tr>
<tr>
<td>8&lt;sup&gt;th&lt;/sup&gt; Limit Dilution Passage</td>
<td>Inoculum @ 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>Mean HA titre = 1029</td>
</tr>
<tr>
<td>Lot 533B (E3/D9)</td>
<td></td>
<td></td>
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<tr>
<td>BVR-26</td>
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</tbody>
</table>
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 10^3 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/AUSTRIA/1359417/2021 (BVR-26) INFLUENZA VIRUS **

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (by DNA sequencing)</td>
<td>6:2 (B/Brisbane/46/2015: B/Austria/1359417/2021) Reassortant B/Austria/1359417/2021 (wild type virus) HA and NA were detected. B/Brisbane/46/2015 (HGP) PB1, PB2, PA, NP, Matrix and NS genes were detected.</td>
</tr>
<tr>
<td>Gene</td>
<td>B/Austria/1359417/2021</td>
</tr>
<tr>
<td>HA</td>
<td>√</td>
</tr>
<tr>
<td>NA</td>
<td>√</td>
</tr>
<tr>
<td>PB1</td>
<td>X</td>
</tr>
<tr>
<td>PB2</td>
<td>X</td>
</tr>
<tr>
<td>PA</td>
<td>X</td>
</tr>
<tr>
<td>NP</td>
<td>X</td>
</tr>
<tr>
<td>M</td>
<td>X</td>
</tr>
<tr>
<td>NS</td>
<td>X</td>
</tr>
</tbody>
</table>

√ - positive by Sequencing  
X - negative by Sequencing

Disclaimer:
The material i.e. high growth reassortant virus BVR-26 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.
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