Influenza Reagent
Influenza anti-B/Phuket/3073/2013-like (B Yamagata lineage) HA serum
NIBSC code: 22/132
Instructions for use
(Version 1.0, Dated 27/02/2024)

1. INTENDED USE
Influenza antisera reagent 22/132 is prepared for single radial diffusion assay of B/Phuket/3073/2013-like antigens using an appropriate NIBSC antigen reagent.

The antisera reagent was prepared in sheep SH792, SH793, SH794, SH795, SH796 and SH797 using the purified HA of a B/Phuket/3073/2013-like virus. The HA antigens were extracted from purified virus by treatment with bromelain and purified by sedimentation on sucrose gradients (Brand, CN and Skehel, JJ, Nature, New Biology, 1972, 238, 145-147).

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.
The immunization schedule for sheep SH792, SH793, SH794, SH795, SH796 and SH797 was as follows: one dose of approximately 100 µg of B/Phuket/3073/2013 virus HA with Freund’s Complete Adjuvant (FCA) was given intramuscularly, followed two weeks later with a 50 microgram dose of B/Phuket/3073/2013 including Freund’s Incomplete Adjuvant (FIA). For SH792, SH795 and SH797 two further 50 microgram doses of B/Phuket/3073/2013 HA including FIA were given after a week. For SH793 and SH796 three further 50 microgram doses of B/Phuket/3073/2013 HA including FIA were given after a week. Six weeks after the initial immunization, serum was collected and sodium azide (0.05% w/v) added.

The antisera was then treated by an APHIS approved method for the inactivation of FMDV.

The antisera obtained from sheep SH792, SH793, SH794, SH795, SH796 and SH797 were pooled, diluted 1 : 5 with PBS buffer containing sodium azide (0.05% w/v), and filled into vials in 2ml volumes.

5. STORAGE
+2-8°C

However, if it is intended to store the reagent for long periods i.e. >2 years, they may be stored at -20°C. The antisera can be frozen and thawed without any adverse impact on its use in the SRD assay.

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
For the assay of antigens containing 20-50 micrograms of HA activity in 1ml, approximately 15-25 µl of the undiluted reagent should be added to 1ml agarose. It may be necessary to change the antisera concentrations depending on the B/Phuket/3073/2013-like antigen standard used or according to local laboratory conditions. Antiserum Reagent 22/132 should be used according to the method described by Wood, JM, Schild, GC, Newman, RW and Seagroatt, VA. Journal of Biological Standardisation, 1977, 5, 2.

8. STABILITY
It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. Once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

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

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES
None

10. ACKNOWLEDGEMENTS
None

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: 1

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Liquid</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): (0.05% w/v)</td>
<td>Contains Sheep Serum and Sodium Azide</td>
</tr>
</tbody>
</table>

| Toxicological properties       |          |
| Effects of inhalation:         | Not established, avoid inhalation |
| 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 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*: United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net weight: 2g</td>
</tr>
<tr>
<td>Toxicity Statement: Non toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable.</td>
</tr>
<tr>
<td>Attached: Yes SH792 SH793 SH794 SH795 SH796 SH797</td>
</tr>
</tbody>
</table>

* 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: 2g
Toxicity Statement: Non toxic
Veterinary certificate or other statement if applicable.
Attached: Yes SH792 SH793 SH794 SH795 SH796 SH797
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 056403038158 [Virology no. SH792], which has been used in the production of blood antiserum between 27th April 2022 and 8th June 2022. Both the ear tag number and the animals' record show that it is of UK origin.

This animal was a breeding Ewe which became surplus to requirements. In my opinion at the time of clinical examination, the ewe was in good health and showed no clinical signs of infectious disease.

Alex McSloy MA VetMB DipACVIM PhD MRCVS
Named Veterinary Surgeon

Date signed: 09 JUNE 22

__________________________
Alex McSloy
Named Veterinary Surgeons (NVS) Group
The Royal Veterinary College, Royal College Street, London NW1 0TU
Tel: +44 (0)1707 666333, E-mail: amcsloy@rvc.ac.uk
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 024126905824 [Virology no. SH793], which has been used in the production of blood antiserum between 27th April 2022 and 8th June 2022. Both the ear tag number and the animals' record show that it is of UK origin.

This animal was a breeding Ewe which became surplus to requirements. In my opinion at the time of clinical examination, the ewe was in good health and showed no clinical signs of infectious disease.

Alex McSloy MA VetMB DipACVIM PhD MRCVS
Named Veterinary Surgeon

Date signed: 07 JUNE 22

Alex McSloy
Named Veterinary Surgeon (NVS) Group
The Royal Veterinary College, Royal College Street, London NW1 0TU
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VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 056171221856 [Virology no. SH794], which has been used in the production of blood antiserum between 27th April 2022 and 8th June 2022. Both the ear tag number and the animals' record show that it is of UK origin.

This animal was a breeding Ewe which became surplus to requirements. In my opinion at the time of clinical examination, the ewe was in good health and showed no clinical signs of infectious disease.

Alex McSloy MA VetMB DipACVIM PhD MRCVS
Named Veterinary Surgeon

Date signed: 07 JUNE 22

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VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 056165630834 [Virology no. SH795], which has been used in the production of blood antiserum between 27th April 2022 and 8th June 2022. Both the ear tag number and the animals' record show that it is of UK origin.

This animal was a breeding Ewe which became surplus to requirements. In my opinion at the time of clinical examination, the ewe was in good health and showed no clinical signs of infectious disease.

Alex McSloy MA VetMB DipACVIM PhD MRCVS
Named Veterinary Surgeon

Date signed: 07 JUNE 22

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VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 010100211220 [Virology no. SH796], which has been used in the production of blood antiserum between 27th April 2022 and 8th June 2022. Both the ear tag number and the animals’ record show that it is of UK origin.

This animal was a breeding Ewe which became surplus to requirements. In my opinion at the time of clinical examination, the ewe was in good health and showed no clinical signs of infectious disease.

Alex McSloy MA VetMB DipACVIM PhD MRCVS
Named Veterinary Surgeon

Date signed: 07 June 22

Alex McSloy
Named Veterinary Surgeons (NVS) Group
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Tel: +44 (0)1707 666333, E-mail: amcsloy@rvc.ac.uk
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 02415124517 [Virology no. SH797], which has been used in the production of blood antiserum between 27th April 2022 and 8th June 2022. Both the ear tag number and the animals' record show that it is of UK origin.

This animal was a breeding Ewe which became surplus to requirements. In my opinion at the time of clinical examination, the ewe was in good health and showed no clinical signs of infectious disease.

Alex McSloy MA VetMB DipACVIM PhD MRCVS
Named Veterinary Surgeon

Date signed: 7 June 22

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