Influenza Reagent
Influenza anti A/Brisbane/10/2007 (H3N2)-Like HA
NIBSC code: 08/246
Instructions for use
(Version 1.0, Dated 21/01/2009)

1. INTENDED USE
Influenza antiserum reagent 08/246 is prepared for single radial diffusion assay of A/Brisbane/10/2007-like antigens using an appropriate NIBSC antigen reagent.

The antiserum reagent was prepared in sheep 493, 495 and 496 to the purified HA of NYMCX-175C. 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 493 was as follows: One dose of approximately 50 µg of NYMCX-175C HA with Freund's Complete Adjuvant (FCA) was given intramuscularly, followed two weeks later with a 10 microgram dose of HA including Freund's Incomplete Adjuvant (FIA). Four weeks after the initial immunization, serum was collected and sodium azide (0.05% w/v) added.
The immunization schedule for both sheep 495 and 496 was as follows: One dose of approximately 50 µg NYMCX-175C HA with Freund's Complete Adjuvant (FCA) was given intramuscularly, followed two weeks later with a 10 microgram dose of including Freund's Incomplete Adjuvant (FIA). Four further 10 microgram doses of NYMCX-175C HA including FIA were given at weekly intervals. Seven weeks after the initial immunization, serum was collected and sodium azide (0.05% w/v) added.
The sera were then pooled and treated by an APHIS approved method for the inactivation of FMDV. It was then diluted 1:3 with PBS buffer containing sodium azide (0.05% w/v) and filled into vials in 2ml volumes.

5. STORAGE
+2-8°C
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING
Vials have a ‘flip-up’ circular cap. Either on the cap or the collar of the vial, there is an indication of the point at which to lever off the cap. This exposes an area of the stopper through which reconstitution and withdrawal of the preparation can be made using a hypodermic needle and syringe. If use of a pipette is preferred, then fully remove the metal collar using, for example, forceps, taking care to avoid cuts by wearing appropriate gloves. Remove the stopper for access. Care should be taken to prevent loss of the contents.

7. USE OF MATERIAL
For the assay of antigens containing 20-50 micrograms of HA activity in 1ml, approximately 15µl of the undiluted Reagent should be added to 1ml agarose. It may be necessary to change the antiserum concentrations according to local laboratory conditions.
Antiserum Reagent 08/246 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. Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

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

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN8 3QG. Tel: +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory
Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

### Physical and Chemical properties

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

### Toxicological properties

<table>
<thead>
<tr>
<th>Route of Exposure</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation</td>
<td>Not established, avoid inhalation</td>
</tr>
<tr>
<td>Ingestion</td>
<td>Not established, avoid ingestion</td>
</tr>
<tr>
<td>Skin absorption</td>
<td>Not established, avoid contact with skin</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**

- **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**: 2g
- **Toxicity Statement**: Non toxic
- **Veterinary certificate or other statement if applicable**: Yes
- **Attached**: SH493 SH493a SH495 SH495a SH496 SH496a

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WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have today examined a Sheep with ear tag number: UK 122414 066 [Virology no. SH493], which has been used in the production of blood antiserum between 8th October 2008 and 5th November 2008. 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.

Kath Hardcastle BVetMed Cert LAS MRCVS
Named Veterinary Surgeon

Kath Hardcastle BVetMed Cert LAS MRCVS
Office: 020 7468 5333  Fax: 020 7468 5378  Mobile: 07809199998
Email: <khardcastle@rvc.ac.uk>
04 December 2008

Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep with ear tag number: UK 122414 066 (Virology no. SH493) has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus (FMDV). The treatment method used was maintenance of pH5.5 or lower for a minimum of 30 minutes.

[Signature]

Dr Philip Minor
Deputy Director
National Institute for Biological Standards and Control
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have today examined a Sheep with ear tag number: UK 281 038 2093[Virology no. SH495], which has been used in the production of blood antiserum between 8th October 2008 and 25th November 2008. 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.

Kath Hardcastle BVetMed Cert LAS MRCVS
Named Veterinary Surgeon

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Office: 020 7468 5333  Fax: 020 7468 5378  Mobile: 07809199998
Email: <kharcastle@rvc.ac.uk>
04 December 2008

Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep with ear tag number: UK 281 038 2093 (Virology no.SH495) has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus (FMDV). The treatment method used was maintenance of pH5.5 or lower for a minimum of 30 minutes.

[Signature]

Dr Philip Minor
Deputy Director
National Institute for Biological Standards and Control
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have today examined a Sheep with ear tag number: UK 281 038 2113 [Virology no. SH496], which has been used in the production of blood antiserum between 8th October 2008 and 25th November 2008. 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.

Kath Hardcastle BVetMed Cert LAS MRCVS
Named Veterinary Surgeon
04 December 2008

Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep with ear tag number: UK 281 038 2113 (Virology no.SH496) has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus (FMDV). The treatment method used was maintenance of pH5.5 or lower for a minimum of 30 minutes.

Dr Philip Minor
Deputy Director
National Institute for Biological Standards and Control