Influenza Reagent
Influenza anti-A/Hong Kong/4801/2014-like HA serum
NIBSC code: 16/182
Instructions for use
(Version 1.0, Dated 02/08/2016)

1. INTENDED USE
Influenza antiserum reagent 16/182 is prepared for single radial diffusion
assay of A/Hong Kong/4801/2014-like antigens using an appropriate
NIBSC antigen reagent.

The antiserum reagent was prepared in sheep 652, 653, 656 and 657
using the purified HA of A/Hong Kong/4801/2014-like viruses. The HA
antigens were extracted from purified virus by treatment with bromelain
and purified by sedimentation on sucrose gradients (Brand, CN and

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 652 was as follows: one dose of
approximately 50 µg of A/Hong Kong/4801/2014-like virus NYMCX-263B
HA with Freund’s Complete Adjuvant (FCA) was given intramuscularly,
followed two weeks later with a 10 microgram dose of NYMCX-263B HA
including Freund’s Incomplete Adjuvant (FIA). Three further 10 microgram
doses of NYMCX-263B HA, including FIA, were given at weekly intervals.
Six weeks after the initial immunization, sera were collected and sodium
azide (0.05% w/v) added.

The immunization schedule for sheep 653, 656 and 657 was as follows:
one dose of approximately 50 µg of A/Hong Kong/4801/2014-like virus NYMCX-263B
HA, with FCA, was given intramuscularly, followed two weeks later with a 10 microgram dose of NYMCX-263B HA including FIA.
Five further 10 microgram doses of NYMCX-263B HA, including FIA, were
given at weekly intervals. Eight weeks after the initial immunization, serum
was collected and sodium azide (0.05% w/v) added.

All sera were then treated by an APHIS approved method for the
inactivation of FMDV.

The sera were pooled and then diluted 1:4 with PBS buffer containing
sodium azide (0.05% w/v) filtered and filled into vials in 2ml volumes.

5. STORAGE
The recommended storage temperature is +2-8ºC.

However, if it is intended to store the reagent for long periods i.e.>2years,
they may be stored at -20ºC. The antiserum can be frozen and thawed
without any adverse impact on 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 20-50µg of HA activity in 1ml, 15µl-25µl
of the undiluted reagent should be added to 1ml agarose. It may be
necessary to change the antiserum concentrations depending on the A/Hong
Kong/4801/2014-like antigen standard used or according to local laboratory
conditions.

Antiserum Reagent 16/182 should be used according to the method
described by Wood, JM, Schild, GC, Newman, RW and Seagroatt, VA.

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 aliquotted,
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/which
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
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: 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): Contains Sheep Serum and Sodium Azide (0.05% w/v)</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>* 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>
<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 SH652 SH653 SH656 SH657</td>
</tr>
</tbody>
</table>

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 841000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 0263 731 00634 [Virology no. SI1652], which has been used in the production of blood antiserum between 9th March 2016 and 20th April 2016. 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.

Olga Woolmer, DVM, MRCVS
Named Veterinary Surgeon

______________________________
Olga Woolmer, DVM, MRCVS Named Veterinary Surgeons (NVS) Group
The Royal Veterinary College, Royal College Street, London NW1 0TU
Mobile: 07809 199998, E-mail: owoolmer@rvc.ac.uk
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 0263 731 00673 [Virology no. SH653], which has been used in the production of blood antisem between 9th March 2016 and 3rd May 2016. 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.

Arturo Fernandez DVM MRCVS
Named Veterinary Surgeon

Arturo Fernandez DVM MRCVS
Named Veterinary Surgeon (NVS) Group
The Royal Veterinary College, Royal College Street, London NW1 0TU
Mobile: 07733 103881, E-mail: arfernandez@rvc.ac.uk
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 2637310587 [Virology no. SH656], which has been used in the production of blood antiserum between 20th April 2016 and 14th June 2016. 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.

Olga Woolmer, DVM, MRCVS
Named Veterinary Surgeon

Olga Woolmer, DVM, MRCVS
Named Veterinary Surgeon (NVS) Group
The Royal Veterinary College, Royal College Street, London NW1 0TU
Mobile: 07809 199998, E-mail: owoolmer@rvc.ac.uk
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 2637310614 [Virology no. SH657], which has been used in the production of blood antiserum between 20th April 2016 and 14th June 2016. 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.

Olga Woolmer, DVM, MRCVS
Named Veterinary Surgeon

______________________________
Olga Woolmer, DVM, MRCVS
Named Veterinary Surgeon

______________________________
Olga Woolmer, DVM, MRCVS Named Veterinary Surgeons (NVS) Group
The Royal Veterinary College, Royal College Street, London NW1 0TU
Mobile: 07809 199998, E-mail: owoolmer@rvc.ac.uk