Influenza Reagent
Influenza anti-A/South Australia/34/2019-like (H3N2) HA serum
NIBSC code: 19/216
Instructions for use
(Version 1.0, Dated 27/11/2019)

1. INTENDED USE
Influenza antiserum reagent 19/216 is prepared for single radial diffusion
assay of H3N2 A/South Australia/34/2019-like antigens using an
appropriate NIBSC antigen reagent.

The antiserum reagent was prepared in sheep 712, 713, 714, 715 and
716 using the purified HA of a A/South Australia/34/2019-like virus. The
HA antigens were extracted from purified virus by treatment with
bromelain and purified by sedimentation on sucrose gradients (Brand, CN

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 712 and 713 was as follows: one
dose of approximately 100 μg of A/South Australia/34/2019 (IVR-197)
HA with Freund’s Complete Adjuvant (FCA) was given intramuscularly, followed two weeks later with a 20 microgram dose
including Freund’s Incomplete Adjuvant (FIA). Three further 20 microgram
doses of A/South Australia/34/2019 (IVR-197) 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 immunization schedule for sheep 714, 715 and 716 was as follows:
one dose of approximately 100 μg of A/South Australia/34/2019 (IVR-197)
HA with Freund’s Complete Adjuvant (FCA) was given intramuscularly, followed two weeks later with a 20 microgram dose
including Freund’s Incomplete Adjuvant (FIA). Two further 20 microgram
doses of A/South Australia/34/2019 (IVR-197) HA including FIA were
given after a week. Five weeks after the initial immunization, serum was
collected and sodium azide (0.05% w/v) added.

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

The antiserum obtained from sheep 712, 713, 714, 715 and 716 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 antiserum 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 antiserum concentrations
depending on the A/South Australia/34/2019-like antigen standard used or
according to local laboratory conditions.

Antiserum Reagent 19/216 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 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
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>Stable: Yes</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
</tr>
<tr>
<td>Flammable: No</td>
</tr>
<tr>
<td>Other (specify):</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

| Country of origin for customs purposes*: United Kingdom |
| Toxicty Statement: Non toxic |
| Net weight: 2g |
| Veterinary certificate or other statement if applicable. Attached: Yes SH712 SH713 SH714 SH715 SH716 |
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 0241269 04783 [Virology no. SH712], which has been used in the production of blood antiserum between 11th October 2019 and 19th November 2019. 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.

Chris Handley BVM MRCVS
Named Veterinary Surgeon

Date signed: 25 MAP

Chris Handley BVM MRCVS
Named Veterinary Surgeons (NVS) Group
The Royal Veterinary College, Royal College Street, London NW1 0TU
Mobile: 07733 101841, E-mail: chandle@rvc.ac.uk
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 0241269 04462 [Virology no. SH713], which has been used in the production of blood antiserum between 11th October 2019 and 19th November 2019. 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.

[Signature]

Chris Handley BVM MRCVS
Named Veterinary Surgeon

Date signed: 25 NOV 2019

*We examined on 18 NOV 2019*
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 0108970 06768 [Virology no. SH714], which has been used in the production of blood antiserum between 16th October 2019 and 20th November 2019. 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.

Chris Handley BVM MRCVS
Named Veterinary Surgeon

Date signed: 25 Nov 2019

Examined on 18 Nov 19

Chris Handley BVM MRCVS
Named Veterinary Surgeons (NVS) Group
The Royal Veterinary College, Royal College Street, London NW1 0TU
Mobile: 07733 103881, E-mail: chandley@rvcollege.ac.uk
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 0100596 00640 [Virology no. SH715], which has been used in the production of blood antiserum between 16th October 2019 and 20th November 2019. 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.


Chris Handley BVM MRCVS
Named Veterinary Surgeon

Date signed: 25 Nov 2019

Chris Handley BVM MRCVS
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The Royal Veterinary College, Royal College Street, London NW1 0TU
Mobile: 07733 103881, E-mail: chandley@rvcl.ac.uk
VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 0241269 04906 [Virology no. SH716], which has been used in the production of blood antiserum between 16th October 2019 and 20th November 2019. 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.

[Signature]
Chris Handley BVM MRCVS
Named Veterinary Surgeon

Date signed: 25 NOV 2019

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