



**Influenza Reagent  
Influenza Anti-A/Perth/16/2009 -Like HA Serum  
NIBSC code: 11/206  
Instructions for use  
(Version 4.0, Dated 03/10/2014)**

### 1. INTENDED USE

Influenza antiserum reagent 11/206 is prepared for single radial diffusion assay of A/Perth/16/09-like antigens using an appropriate NIBSC antigen reagent.

The antiserum reagent was prepared in sheep 548, 549, 550 and 551 to the purified HA of A/Perth/16/09-like (NIB65 and NYMCX-187) influenza strains. 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 schedules for both sheep 548 and 549 were as follows: One dose of approximately 50µg of A/Victoria/210/2009 (NIB65) 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). Ten further 10 microgram doses of A/Victoria/210/2009 (NIB65) HA including FIA were given at weekly intervals. Twelve weeks after the initial immunization, serum was collected and sodium azide (0.05% w/v) added. The sera were then treated by an APHIS approved method for the inactivation of FMDV.

The schedule for sheep 550 was as follows: One dose of approximately 50µg of A/Victoria/210/2009 (NYMCX-187) HA with Freund's Complete Adjuvant (FCA) was given intramuscularly, followed two weeks later with a 10 microgram dose of A/Victoria/210/2009 (NYMCX-187) including Freund's Incomplete Adjuvant (FIA). One further 10 microgram dose of A/Victoria/210/2009 (NYMCX-187) HA including FIA was given one week later. Four weeks after the initial immunization, serum was collected and sodium azide (0.05% w/v) added. The serum was then treated by an APHIS approved method for the inactivation of FMDV.

The schedule for sheep 551 was as follows: One dose of approximately 50µg of A/Victoria/210/2009 (NYMCX-187) HA with Freund's Complete Adjuvant (FCA) was given intramuscularly, followed two weeks later with a 10 microgram dose of A/Victoria/210/2009 (NYMCX-187) including Freund's Incomplete Adjuvant (FIA). Two further 10 microgram dose of A/Victoria/210/2009 (NYMCX-187) HA including FIA were given at one week intervals. Five weeks after the initial immunization, serum was collected and sodium azide (0.05% w/v) added. The serum was then treated by an APHIS approved method for the inactivation of FMDV.

The sera were pooled and then diluted 1:2.4 with PBS buffer containing sodium azide (0.05% w/v) and filled into vials in 2ml volumes.

### 5. STORAGE

The recommended storage temperature is +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 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 12µl 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 11/206 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](mailto: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](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](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

Physical and Chemical properties	
Physical appearance: Liquid	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains Sheep Serum and Sodium Azide (0.05% w/v)
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](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

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> 2g
<b>Toxicity Statement:</b> Non toxic
<b>Veterinary certificate or other statement</b> if applicable. <b>Attached:</b> Yes SH548 SH548A SH549 SH549A SH550 SH550A SH551 SH551A



**RVC**

Royal Veterinary College  
University of London

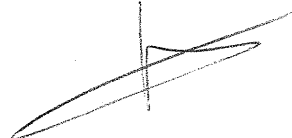
**Dr Jean-Philippe Mocho MRCVS**  
Named Veterinary Surgeon  
Royal Veterinary College  
Royal College Street  
LONDON  
NW1 0TU

---

### VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a Sheep with ear tag number: UK 028 8289 1346 [Virology no. SH548], which has been used in the production of blood antiserum between 20<sup>th</sup> July 2011 and 19<sup>th</sup> October 2011. 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.



Dr Jean-Philippe Mocho MRCVS  
Named Veterinary Surgeon

---

Dr Jean-Philippe Mocho MRCVS  
Phone/Fax 020 7468 5333 Mobile: 07809 099 458  
Email: <jmocho@rvc.ac.uk>



### Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep no. UK 028 8289 1446 [Virology no.SH 548] has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus. 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



Royal Veterinary College  
University of London

**Dr Jean-Philippe Mocho MRCVS**  
Named Veterinary Surgeon  
Royal Veterinary College  
Royal College Street  
LONDON  
NW1 0TU

---

### VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have examined a sheep with ear tag number: UK 028 8289 0628 [Virology no. SH549], which has been used in the production of blood antiserum between 20<sup>th</sup> July 2011 and 19<sup>th</sup> October 2011. 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.

Dr Jean-Philippe Mocho MRCVS  
Named Veterinary Surgeon

---

**Dr Jean-Philippe Mocho MRCVS**  
Phone/Fax 020 7468 5333 Mobile: 07809 099 458  
Email: <jmocho@rvc.ac.uk>



### Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep no. UK 028 8289 0628 [Virology no.SH 549] has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus. 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



Royal Veterinary College  
University of London

Lucy Whitfield  
Named Veterinary Surgeon  
Royal Veterinary College  
Royal College Street  
LONDON  
NW1 0TU

---

### VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have today examined a sheep with ear tag number: UK 028 8289 8149 [Virology no. SH550], which has been used in the production of blood antiserum between 5<sup>th</sup> October 2011 and 2<sup>nd</sup> November 2011. Both the ear tag number and the animal's record show that she 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.

Lucy Whitfield MA VetMB DLAS MRCVS  
Named Veterinary Surgeon

---

Lucy Whitfield MA VetMB DLAS MRCVS  
Phone/Fax 020 7468 5333 Mobile: 07778 332464  
Email: <lwhitfield@rvc.ac.uk>



### Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep no. UK 028 8289 8149 [Virology no.SH 550] has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus. 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





Royal Veterinary College  
University of London

Lucy Whitfield  
Named Veterinary Surgeon  
Royal Veterinary College  
Royal College Street  
LONDON  
NW1 0TU

---

### VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have today examined a sheep with ear tag number: UK 028 8289 1542 [Virology no. SH551], which has been used in the production of blood antiserum between 5<sup>th</sup> October 2011 and 9<sup>th</sup> November 2011. Both the ear tag number and the animal's record show that she 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.

Lucy Whitfield MA VetMB DLAS MRCVS  
Named Veterinary Surgeon

---

Lucy Whitfield MA VetMB DLAS MRCVS  
Phone/Fax 020 7468 5333 Mobile: 07778 332464  
Email: <lwhitfield@rvc.ac.uk>



### Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep no. UK 028 8289 1542 [Virology no.SH 551] has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus. 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