



**Influenza Reagent**  
**Influenza Antiserum A/HongKong/1073/99 Like (H9)**  
**NIBSC code: 07/146**  
**Instructions for use**  
**(Version 3.0, Dated 25/08/2011)**

#### 1. INTENDED USE

Influenza antiserum reagent 07/146 is prepared in sheep for single-radial-diffusion assay of A/Hong Kong/1073/99 antigens. An appropriate NIBSC antigen reagent should be included in each assay.

#### 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 antiserum was prepared in SHEEP (SH453 and SH455) to the purified haemagglutinin (HA) of NIB-44 virus (H9N1). NIB-44 is an antigenic reassortant of A/quail/Hong Kong/G1/99 (A/qu/HK) and A/Puerto Rico/34 viruses and has HA derived from A/qu/HK and NA from A/Puerto Rico/34. The HA of A/qu/HK is antigenically similar to the HA of A/Hong Kong/1073/99 virus. The HA antigen was 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). The immunization schedule for SH453 was as follows: one dose of approximately 50µg of HA with Freund's complete adjuvant (FCA) was given intramuscularly, followed two weeks later with a 25µg dose with Freund's incomplete adjuvant (FIA). Five further 25µg doses with FIA were administered at weekly intervals.  
Eight weeks after the initial immunization, serum was collected and sodium azide (0.05%) was added.

The immunization schedule for SH453 was as follows: one dose of approximately 50µg of HA with Freund's complete adjuvant (FCA) was given intramuscularly, followed two weeks later with a 25µg dose with Freund's incomplete adjuvant (FIA). Five further 25µg doses with FIA followed by seven 50µg doses with FIA were administered at weekly intervals.

Sixteen weeks after the initial immunization, serum was collected and sodium azide (0.05%) was added.

The sera were pooled, diluted 1:2 with phosphate buffered saline containing sodium azide (0.05% w/v) and filled into vials in 2ml volumes. The mean weight of 56 vials test weighed was 2.0923g with a coefficient of variation of 1.224%.

#### 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 20-50µg of HA activity in 1ml, 21µl of the undiluted Reagent should be added to 1ml of agarose. Antigens of lower concentration (5-20µg HA/ml) are assayed by adding 10.5µl of the Reagent to 1ml of agarose. It may be necessary to change the antiserum concentrations according to local laboratory conditions.

Antiserum reagent 07/146 should be used according to the method described by Wood JM, Schild GC, Newman RW and Seagroatt VA. Journal of Biological Standardisation, 1977, 5, 237- 247.

#### 8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label, please also see attached storage information sheet.

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

#### 9. REFERENCES

N/A

#### 10. ACKNOWLEDGEMENTS

This reagent was produced on behalf of the FLUSECURE project with funding from the EU.

#### 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](mailto: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

<b>Physical and Chemical properties</b>	
Physical appearance: straw coloured 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	
<b>Toxicological properties</b>	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
<b>Suggested First Aid</b>	
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.
<b>Action on Spillage and Method of Disposal</b>	
Spillage of 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 SH453 and SH455 plus Storage Information sheet



## STORAGE OF REAGENT 07/146

NIBSC has prepared a number of reagents for single radial diffusion assay of influenza subtypes of pandemic potential.

Since it is not known when these reagents may be required, it is desirable that they have an indefinite shelf life and they are stored at NIBSC in colder conditions than reagents prepared for the assay of epidemic strains. Therefore the recommended storage temperature marked on the label for reagent 07/146 is  $-20^{\circ}\text{C}$ .

However it is assumed that a customer ordering this reagent, will use it within a short period similar to that for a conventional reagent. Consequently, this reagent is not normally shipped frozen and **the recommended storage temperature is  $+4^{\circ}\text{C}$ .**



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### Veterinary Certificate

This is to certify that Sheep no. 4107 [Virology No. SH 453] was used for the production of blood antiserum between 9<sup>th</sup> March 2005 and 4<sup>th</sup> May 2005.

This sheep was a ewe that was surplus to breeding requirements, in overt good health, and showed no signs of clinical disease.

The ear tag identifying the animal indicated that it was of UK origin.

*R.M. Hull*  
*11 May 2005*  
R.M. Hull  
BVSc, PhD, MRCVS  
Named Veterinary Surgeon



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## Veterinary Certificate

— This is to certify that Sheep no. 4109 [Virology No. SH 455] was used for the production of blood antiserum between 4<sup>th</sup> May 2005 and 24<sup>th</sup> August 2005.

This sheep was a ewe that was surplus to breeding requirements, in overt good health, and showed no signs of clinical disease.

The ear tag identifying the animal indicated that it was of UK origin.

*R.M. Hull 30-August 2005*

— R.M. Hull  
BVSc, PhD, MRCVS  
Named Veterinary Surgeon



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