



**Influenza Reagent**  
**Influenza anti A/Equine/Newmarket/2/93 (H3N8) HA Serum (**  
**NIBSC code: 97/614**  
**Instructions for use**  
**(Version 4.0, Dated 29/03/2008)**

### 1. INTENDED USE

Influenza antiserum reagent 97/614 is prepared in sheep for the single radial diffusion assay of A/Equine/Newmarket/2/93 antigens.

### 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 a SHEEP (SH376) to the purified HA of A/Equine/Newmarket/2/93 (H3N8) virus. The HA antigen was extracted from purified virus by treatment with bromelain and purified by sedimentation on sucrose gradients (Brand, C N and Skehel JJ, Nature, New Biology, 1972, 238, 145-147). One dose of approximately 50 micrograms of HA with Freund's complete adjuvant (FCA) was given intramuscularly, followed two weeks later with a 10 microgram dose and two further 10 microgram doses at weekly intervals.

Five weeks after the initial immunization, serum was collected, diluted 1:4 with PBS buffer containing sodium azide (0.05% w/v) and filled into vials in 2 ml volumes. The mean weight of 13 vials test weighed was 2.02g with a coefficient of variation of 0.46%.

### 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

**No attempt should be made to weigh out any portion of the material**

For the assay of virus antigens containing 20-50 micrograms of HA activity in 1ml, 20µl of the undiluted Reagent should be added to 1ml agarose. Antigens of lower concentration (5-20 micrograms HA/ml) are assayed, by adding 10µl of the Reagent to 1ml agarose.

It may be necessary to change the antiserum concentrations according to local laboratory conditions. The clarity of the SRD zones may be improved by washing the gels with PBS before pressing and staining.

Antiserum Reagent 97/614 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. For the assay of antigens containing 20-50 micrograms of HA activity in 1ml, approximately 10µl of undiluted reagent should be added to 1ml agarose. Antigens of lower concentration (5-20 micrograms HA/ml) are assayed by adding 5µl of the reagent to 1ml agarose. It may be necessary to change these antiserum concentrations according to local laboratory conditions.

Antiserum Reagent 00/442 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

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](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

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:	Avoid inhalation
Effects of ingestion:	Avoid ingestion
Effects of skin absorption:	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> No