**Influenza Reagent**  
**Influenza anti-A/Philippine/2/82 (H3N2)(X79) serum**  
**NIBSC code: 83/545**  
**Instructions for use**  
(Version 4.0, Dated 26/03/2008)

1. **INTENDED USE**  
Influenza antiserum reagent 83/545 is prepared in sheep for the single radial diffusion assay of A/Philippines/2/82 antigens. An appropriate NIBSC antigen reagent should be used 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 (SH45) to the purified HA of A/Philippines/2/82 virus (X79). The HA antigen was extracted from purified virus by treatment with bromelain and purified by sedimentation on sucrose gradients (Brand, C.N. and Skehel, J.J., Nature, New Biology, 1972, 238, 145-147). One dose of approximately 50 µg HA with Freund's complete adjuvant (FCA) was given intramuscularly, followed 2 weeks later by 4 further doses of 25 µg HA with FCA at 1 week intervals. Six weeks after the initial immunisation, serum was collected, diluted 1:4 with PBS and processed for freeze-drying in 1ml volumes as described by Campbell, P.J., Journal of Biological Standardization 1974, 2,249-267. The mean weight of 73 ampoules, test weighed was 1.004g (±0.3%).

5. **STORAGE**  
-20°C

   Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. **DIRECTIONS FOR OPENING**  
Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position ‘A’, shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point ‘B’. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.

   Side view of ampoule opening device containing an ampoule positioned ready to open. ‘A’ is the score mark and ‘B’ the point of applied pressure.

7. **USE OF MATERIAL**  
No attempt should be made to weigh out any portion of the material

   Reconstitute the total contents of one ampoule of reagent with 1ml of distilled water. Allow to stand for a minimum of 5 minutes before use to allow for complete solution of the freeze-dried material. For the assay of antigens containing 20-50 micrograms of HA activity in 1ml, approximately 18µl of undiluted reagent should be added to 1ml agarose. Antigens of lower concentration (5-20 micrograms HA/ml) are assayed by adding 9µl of the reagent to 1ml agarose. It may be necessary to change these antiserum concentrations according to local laboratory conditions.

   Antiserum Reagent 83/545 should be used according to the method described by Wood, JM, Schild, GC, Newman, RW and Seagroatt, VA Jornal 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  
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
code number, and the name and address of NIBSC are cited correctly.

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: white powder</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
</tr>
<tr>
<td>Flammable: No</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: 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 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

* 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.

Net weight: 1g

Toxicity Statement: Non toxic

Veterinary certificate or other statement if applicable.

Attached: No