Second International Standard for HBsAg, subtype adw2, genotype A
NIBSC code number: 00/588

Instructions for Use (Version 1, 24 February 2004)

1. INTRODUCTION

This preparation contains inactivated HBsAg, subtype adw2, genotype A, and has been calibrated in International Units in an international collaborative study. It was calibrated with the First International Standard for HBsAg, subtype ad, along with several commonly used national and working standards.

2. UNITAGE

The Second International Standard for HBsAg has an assigned unitage of 33IU/vial. Uncertainties associated with this assigned unitage are addressed in the report of the international collaborative study (1), available on the internet at: http://www.who.int/biologicals/. The coefficient of variation of the fill volume was determined to be 0.49%.

3. CONTENTS

Each vial contains a freeze-dried residue comprising plasma derived HBsAg subtype adw2, genotype A, small particles, in recalcified plasma under an atmosphere of nitrogen. The HBsAg was purified at the Central Laboratory of the Netherlands Red Cross by PEG precipitation and ultracentrifugation to remove Dane particles and inactivated by heating at 101-103°C for 90 seconds followed by pasteurisation at 65°C for 10h (2). This antigen preparation was diluted in recalcified plasma that has been shown to be negative for anti-HCV, anti-HIV 1+2, HBsAg, anti-HBs as well as negative for HCV RNA, HBV DNA and HIV RNA. 0.05% Merthiolate has been added to the serum as preservative.

4. CAUTION

4.1 THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.
4.2 A 'Safety Data Sheet' is included as the last page of these Instructions.
4.3 This preparation contains HBsAg which has been inactivated by validated procedures (2). It also contains plasma of human origin, which has been tested and found negative for anti-HIV 1+2, anti-HCV, HBsAg, anti-HBs, HCV RNA and HIV RNA. 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 probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening vials, to avoid cuts.
5 DIRECTIONS FOR OPENING VIALS

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.

6 USE OF THE STANDARD

Vials should be stored at –20ºC on receipt. The contents of vials should be reconstituted with 1ml distilled water using safety precautions as described above.

This material is for use in the calibration of secondary reference materials for HBsAg. The material does not contain pre-S and is not suitable for evaluation of assays depending on pre-S epitopes.

A series of dilutions of the International Standard and the material to be calibrated should be prepared in human serum negative for HBsAg and anti-HBs. Should any other diluent be used, it is important that users satisfy themselves that the matrix chosen is satisfactory, as different diluent matrices may affect results.

7 LIMITATIONS OF USE

This preparation is intended as a quantitative reference standard for HBsAg subtype adw2, genotype A. The use of secondary reference materials calibrated against this standard will give an indication of the analytical sensitivity of an assay. However, as a single specific reagent, this preparation cannot represent all types and antigenic forms. The evaluation of test kits for HBsAg should include performance testing of seroconversion panels, difficult samples, and local samples representing the prevalent HBV genotypes and variants in that target region.

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. For information specific to a particular biological standard, contact the Technical Information Officer or, where known, the appropriate NIBSC scientist.

It is recommended that reconstituted material is held for no longer than 1 month. The reconstituted standard should not be frozen. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.
9. CITATION

In all publications, including data sheets, in which this material is reference, it is important that the WHO status of the preparation, specified by the title of the preparation, the name and address of the WHO International Laboratory for Biological Standards at NIBSC and the NIBSC code number are cited and cited correctly.

10 PRODUCT LIABILITY

Information emanating from NIBSC is given after the exercise of all reasonable care and skill in its compilation, preparation and issue but is provided without liability in its application and use.

This product is intended for use as a standard or reference material in laboratory work in relation to biological research, manufacturing or quality control testing of biological products or in the field of *in vitro* diagnostics. It is the responsibility of the user to ensure that he/she has the necessary technical skills to determine the appropriateness of this product for the proposed application. Results obtained from this product are likely to be dependent on conditions of use and the variability of materials beyond the control of NIBSC.

NIBSC accepts no liability whatsoever for any loss or damage arising from the use of this product, whether loss of profits, or indirect or consequential loss or otherwise, including, but not limited to, personal injury other than as caused by the negligence of NIBSC. In particular, NIBSC accepts no liability whatsoever for:
(i) results obtained from this product; and/or
(ii) non-delivery of goods or for damages in transit.

In the event of any replacement of goods following loss or damage a customer accepts as a condition of receipt of a replacement product, acceptance of the fact that the replacement is not to be construed as an admission of liability on NIBSC's behalf.

11 REFERENCES

12. MATERIAL SAFETY DATA SHEET

Physical properties (at room temperature)

<table>
<thead>
<tr>
<th>Physical appearance</th>
<th>Freeze dried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fire hazard</td>
<td>None</td>
</tr>
</tbody>
</table>

Chemical properties

<table>
<thead>
<tr>
<th>Stable</th>
<th>Yes</th>
<th>Corrosive</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hygroscopic</td>
<td>No</td>
<td>Oxidising</td>
<td>No</td>
</tr>
<tr>
<td>Flammable</td>
<td>No</td>
<td>Irritant</td>
<td>No</td>
</tr>
</tbody>
</table>

Other (specify) Contains material of human origin; contains 0.05% Merthiolate

Handling: See precautions in section 4.3

Toxicological properties

<table>
<thead>
<tr>
<th>Effects of inhalation</th>
<th>No adverse effects reported for this material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of ingestion</td>
<td>No adverse effects reported for this material</td>
</tr>
<tr>
<td>Effects of skin absorption</td>
<td>No adverse effects reported for this material</td>
</tr>
</tbody>
</table>

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

Spillages of vial contents should be taken up with absorbant material wetted with a viricidal agent. Rinse area with a viricidal agent followed by water.

Absorbant material used to treat spillages should be treated as biologically hazardous waste.