WHO HBsAg subtype adw2, genotype A Reference Panel
NIBSC code number: 03/262
Instructions for Use (Version 1 Initial Version 21 April 2004)

1 INTRODUCTION
The panel is a series of four-fold dilutions of HBsAg may be of use to National Regulatory Authorities in evaluating the analytical sensitivity of tests for the detection of HBsAg. The panel contains four specimens, A – D at dilutions which encompass the sensitivities of most kits available at present. The panel also includes a negative control sample, sample E, which contains no HBsAg. The panel has been assessed for suitability in an international collaborative study.

2 UNITAGE
The panel members are a series of fourfold dilutions, the most concentrated of which, panel member A, is a fourfold dilution of the Second International Standard (IS) for HBsAg, genotype A, subtype adw2, (00/588), panel member B is a 1 in 16 dilution of the IS, panel member C a 1 in 64 dilution of the IS and panel member D is a 1 in 256 dilution IS. The Second International Standard for HBsAg has an assigned unitage of 33IU/vial (1). Panel member E is a preparation of normal re-calcified plasma.

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. This antigen preparation was diluted in recalcified plasma which 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 THESE PREPARATIONS ARE NOT FOR ADMINISTRATION TO HUMANS.
4.2 A 'Safety Data Sheet' is included as the last page of these Instructions.
4.3 These preparations contain HBsAg which has been inactivated by validated procedures (2). They also contain-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 avoids cuts by wearing appropriate gloves. Remove the stopper for access.

6  USE OF REFERENCE PANEL
Vials should be stored at –20°C on receipt. The contents of vials should be reconstituted with 1ml distilled water using safety precautions as described above.

The proposed HBsAg panel may be used as an aid to determining the analytical sensitivity assay kits for the detection of HBsAg. They should be used following reconstitution without further dilution, other than as required in individual test procedures.

Panel member E should be scored negative on every occasion.

7 LIMITATIONS OF USE
This material should be used in addition to the positive and negative controls supplied with each lot of kit. Only the controls supplied with each lot of kit by the manufacturer are used to determine the validity of assays and to calculate the cut-off value which forms the basis for donor screening or diagnosis.

The use of this panel will give an indication of the sensitivity of detection of assay kits for the detection of HBsAg but its use does not replace a more comprehensive evaluation of fitness for purpose which may involve testing a range of samples including sero-conversion panels, ‘difficult’ samples, local HBV genotypes and variants. The material does not contain pre-S and is not suitable for evaluation of assays depending on pre-S epitopes.

These materials are not for use in the calibration of secondary reference materials. The Second International Standard for HBsAg (NIBSC code 00/588) is available for calibration of in-house or working standards.

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.

National Institute for Biological Standards and Control

The Institute's UKAS accreditation relates only to our programme of testing for biological medicines. Full details can be found on our Schedule of Accreditation, which is available on request.
It is recommended that reconstituted material is held for no longer than 1 month. Unused contents should not be frozen. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9 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.

10 REFERENCES

## 11. MATERIAL SAFETY DATA SHEET

<table>
<thead>
<tr>
<th>Physical properties (at room temperature)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance</td>
<td>Freeze dried</td>
</tr>
<tr>
<td>Fire hazard</td>
<td>None</td>
</tr>
</tbody>
</table>

### Chemical properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Status</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>Yes</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Hygroscopic</td>
<td>No</td>
<td>Oxidising</td>
</tr>
<tr>
<td>Flammable</td>
<td>No</td>
<td>Irritant</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>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation</td>
<td>No adverse effects reported for this material</td>
</tr>
<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

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Ingestion</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes</td>
<td>Wash with copious amounts of water. Seek medical advice.</td>
</tr>
<tr>
<td>Contact with skin</td>
<td>Wash thoroughly with water.</td>
</tr>
</tbody>
</table>

### 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.

Compiled by: Morag Ferguson  
Date: October 26, 2004