



**WHO International Standard
Second International Standard (2003) for HBsAg, subtype adw2,
genotype A
NIBSC code: 00/588
Instructions for use
(Version 4.0, Dated 12/04/2013)**

1. INTENDED USE

This preparation contains inactivated HBsAg, subtype adw2, genotype A, and has been calibrated in International Units in an international collaborative study (1). It was calibrated against the First International Standard for HBsAg, subtype ad, along with several commonly used national and working standards.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

00/588 contains 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 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

The Second International Standard for HBsAg has an assigned unitage of 33IU/vial (1). 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%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
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° for 90 seconds followed by pasteurisation at 65° 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.

5. STORAGE

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.

It is recommended that reconstituted material is held for no longer than 1 month. Unused contents should not be frozen.

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 freeze-dried material prior to reconstitution.

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 (3).

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 suitable, as different diluent matrixes may affect results.

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

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

Degradation studies on 00/588 indicate that the freeze-dried material is stable and suitable for long-term storage. Reconstituted material may be stored at 4°C for up to 1 month. Do not re-freeze reconstituted material (1).

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

9. REFERENCES

1. WHO/BS/03.1987
WHO Working Group on Hepatitis and HIV Diagnostic Kits
Report of a collaborative study to 1) assess the suitability of a candidate replacement International Standard for HBsAg and a reference panel for HBsAg and 2) to calibrate the candidate standard in IU. Available on the internet at: <http://www.who.int/biologicals/>.
2. Lelie PN, Reesink HW, Niessen J, Brotman B, Prince AM. Inactivation of 1015 chimpanzee infectious doses of hepatitis B virus during preparation of a heat-inactivated hepatitis B vaccine. *Journal of Medical Virology* 1987; 23: 289-295.
3. Schuttler CG, Wend UC, Faupel FM, Lelie PN, Gerlich WH: Antigenic and physicochemical characterization of the 2nd International Standard for hepatitis B virus surface antigen (HBsAg). *J Clin Virol* 2010, 47(3):238-242.

10. ACKNOWLEDGEMENTS

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 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: Freeze dried	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains material of human origin; Contains 0.05% Merthiolate
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, 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 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

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biol_standardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.