WHO International Standard
Third International Standard for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2)
NIBSC code: 12/226
Instructions for use
(Version 2.0, Dated 30/01/2017)

1. INTENDED USE
This preparation contains inactivated HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2) and has been calibrated in International Units (IU) in an international collaborative study (1). It was calibrated against the 2nd international standard (IS) for HBsAg (A2, adw2) along with additional study samples representing different HBV genotypes. The 3rd WHO IS for HBsAg is intended to be used for the determination of analytical sensitivity of HBsAg assays and for the calibration of secondary references for HBsAg.

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

The preparation contains material of human origin. The source material is human plasma obtained from asymptomatic carriers positive for HBsAg. The HBsAg was purified and inactivated using validated methods for the manufacture of plasma-derived Hepatitis B vaccine. (http://whqlibdoc.who.int/trs/WHO_TRS_858.pdf ) (2).

The HBsAg bulk is negative for antibodies to HIV and HCV RNA. The inactivated HBsAg bulk is positive for HBV DNA. 12/226 was formulated by diluting the HBsAg vaccine bulk in thrombinized and declotted plasma which had been shown to be negative for anti-HCV, anti-HIV 1+2, HBsAg, anti-HBs, HCV RNA, HBV DNA 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 3rd International Standard for HBsAg has an assigned unitage of 47.3 IU/ampoule (1).

4. CONTENTS
Country of origin of biological material: Vietnam (HBsAg vaccine bulk) and UK (thrombinized and declotted plasma).

Each ampoule contains the freeze-dried equivalent of 1.0 mL HBsAg in thrombinized and declotted plasma containing 0.05% Bronidox as preservative. The international collaborative study for calibrating 12/226 indicated an overall geometric mean potency of 47.3 IU/ampoule (1).

The source material used to produce 12/226 is a non-adjuvanted HBsAg vaccine bulk derived from human plasma obtained from viremic carriers. During its manufacture, the purified HBsAg vaccine bulk had been rendered non-infectious by heat treatment and formaldehyde inactivation steps. Sequence analysis of the source HBsAg bulk identified at least two different HBV strains, both with genotype B4. The B4 genotype is in good correlation to the prevalent HBV genotypes in Vietnam where the plasmas were collected and purified. As a consequence of pooling plasma from multiple donors, the candidate HBsAg subtype is a heterogeneous population of ayw1 and adw2 (1).

5. STORAGE
12/226 should be stored at -20°C on receipt. Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING
DIN ampoules have an ‘easy-open’ coloured stress point, where the narrow ampoule stem joins the wider ampoule body.

Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution. The contents of the ampoule should be reconstituted with 1mL distilled water using safety procedures as described in Section 2.

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. NIBSC follows the policy of WHO with respect to its reference materials. Users should determine the stability of 12/226 according to their own method of preparation, storage and use. Stability studies indicate that 12/226 is adequately stable for long-term storage at -20°C and is suitable for transportation at ambient temperatures (1).

Additional stability assessments undertaken at NIBSC indicate that the reconstituted material is stable for up to two months when stored at +4°C. The reconstituted material is able to undergo three freeze-thaw cycles showing no significant loss of potency.

9. REFERENCES
1. WHO/BS/2014.2241

10. ACKNOWLEDGEMENTS
We gratefully acknowledge the important contributions of the collaborative study participants. We also thank Professor Nguyen Thu Van, VABIOTECH, Hanoi, Vietnam, for the kind donation of the HBsAg bulk material.

11. FURTHER INFORMATION
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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Freeze-dried</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Contains material of human origin. Contains 0.05% Bronidox.</td>
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<table>
<thead>
<tr>
<th>Toxicological properties</th>
<th></th>
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<tbody>
<tr>
<td>Effects of inhalation: Not established, avoid inhalation</td>
<td></td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
<td></td>
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<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
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</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**

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

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*: United Kingdom</th>
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<tbody>
<tr>
<td>* 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.</td>
</tr>
<tr>
<td>Net weight: 1g</td>
</tr>
<tr>
<td>Toxicity Statement: Non-toxic</td>
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<tr>
<td>Veterinary certificate or other statement if applicable.</td>
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<tr>
<td>Attached: No</td>
</tr>
</tbody>
</table>

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_refstandardsrev2004.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.