Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

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 -20ºC 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. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

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 -20ºC 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 +4ºC.
The reconstituted material is able to undergo three freeze-thaw cycles showing no significant loss of potency.

9. REFERENCES

1. WHO/BS/2014.2241
2. WHO Expert Committee on Biological Standardization. Forty-fifth report. World Health Organ Tech Rep Ser, 1995. 858: p. 1-101.

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

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

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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% Bronidox.		

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 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

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_biolefstandardsrev2004.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.