Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
Vitamin B12, Serum Folate and HoloTC
NIBSC code: 03/178
Instructions for use
(Version 3.0, Dated 26/10/2015)

1. INTENDED USE

The assay of blood levels of the vitamins B_{12} and folate is the current routine procedure for determining deficiency of these vitamins. Deficiency can result in a number of clinical conditions including megaloblastic and pernicious anaemia.

The International Standard (IS) for serum B_{12} and serum folate, 03/178, was assayed using a wide range of methods in 24 laboratories in 7 countries. Methods included a range of commercial analysers and, for serum folate, candidate reference methods of isotope-dilution tandem mass spectrometry coupled to liquid chromatography (LC/MS/MS). The inclusion of three serum samples in the study, with different B_{12} and folate levels, demonstrated a considerable reduction in inter-laboratory variability when the B_{12} and folate levels in the samples were determined relative to the IS with assigned B_{12} and folate values rather than to the analysers' calibration.
Since the IS was first established, commercial assays for holotranscobalamin (holoTC), the active portion of B12, have become available. There is evidence that holoTC is a better marker of early B12 deficiency than total B12. This prompted another international collaborative study to assign a holoTC value to the IS for B12 and serum folate, 03/178. Twelve laboratories in 8 countries participated.

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV 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 IS for serum B_{12} and serum folate, 03/178, has an assigned value of 12.1 nmol/L total folate, made up of 9.75 nmol/L 5MeTHF (5-methyltetrahydrofolate; coefficient of variation (CV) 5.5%), 1.59 nmol/L 5FoTHF (5-formyltetrahydrofolate; CV 4.2%) and 0.74 nmol/L FA (folic acid; CV 31.6%), when reconstituted with 1.0 mL distilled/deionised water, as determined using LC/MS/MS. The total folate content of 12.1 nmol/L is equivalent to 5.33 ng/mL, using a conventional conversion factor of 2.266.

The IS for serum B_{12} and serum folate, 03/178, has an assigned consensus value of 480 pg vitamin B_{12} (480 pg/mL when reconstituted with 1.0 mL distilled/deionised water). The preparation will be re-evaluated when a reference measurement procedure has been established.

The IS 03/178 has an assigned consensus holoTC value of 107 pmol/L when reconstituted with 1.0 mL (0.107 pmol/ampoule).

The variance in the ampoule contents was determined to be 0.08%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the lyophilized residue of ~1 mL human serum.

5. STORAGE

Unopened ampoules should be stored at -20°C or below.
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
Reconstitute the contents with 1.0 mL distilled/deionised water on the day of assay.

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.

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

Accelerated degradation studies on 03/178 indicate that the lyophilized material will be adequately stable at -20°C with respect to B_{12}, holoTC and folate content. Once reconstituted, users should determine the stability of the material according to their own conditions of storage and use.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

SJ Thorpe, A Heath, S Blackmore, A Lee, M Hamilton, S O'Broin, BC Nelson and C Pfeiffer. An international standard for serum vitamin B_{12} and serum folate: international collaborative study to evaluate a batch of lyophilized serum for B_{12} and folate content. Clin Chem Lab Med 45, 380-386 (2007).

10. ACKNOWLEDGEMENTS

We thank the participants of the collaborative studies.

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

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

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: Lyophilisate		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	Contains material of human origin		

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: 0.08g
Toxicity Statement: Toxicity not assessed
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.