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 B₁₂ 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 B₁₂ 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 B₁₂ and folate levels, demonstrated a considerable reduction in inter-laboratory variability when the B₁₂ and folate levels in the samples were determined relative to the IS with assigned B₁₂ and folate values rather than to the analysers’ calibration.

When 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 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.

3. UNITAGE
The IS for serum B₁₂ 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 5.53 ng/mL, using a conventional conversion factor of 2.266.

The IS for serum B₁₂ and serum folate, 03/178, has an assigned consensus value of 480 pg vitamin B₁₂ (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.

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.

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 B₁₂, 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

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/

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

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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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Lyophilisate</td>
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<tr>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
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<tr>
<td>Oxidising: Yes</td>
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<tr>
<td>Hygroscopic: No</td>
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<tr>
<td>Irritant: No</td>
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<tr>
<td>Flammable: No</td>
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<tr>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Contains material of human origin</td>
</tr>
</tbody>
</table>

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_biol_efstandardsrev2004.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.

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WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory