WHO International Standard
1st International Standard for Tetanus Immunoglobulin, Human
NIBSC code: TE-3
Instructions for use
(Version 8.0, Dated 21/08/2013)

1. INTENDED USE
The standard preparation was established in October 1992 [2]. This standard replaces the Second International Standard for Tetanus antitoxin, Equine, for testing of human anti-tetanus immunoglobulin preparations used clinically. It can also be used for titration of human serum samples for tetanus antitoxin. This material has been characterised by the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK.

For details of this International Standard, please refer to the enclosed package insert from the Statens Serum Institut.

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
By definition, each ampoule contains 120 International Units (IU) of Tetanus Immunoglobulin, Human. Calibration of the standard was based on PhEur method against the 2nd International Standard for Tetanus Antitoxin (16/4).

4. CONTENTS
Country of origin of biological material: Germany.
Each ampoule contains a volume of 1ml of 5% solution of purified human IgG, freeze-dried.

5. STORAGE
Unopened ampoules should be stored at -20°C. Please note: because of the inherent stability of lyophilized material, NIBSC may ship these preparations at ambient temperature.

6. DIRECTIONS FOR OPENING
Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position ‘A’; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point ‘B’. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.
The entire contents of each ampoule should be completely resuspended in an accurately measured amount of a suitable solution (e.g. PBS). A suspension of the total content of an ampoule will contain 120 IU in the total volume. The suspension can be stored frozen.

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. It is the policy of WHO not to assign an expiry date to their International Reference Materials.

There was no detectable loss of potency after storage of the freeze-dried material at -20°C for 3.7 years [2].

When reconstituted in 12ml PBS, TE-3 has been confirmed to be stable for up to 3 years in ELISA assay at NIBSC when stored as 50µl aliquots at -20°C. However, once reconstituted, diluted and/or divided into aliquots, users are encouraged to determine the stability of the material according to their own methods of preparation, storage and use.

Users who have data supporting any changes in the characteristics of this material are encouraged to contact NIBSC.

9. REFERENCES

10. ACKNOWLEDGEMENTS
The standard preparation has been calibrated in an International Collaborative Study in 15 laboratories in 15 countries.

11. FURTHER INFORMATION
Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biosafety/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
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>Corrosive</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried powder</td>
<td>Oxidising</td>
<td>No</td>
</tr>
<tr>
<td>Stable</td>
<td>Irritant</td>
<td>No</td>
</tr>
<tr>
<td>Hygroscopic</td>
<td>Handling</td>
<td>See caution, Section 2</td>
</tr>
<tr>
<td>Flammable</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Other (specify):</td>
<td>Contains material purified from human blood</td>
<td></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 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*: Germany
* 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.

<table>
<thead>
<tr>
<th>Net weight:</th>
<th>1 ml</th>
</tr>
</thead>
</table>

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_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.
WHO International Laboratory for Biological Standards

THE INTERNATIONAL STANDARD
for
TETANUS ANTI TOXIN, HUMAN
(third international standard preparation)

1. THE STANDAR D PREPARATION
This standard preparation was established in 1977. It is prepared from a pool of human plasmas from 10,428 donations by cold ethanol fractionation according to the Colin method. The final bulk, which contained 25.6 g protein per litre was filled to 1 ml amounts into ampoules which were then freeze-dried and packaged under nitrogen. During the investigations the batch was identified as batch no. 26 468. The established standard preparation is identified by the abbreviation: TH-3.

A European reference material was established from the same freeze-drying batch.

2. AMPOULE CONTENTS
By definition, the total dry material of an ampoule contains 126 International Units (IU) of Tetanus Immunogobulin, Human. This potency has been defined on the basis of the results of an international collaborative study, where batch no. 26 468 was compared with the second International Standard for Tetanus Antitoxin in different assay systems. The potency definition is primarily based on the results obtained by in vivo toxin neutralization assays in mice and following the methods outlined in the European Pharmacopoeia using either death (% mortality) or the onset of paralysis (1p/10) as the end-point. The IU of Tetanus Immunoglobulin, Human and the IU of Tetanus Antitoxin defined by the International Standard for Tetanus Antitoxin are identical in assays in mice and in most other assay systems. The two international standard preparations cannot be compared in conventional enzyme immunoassay (ELISA) assays, because these assays are species specific.

Apart from the ELISA test nothing indicates that animal antitoxin and human antitoxin are not comparable in most types of tests and that the two standard preparations define the same unit, the International Unit of Tetanus Antitoxin.

3. USE OF THE STANDARD
For the potency assay of tetanus antitoxin preparations of human or animal origin.

4. INJECTION AGENTS
Individual diluted solutions were tested for HBsAg and for antibodies against HIV, by Western blot methods and ELISA, respectively. They were tested for antibodies to HIV and HCV.

The final product (batch 26 468) was subjected to tests for presence of HBsAg and for HIV and HCV antibodies. All tests were negative.
5. WARNING

International reference materials of human origin might constitute a risk in regard to transmission of blood borne infections. All samples stored therefore be treated as if capable of causing infection.

It should also be noted that distribution of international reference materials is being performed as a public service. Consequently, by accepting delivery and proceeding to use the reference material concerned, the recipient agrees not to hold the Statens Serum Institute, Copenhagen, or WHO liable for the consequences of any injury or illness attributable to infection acquired from the reference material.

If a recipient does not agree to this condition on use of the reference materials, he must immediately return the reference material to the Statens Serum Institute, Copenhagen.

6. GENERAL REMARKS ABOUT INTERNATIONAL REFERENCE MATERIALS

International biological standards and international biological reference reagents provide a means of ensuring uniformity throughout the world in the designation of the potency or activity of preparations used in the prophylaxis, therapy, or diagnosis of disease, where this cannot be expressed in terms of physical or chemical quantities. The International Units are units of quantities of "effective constituent".

The standard is the material as it exists in the ampoules; the "material" thus includes the effective constituents together with all the other constituents that may be present (moisture, carrier, buffer, salt etc., according to the form in which the standard is available).

International biological reference materials are intended for use in the calibration of the contents of "effective constituent" in national or working standard preparations and for the expression of these contents in the respective International Units. For routine use in the laboratory the national or working standards should be used in order to save as much as possible the international reference materials. These are only sent to individual laboratories in very limited amounts. International Reference Materials are distributed free of charge to National Control Laboratories of Member States of the World Health Organization. Other laboratories are due to pay a handling charge.

August 1996

7. REFERENCES

