WHO International Standard
1st International Standard for tPA antigen in plasma
NIBSC code: 94/730
Instructions for use
(Version 3.0, Dated 02/04/2008)

1. INTENDED USE
Tissue plasminogen activator (tPA) is a marker for endothelial cell dysfunction and may be raised in subjects suffering from cardiovascular disease. The standard is intended to be used to calibrate methods designed to study tPA antigen in plasma by ELISA methods. A report describing the establishment of this standard is available from WHO (WHO/BS/07.2068).

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
A collaborative study was conducted which included 8 different commercial or in-house ELISA methods and a consensus geometric mean value from these methods determined that each ampoule of the standard contains 25 ng of tPA antigen in 1ml of human plasma.

4. CONTENTS
Country of origin of biological material: United Kingdom. Approximately 2L of human plasma from 10 donors was kindly supplied as fresh frozen units by Bio Products Laboratory, Elstree, Hertfordshire. Plasma from each individual donation was tested and found negative for anti-HIV 1 and 2, anti-HCV and HBsAg. Samples of plasma before addition of tPA were kept and when analysed repeatedly for tPA antigen by ELISA at NIBSC were found to contain approximately 5 ng/ml. The pool was supplemented with recombinant CHO cell-derived tPA (Actilyse, provided by Boehringer Ingelheim, Germany) to a final concentration of 20 ng/ml. This solution was dispensed into ampoules in 1ml aliquots (mean weight 1.0146 g with a cv = 0.125%) and freeze dried. Repeated assays of 94/730 since manufacture indicate the material is stable (see WHO/BS/07.2068).

5. STORAGE
Unopened ampoules should be stored at -20°C. Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials 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 layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point ‘B’. The ampoule will snap open.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

The total contents of the ampoule should be reconstituted with 1.0ml distilled water and dissolved gently by swirling to avoid froth. The reconstituted reagent should be used as soon as possible after reconstitution.

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.

Repeated assays of 94/730 since manufacture indicate the material is stable (see WHO/BS/07.2068).

9. REFERENCES

10. ACKNOWLEDGEMENTS

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
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: Freeze dried powder</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
</tr>
<tr>
<td>Flammable: No</td>
</tr>
<tr>
<td>Other (specify):</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*: United Kingdom
- Net weight: 10mg
- Toxicity Statement: Toxicity not assessed
- Veterinary certificate or other statement if applicable: 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_biolstandardsrev2004.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.