1. INTENDED USE
This material was established as the 3rd International Standard (IS) for Thromboplastin Human, Recombinant, Plain by the WHO Expert Committee on Biological Standardization (ECBS) in 1996 and consists of ampoules containing freeze-dried recombinant tissue factor (coded rTF/95). Details of the collaborative study can be found in document WHO/BS/96.1846 Rev 1 available on the following link: http://www.who.int/bloodproducts/publications/en/96-1846rev1.pdf.

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

This preparation does not contain any material of human origin (see Section 4 for detailed description of contents). 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. INTERNATIONAL SENSITIVITY INDEX (ISI) AND COLLABORATIVE STUDY
The International Standard has been assigned an ISI value of 0.94

The ISI value was determined in a collaborative study against all the available WHO International Reference Preparations of tissue factor from human, rabbit and bovine origin (1). This involved 19 laboratories from Europe, Canada, Argentina and Australia (1). The 3rd International Standard, rTF/95, and the other WHO International Reference Preparations of human, rabbit and bovine origin (i.e., BCT/253, RBT/90 and QBT/79) were tested in each laboratory by the same expert operator using the manual tilt tube technique. Test plasmas were freshly prepared from healthy subjects and patients on long term anticoagulant therapy. Participants selected patient plasmas with prothrombin times (PT) corresponding to an interval of International Normalized Ratios (INR) from 1.5 to 4.5. To account for the effect of inter-day variation, PT measurements were performed in each laboratory on ten different days (not necessarily consecutive). Participants included on each day plasmas from 2 healthy individuals and 6 anticoagulated patients, using plasmas of different healthy subjects and patients on each working day. To minimize the effect of possible plasma instability on the prothrombin times, the order of testing was changed each day. Each plasma was to be tested with each thromboplastin before proceeding to the next. Plasmas were tested on each day according to the following order: - normal plasma 1, patient plasma 1 through 6 and normal plasma 2.

4. CONTENTS
Country of origin of biological material: United Kingdom. THROMBOPLASTIN (lyophilised portion rTF/95), the residue of 1.0 ml of a solution containing: 
Mixed Phospholipids. Individual phospholipid components were manufactured from soybean and other plant sources and are >99.9%

5. STORAGE
Unopened ampoules of rTF/95 should be stored in the dark at -20 °C or below. Store reconstitution fluid at 2 - 8 °C. 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.
Equilibrate ampoules at room temperature for at least 15 minutes before reconstitution.

Each ampoule of the freeze-dried material is to be reconstituted with exactly 1.0 ml of the provided reconstitution fluid (07/284).
Do not attempt to mix the contents by placing the thumb over the open end of the ampoule.
Leave the ampoule undisturbed for 20 minutes at room temperature and then swirl gently to dissolve the contents. Ensure that the entire freeze-dried residue is dissolved. Pool the contents of ampoules if more than one is needed to complete any one calibration session. Leave the reconstituted thromboplastin at room temperature and use within 2 hours of reconstitution. Unused material should be discarded.

CALIBRATION PROCEDURE TO BE USED WITH rTF/95
According to the WHO Guidelines (2) calibration of thromboplastins should be performed on plasmas from 20 healthy subjects and 60 patients on stabilized oral anticoagulant therapy. The whole calibration procedure can be conveniently split into ten working sessions, not necessarily consecutive. Schedule of one-day calibration During the first 2 hours collect the blood, centrifuge and separate the platelet-poor plasma, and reconstitute thromboplastins according to the instructions. During the next 2-3 hours perform the actual testing of plasmas according to the design provided (see below).

Selection of healthy subjects and patients
Healthy subjects must be ambulant adults (females taking oral contraceptives can be included). On each working day use one male and one female (if it is possible) and select a different pair each day. Patients must be different on each day and chosen among those who are in good health (outpatients) and have been stabilized for at least 6 weeks in the range of treatment between 1.5 and 4.5 INR, according to the routine reagent of the laboratory. Select patients covering the whole range of anticoagulation from 1.5 to 4.5. To avoid bias all results obtained with the chosen patients’ plasmas must be recorded.

Blood collection and plasma preparation
At the beginning of each working day collect blood from 2 healthy subjects and 6 patients stabilized on oral anticoagulant treatment. Blood will be collected by clean venipuncture in a plastic (or glass siliconized vacuum) tube containing trisodium citrate solution within the range of concentration...
Preparation of thromboplastins

On each working day:
Equilibrate a suitable number of ampoules of rTF/95 and the provided reconstitution fluid at room temperature for at least 15 minutes before reconstitution.
Equilibrate a suitable number of ampoules of thromboplastin to be calibrated and its reconstitution fluid (if any) at room temperature for at least 15 minutes before reconstitution.
Reconstitute ampoules of rTF/95 following instruction (see sections 6 and 7 above).
Reconstitute ampoules of thromboplastin to be calibrated following instructions. **Discard the remaining reconstituted thromboplastins at the end of each working day.**

Testing procedure

Test the 8 plasma samples (2 normal and 6 patients stabilized on oral anticoagulant therapy) with the two thromboplastins according to the design given below. **Testing must be done as single determinations.**
Calibrations using rTF/95 must be performed exclusively by using automatic (either tilt tube or Koller-Hook) technique whereas a coagulometer may be used for testing with the thromboplastin to be calibrated with a secondary standard as appropriate. With the tilt tube technique, test tubes must be immersed in the water bath as deeply as possible to ensure optimal temperature control. Tilt the tubes back and forth at regular intervals. To avoid prolongation of removal of tubes from the water, the use of an illuminated water-bath is recommended. The order of testing normal and patient plasmas will be random and must reflect the order of blood collection if this is considered random. As an example collect first the normal 1 (which will be tested first) then the 6 patients on oral anticoagulant treatment and finally the normal 2 (which will be tested last). In any case the order of testing should not be related to the prolongation of the clotting time of the patient plasma. The order of testing on each working day shall be as follows:

**Normal 1 Patient 1 Patient 2 Patient 3 Patient 4 Patient 5 Patient 6 Normal 2**

Each plasma shall be tested with both thromboplastins before proceeding to the next if both are used with the manual technique. If the prothrombin time system to be calibrated involves the use of an automated instrument, it is not practical to test each plasma with both thromboplastins before proceeding to the next. In that case, all plasmas can be tested with each thromboplastin consecutively and more or less simultaneously with both thromboplastins. The same expert operator shall be in charge to carry out the whole calibration.

**Actual testing with rTF/95**

Place glass test tubes in the water bath and wait at least 5 minutes to reach 37°C.

Pipette 0.2 ml of rTF/95 and incubate for at least 2 minutes to reach 37°C.

Pipette 0.1 ml not pre-warmed test plasma and start a stopwatch immediately.

Shake to mix the content and tilt the tube regularly back and forth until clot forms.

Record the clotting time in seconds and 1/10 seconds.

**Equipment**

Calibrated pipettes to reconstitute thromboplastins and to deliver thromboplastin and plasma samples for actual testing. If automated micro-pipettes are used, tips must be changed for each test.
Non-contact tubes with non-contact stoppers (no rubber) to store blood and plasma.
Non-contact pipettes to transfer plasmas for storage and to dispense plasmas for testing.
Glass tubes for testing

Water-bath thermostatted at 37°C ± 0.5.
Stopwatches

**Statistical analysis and ISI determination**

For statistical analysis and ISI determination refer to the WHO Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy (2). These requirements are available on request from the Biologicals Unit, WHO, CH-1211 Geneva 27, Switzerland.

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.

9. REFERENCES


10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to the participants in the collaborative study. This study was organized and carried out under the auspices of the Scientific and Standardization Committee (SSC) (Subcommittee on Control of Anticoagulation), of the International Society on Thrombosis and Haemostasis (ISTH). Grateful acknowledgements are also due to Ortho Clinical Diagnostics, Raritan N.J. and Dade International, Miami Fl., who donated the candidate materials for the collaborative study.

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): Contains recombinant protein, stabilizers and preservative (azide)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation: Not established, avoid inhalation</td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

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: 0.100 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

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