WHO International Standard
WHO INTERNATIONAL STANDARD THROMBOPLASTIN, RABBIT, PLAIN
NIBSC code: RBT/05
Instructions for use
(Version 3.0, Dated 13/12/2012)

1. INTENDED USE
This material was established as the International Standard for Thromboplastin Rabbit, Plain by the WHO Expert Committee on Biological Standardization (ECBS) in 2005 and consists of ampoules containing freeze-dried rabbit brain thromboplastin (coded RBT/05). Details of the collaborative study can be found in document WHO/BS/05.2029.

This STANDARD MUST BE RECONSTITUTED USING THE RECONSTITUTION FLUID (04/210) PROVIDED.

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

The preparation consists of a tissue extract from rabbit brain which contains tissue factor and phospholipids; it does not contain material of human origin. 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. UNTAGE
The International Standard has been assigned an ISI value of 1.15

The ISI value was determined in a collaborative study against all the available WHO International Standards from human, and bovine origin and the certified reference materials for rabbit thromboplastin available from the Institute for Reference Materials and Measurements (IRMM) of the European Commission and the European Action on Anticoagulation (EAA) (1). The collaborative study involved 21 laboratories from Europe, Canada, South America and Asia (1). RBT/05 and the other WHO, IRMM and EAA standards from human, rabbit and bovine origin (i.e., rTF/95, ERM-AD149, EUTHR-01 and OBT/79) were tested in each laboratory by the same expert operator with the manual (till tube) technique. Test plasmas were freshly prepared from healthy subjects and patients stabilized on long term oral anticoagulant therapy. Participants were instructed to select patient plasmas with PT corresponding to an interval of 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 were instructed to include on each day plasmas from 2 healthy individuals and 6 anticoagulated patients. Healthy subjects and patients had to be different on each working day. To minimize the effect of plasma instability on the relationship between the thromboplastins, 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.

The material was supplied by a manufacturer of commercial reagent and consists of a tissue extract from rabbit brain which contains tissue factor and phospholipids.

5. STORAGE
Store unopened ampoules of RBT/05 in the dark 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.

Equilibrate ampoules at room temperature for at least 15 minutes before reconstitution. Each ampoule of the lyophilized material is to be reconstituted with exactly 1.0 ml of the provided reconstitution fluid (coded 04/210). Leave the ampoule undisturbed 20 minutes at room temperature and then swirl gently to dissolve the content. Pool the contents of ampoules if more than one is needed to complete one calibration session. Leave thromboplastin at room temperature and do not use the reconstituted material for longer than 2 hours. This thromboplastin does not contain calcium chloride.

CALIBRATION PROCEDURE TO BE USED WITH RBT/05

According to the WHO Guidelines (2) full 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 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 take 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 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 105-109 mmol/L (9 volumes of blood/1 volume of sodium citrate anticoagulant). The tube must be inverted several times to ensure complete mixing of blood and anticoagulant. Citrated blood will be centrifuged immediately after collection at least 2,500g for 10 minutes at a controlled
Preparation of thromboplastins

On each working day:

- Equilibrate a suitable number of ampoules of RBT/05 and of 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 RBT/05 following instructions (see sections 6 and 7 above).
- Reconstitute ampoules of thromboplastin to be calibrated following instructions. Note: 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.

- Testing with RBT/05 must be performed exclusively by using manual (tilt tube) technique, whereas a coagulometer may be used for testing with the thromboplastin to be calibrated. With the tilt tube technique, test tubes must be immersed in the water as deeply as possible to ensure optimal temperature control. Tilt the tubes back and forth at regular intervals. To avoid prolonged 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 RBT/05

- Pipette 0.1 ml RBT/05 into glass tube at 37° C and incubate for 2 minutes.
- Pipette 0.1 ml not pre-warmed test plasma, mix well and incubate for 1 minute.
- Pipette 0.1 ml pre-warmed (at 37°C) 25 mmol/L calcium chloride 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 test 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).

8. STABILITY

Reference materials are held at NIBSC within assured temperature-controlled storage facilities and they 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. Stability studies have indicated that this material is suitably stable, when stored at -20 °C or below, for the assigned values to remain valid until the material is withdrawn or replaced. These studies have also shown that the material is suitably stable for shipment at ambient temperature without any effect on the assigned values. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES


10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to the participants in the collaborative study and to the manufacturers (in alphabetical order) of RBT/05 and the other candidate which was included in the collaborative study (Axis-Shield, Norway and South Manchester University Hospitals Trust, UK).

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:
  - Derivation of International Units:
    - http://www.nibsc.org/standardisation/international_standards.aspx
  - Ordering standards from NIBSC:
  - 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

Physical and Chemical properties

<table>
<thead>
<tr>
<th>Physical appearance: Freeze-dried powder Corrosive: No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable: Yes Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: Yes Irritant: No</td>
</tr>
<tr>
<td>Flammable: No Handling: See caution, Section 2</td>
</tr>
</tbody>
</table>

Other (specify): Material extracted from rabbit brain

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

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*: United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>* 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</td>
</tr>
<tr>
<td>Net weight: 0.100 g</td>
</tr>
<tr>
<td>Toxicity Statement: Non-toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable. Attached: No</td>
</tr>
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</table>

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_bioefstandardsrev2004.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.

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