THROMBOPLASTIN HUMAN RECOMBINANT PLAIN

3rd International Standard
(rTF/95)
Instructions for Use

1. INTRODUCTION

This material was established as the 3rd International Standard (IS) for thromboplastin human, recombinant plain by the WHO Expert Committee on Biological Standardization in 1996. It consists of a batch of ampoules containing freeze-dried rTF (coded rTF/95), and a batch of ampoules with diluent to reconstitute the rTF/95.

2. AMPOULE CONTENTS

2.1. THROMBOPLASTIN (lyophilized portion rTF/95), the residue of 1.0 ml of a solution containing:


2.1.2. Mixed Phospholipids. Individual phospholipid components were manufactured from soybean and other plant source and are > 99.9% pure. An antioxidant is included in the final lipid blend to prevent oxidation.

2.1.3. Stabilizers. A sugar is used as a stabilizer of the lyophilized product.

2.1.4. Preservatives. Sodium Azide (0.04%) is used as preservative in the lyophilized product.

2.2. DILUENT (liquid portion) which contains:

2.2.1. Calcium Chloride (12.04 mmol/L) and Proxel GXL (0.03%) as preservative.

3. INTERNATIONAL SENSITIVITY INDEX (ISI) AND COLLABORATIVE STUDY

3.1. ISI.

The ISI of this International Standard is 0.94 and was determined in a collaborative study against the other WHO International Reference Preparations of tissue factor of human, rabbit and bovine origin (1).

3.2. Collaborative Study.

This involved 19 laboratories from Europe, Canada, Argentina and Australia (1).
3.3. Design of the study.

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 OBT/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. STORAGE OF AMPOULES

The lyophilized portion of rTF/95 should be stored at -20 °C and the diluent at 4°C.

5. RECONSTITUTION OF THE 3rd INTERNATIONAL STANDARD

Equilibrate ampoules of the 3rd International Standard, freeze-dried thromboplastin (rTF/95) and its reconstitution fluid, at room temperature 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 diluent. Leave the ampoule undisturbed for 20 minutes at room temperature and then swirl gently to dissolve the content. 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. The material reconstituted, which is kept at room temperature, should be used only within 2 hours of reconstitution. Unused material remaining should be discarded.

No attempt should be made to weigh out any portion of the freeze-dried material.

6. CALIBRATION PROCEDURE TO BE USED WITH rTF/95

According to the WHO Requirements (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.

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6.1. 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).

6.2. Selection of healthy subjects and patients

6.2.1. Healthy subjects must be ambulant adults (females taking oral contraceptives may be included). On each working day use one male and one female (if it is possible) and select a different pair each day.

6.2.2. Different patients must be chosen on each day among outpatients who have been stabilized on oral anticoagulants 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 INR. To avoid bias, all results obtained with the chosen patients' plasmas must be recorded.

6.3. 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 should be collected by clean venipuncture in a plastic (or glass siliconized vacuum) tube containing 109 mmol/L trisodium citrate solution (9 volume of blood/1 volume of anticoagulant). The tube must be inverted several times to ensure complete mixing of blood and anticoagulant. Citrated blood should be centrifuged immediately after collection at least 2,500 g for 10 minutes at a controlled room temperature. Platelet-poor plasmas are transferred into plastic tubes and stored capped at room temperature until tested.

6.4. Preparation of thromboplastins

On each working day:

6.4.1. Equilibrate a suitable number of ampoules of rTF/95 and its diluent at room temperature for at least 15 minutes before reconstitution.

6.4.2. Equilibrate a suitable number of ampoules of thromboplastin to be calibrated and its diluent (if any) at room temperature for at least 15 minutes before reconstitution.

6.4.3. Reconstitute ampoules of rTF/95 following instruction (see section 6, above).

6.4.4. Reconstitute ampoules of thromboplastin to be calibrated following instructions.

Note: Discard the remaining reconstituted thromboplastins at the end of each working day.
6.5. 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 manual (either tilt tube or Kolle-Hook) technique, whereas a coagulometer may be used for testing the thromboplastin to be calibrated with a secondary standard as appropriate. If the tilt tube technique is used, 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
2
3
4
5
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. The same expert operator shall be in charge to carry out the whole calibration.

6.6. Actual testing with rTF/95

6.6.1. Place glass test tubes in the water bath and wait at least 5 minutes to reach 37° C.
6.6.2. Pipette 0.2 ml of rTF/95 and incubate for at least 2 minutes to reach 37° C.
6.6.3. Pipette 0.1 ml not prewarmed test plasma and start a stopwatch immediately.
6.6.4. Shake to mix the content and tilt the tube regularly back and forth until clot forms.
6.6.5. Record the clotting time in seconds and 1/10 seconds.
7. EQUIPMENT

7.1. Calibrated pipettes to reconstitute thromboplastins and to deliver thromboplastins and plasma samples for actual testing. If automated micro-pipettes are used, tips must be changed for each test.
7.2. Non-contact tubes with non-contact stoppers (no rubber) to store blood and plasma.
7.3. Non-contact pipettes to transfer plasmas for storage and to dispense plasmas for testing.
7.4. Glass tubes for testing
7.5. Water-bath thermostatted at 37° C ± 0.2.

8. STATISTICAL ANALYSIS AND ISI DETERMINATION

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

9. CITATION

It is important that the name of the preparation, ampoule codes, name and address of the supplier are cited correctly in all publications (or data sheets for kits) which refer to the use of this International Standard as the primary reference material for calibration.

10. CAUTION

**THIS PREPARATION IS FOR LABORATORY USE ONLY AND SHOULD NOT BE ADMINISTERED TO HUMANS.**

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 probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

11. PRODUCT LIABILITY

Information emanating from CLB is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but is provided without liability in its application and use.

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12. REFERENCES


13. ACKNOWLEDGEMENTS

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