Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
5th International Standard 2016
Thromboplastin, Human, Recombinant, Plain
NIBSC code: rTF/16
Instructions for use
(Version 2.0, Dated 03/02/2021)

1. INTENDED USE

This standard is used for the ISI calibration of human thromboplastin reagents. The standard was established as the WHO 5th International Standard (IS) for Thromboplastin Human, Recombinant, Plain by the WHO Expert Committee on Biological Standardization (ECBS) in 2016 and consists of ampoules containing lyophilised recombinant human tissue factor reagent (coded 14/324) and ampoules of diluent for reconstitution (coded 14/326). Details of the collaborative study can be found in document WHO/BS/2016.2294.

THIS STANDARD MUST BE RECONSTITUTED USING THE DILUENT (14/326) PROVIDED

2. CAUTION

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

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

INTERNATIONAL SENSITIVITY INDEX (ISI) AND COLLABORATIVE STUDY

The International Standard has been assigned an ISI value of 1.11

The ISI value was determined in a collaborative study against the WHO International Reference Preparations of tissue factor from human (rTF/09) and rabbit origin (RBT/05). The study involved 20 laboratories from Europe, North America, South America, Australia and Asia. The candidate 5th International Standard and the WHO International Reference Preparations of human and rabbit origin (ie. rTF/09 and RBT/05) 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 States of America.
THROMBOPLASTIN REAGENT (lyophilised portion coded 14/324), the residue of a solution containing:
Tissue Factor (TF). A human recombinant membrane-spanning protein, expressed in a baculovirus expression vector and purified using ion exchange and size exclusion chromatography.
Mixed Phospholipids. Individual phospholipid components were prepared synthetically and are >99.9% pure. An antioxidant is included in the final lipid blend to prevent oxidation.
Stabilizers. A sugar is used as a stabilizer of the lyophilized product.
Preservatives. Sodium Azide (0.04 %) is used as preservative in the lyophilized product

DILUENT FOR RECONSTITUTION (coded 14/326) A liquid preparation which contains: calcium chloride, heparin neutralizing agent (polybrene) and preservative.

5. STORAGE

Unopened ampoules of lyophilised rTF/16 reagent (14/324) should be stored in the dark at -20 °C or below. Unopened ampoules of the diluent (14/326) should be stored in the dark between 2 to 8 °C - do not freeze.

Please note: NIBSC may ship these materials with cooling packs to ensure stability in transit.

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 lyophilised rTF/16 reagent (14/324) is to be reconstituted with exactly 1.0 ml of the provided diluent (14/326).
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 lyophilised reagent is dissolved. Pool the contents of ampoules if more than one is needed to complete any one calibration session. Leave the reconstituted thromboplastin (rTF/16) at room temperature and use within 4 hours of reconstitution. Unused material should be discarded.

CALIBRATION PROCEDURE TO BE USED WITH rTF/16

According to the WHO Guidelines (1) 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 five or more 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 male and female subjects (if it is possible) and select different subjects 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 healthy subjects and 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 room temperature. Platelet-poor plasmas are transferred into plastic tubes and stored capped **at room temperature**, until tested.

Preparation of thromboplastins
On each working day:
Equilibrate a suitable number of ampoules of lyophilised rTF/16 reagent (14/324) and the provided diluent (14/326) 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 lyophilised rTF/16 reagent (14/324) following instructions (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
If testing is performed in 10 working sessions, the following procedure is used in each session. 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/16 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

If testing is performed in 5 working sessions, the number of samples used in each session is 16 (4 normal and 12 patients). The order of testing on each working day shall be as follows:

Normal 1, Normal 2, Patient 1 - 12, Normal 3, Normal 4

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/16
Place glass test tubes in the water bath and wait at least 5 minutes to reach 37ºC.
Pipette 0.2 ml of rTF/16 and incubate for 2 minutes to reach 37ºC.
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 (1). 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 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 with cooling packs 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
1 WHO Expert Committee on Biological Standardization. Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy with vitamin K antagonists. WHO Technical Report Series 2013; No. 979: 271-316.

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 Instrumentation Laboratory (Orangeburg, NY) and Technoclone GmbH (Vienna, Austria), who donated the candidate materials and control samples 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

Physical and Chemical properties	
Physical appearance: Freeze-dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains recombinant protein, stabilizers and preservative (azide)	
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*: USA * 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

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_biolefstandardsrev2004.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.