THROMBOPLASTIN BOVINE, COMBINED
2nd International Reference Preparation
(code OBT/79)

1. INTRODUCTION

This material which was established as the 2nd IRP for thromboplastin bovine, combined by the WHO Expert Committee on Biological Standardization (ECBS) in 1983 consists of a batch of ampoules (coded OBT/79).

2. AMPOULE CONTENTS

The IRP is composed of thromboplastin which is the residue of 2.2 ml of a solution of tissue extract from bovine brain which contains Tissue Factor, phospholipids and adsorbed bovine plasma as source of fibrinogen and factor V.

3. INTERNATIONAL SENSITIVITY INDEX (ISI) AND COLLABORATIVE STUDY

3.1. ISI. The ISI of the IRP is 1.0 and has been determined in a collaborative study against the 1st WHO IRP 67/40 from human, origin (1).

3.2. Collaborative Study. This involved 10 laboratories from Europe and United States (2).

3.3. Design of the study. OBT/79 and the WHO IRP from human, origin (i.e., 67/40) were tested in each laboratory by the same expert operator with the manual (tilt tube) technique. Test plasmas were freshly prepared from healthy subjects and patients stabilized on long term anticoagulant therapy. Participants were instructed to select patient plasmas with PT corresponding to an interval of INR from 1.5 to 5.0. To account for the effect of inter-day variation, PT measurements were performed in each laboratory on 10 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. CAUTION
This preparation is not for administration to humans.

5. STORAGE OF AMPOULES

Ampoules of OBT/79 shall be stored at -20 °C.

6. RECONSTITUTION OF OBT/79

Equilibrate ampoules of OBT/79 and the diluent (3.2 mmol/L CaCl₂) at room temperature at least 15 minutes before reconstitution. Each ampoule of the lyophilized material is to be reconstituted with exactly 2.2 ml of 3.2 mmol CaCl₂. Leave the ampoule undisturbed 20 minutes at room temperature and then swirl gently to dissolve the content. Shake vigorously 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.

7. CALIBRATION PROCEDURE TO BE USED WITH OBT/79

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.

7.1. 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).

7.2. Selection of healthy subjects and patients

7.2.1. 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.

7.2.2. 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 INR. To avoid bias all results obtained with the chosen patients must be recorded.
7.3. 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 will 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 will be centrifuged immediately after collection at least 2,500 g for 10 minutes at a controlled room temperature. Plasma will be transferred into plastic tubes and stored capped at room temperature until testing.

7.4. Preparation of thromboplastins

On each working day:

7.4.1. Equilibrate a suitable number of ampoules of OBT/79 and its diluent (3.2 mmol/L CaCl₂) at room temperature for at least 15 minutes before reconstitution.

7.4.2. 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.

7.4.3. Reconstitute ampoules of OBT/79 following instructions (see section 6, above).

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

Note: Discard the remaining reconstituted thromboplastins at the end of each working day.

7.5. 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. Testing with OBT/79 must be performed exclusively by using manual (either tilt tube or Kolle-Hook) technique, whereas a coagulometer may be used for testing with the thromboplastin to be calibrated. 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 thromboplatin consecutively, and more or less simultaneously with both thromboplastins. The same expert operator shall be in charge to carry out the whole calibration.

7.6. Actual testing with OBT/79

7.6.1. Place glass test tubes in the water bath and wait at least 5 minutes to reach $37^\circ$ C.

7.6.2. Pipette 0.4 ml of OBT/79 and incubate for at least 2 minutes to reach $37^\circ$ C.

7.6.3. Pipette 0.05 ml not prewarmed test plasma and start a stopwatch immediately.

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

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

8. EQUIPMENT

8.1. 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.

8.2. Non-contact tubes with non-contact stoppers (no rubber) to store blood and plasma.
8.3. Non-contact pipettes to transfer plasmas for storage and to dispense plasmas for testing.

8.4. Glass tubes for testing

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

9. 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 (3).

10. ACKNOWLEDGEMENT

Grateful acknowledgements are due to the participants in the collaborative study and the manufacturer of OBT/79 (Nyegaard, now Nycomed Pharma, Oslo, Norway).

11. REFERENCES


11.3. Requirement for thromboplastins and plasma used to control oral anticoagulant therapy 1997 WHO, Geneva, Switzerland.