6. Quality Assurance

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6.1 Introduction

Quality assurance is to ensure the reliability of the laboratory tests. The objective is to achieve precise and accurate test results.

Precision can be achieved by replicate tests and by repeated tests on previously measured specimens. Accuracy is checked by the use of reference material which has been assayed by reference methods.

Internal quality control

Monitoring the haematology test procedures that are performed in the laboratory by

- Measurement on specially prepared material.
- Reported measurements on routine specimen
- Statistical analysis.

External quality assessment

Is the evaluation of the laboratory performance by an outside agency by specifically supplied samples.

Analysis of performances is retrospective.

The objective is to achieve inter laboratory or inter methodological comparability.

6.2 Quality control material

Blood collected in to ACD or CPD.
And pass through blood infusion sets to remove clots.
All handling should be aseptic
Broad spectrum antibiotic should be added–
-1mg penicillin
-5mg gentamicin
Human blood should be negative for help B, C, D, & HIV.

Preparation method

- Collect blood into sterile container with ACD or CPD
- Leave for 2-3 days at 40C & centrifuge the blood at 2000g. Separate the plasma & buffy coat & transfer to 500ml bottles.
- Mix 3 volumes of the red cells with 1.5 volumes of 9g/l NaCl, centrifuge for 20min at 2000g and remove the supernatant and upper layer of the red cells by suction
- Repeat step 3.
- Dilute 5 volumes of the plasma with 2 volumes of 9g/l NaCl and add an antibiotic.
- Add the diluted plasma from step 5 to the red cell concentrate at an appropriate ratio to obtain a preparation suitable for use as red cell count control.
- Mix well with continuous mixing dispense in aliquot volumes into clean sterile vials and cap tightly. Store at 40c.

Assign values for Hb, RBC count and PCV by at least 5 replicate measurements, using the counter on which the subsequent tests will be performed.

Before analysis, mix the sample
- Roller mixer
- Continuously by hand for 5 min.

The coefficient of variation (CV) between two tests results should not exceed 2%.

Unopened vials of human blood could be kept in good condition for about 3 weeks at 40c, equine blood for up to 2 months.

6.3 Preparation of lysate.

- Collect blood into a blood transfusion donor bag. Out of date donor blood can be used, provided that it is not lysed.Centrifuge at c 2000g for 20 min and discard the plasma and buffy coat.

- Add an equal volume of 9g/l NaCl, mix well, transfer to a sterile centrifuge bottle and re-centrifuge. Discard the supernatant.
- Repeat the saline wash three times to ensure complete removal of the plasma, leucocytes and platelets.

- To each 10ml volumes of washed cells, add 6 volume of H_2O + 4 volumes of toluene, cap and shake vigorously on a mechanical shaker or vibrator for 1h. Then keep overnight at 40c to

allow the lipid / cell debris to form a semi- solid surface between the toluene and lysate.

- On the following day, centrifuge at c 2000g for 20 min remove the lysate layers and pool them in a clean bottle.

- Using gentle vacuum suction e.g. by water pump, fitter lysate through coarse fitter paper (whatman No. 1) in a Buchner funnel.

- Repeat filtration using 0.22 µm micropore or fine filter paper (whatman No. 42), changing the paper whenever the filtration slows down.

- To each 10 ml of lysate, add 30 ml, of glycerol and broad spectrum antibiotic. If a lower Hb is requested add 30% glycerol in saline. Mix well, dispense into sterile containers and cap tightly.

- Assign a value for Hb concentration by the spectrophotometric method, carry out 10 replicate tests, taking samples at random from several vials of the batch.

- The CV should be less than 2% .
  Stored at 40c the product should retain it's assigned value for at least several months or for 1 to 2 years if kept at 20^0c .

6.4 Preparation of stabilized whole blood control

Reagent

formaldehyde	37-40%	6.75ml
Glutaraldehyde	50%	0.75ml
Trisodium citrate		26 g
Water to		100ml

Method

- Obtain whole blood in CPD or ACD. This should be as fresh as possible and never more than 48h old. Filter through a 40µm blood filter into a series of plastic bottle.

- If an increased red cell count is required, centrifuge one (or more) of the sample and remove part of the plasma; if a lower red cell count is required, add to another sample the plasma which was removed. If paired bottles are gently centrifuged (c 1500g) for 15 minutes to produce buffy coats, those can be manipulated in a similar way to provide different levels of leucocytes and platelet counts. Add broad spectrum antibiotics to each sample.

- Mix well and add 1 volume of reagent to 50 volumes of the cell suspension. Mix on a mechanical mixer for 1h at room temperature and leave for 24h at 40C.

- With continues mixing, dispense in to sterile containers, cap tightly and seal with plastic tape. Refrigerate at 40C until needed. For analysis. Sample should be gently mixed on a roller mixer or by hand before opening. Assign values for Hb and cell counts by at least five replicate tests and check between sample homogeneity by repeated counts on five randomly selected vials. CV should not exceed 2%. Note, however that the PCV by centrifugation will be c 10% lower than the haematocrit obtained by automated count.

6.5 Simple method for blood quality control preparation

This provides a suitable preparation for control of total red cells, leucocytes and platelet counting by semi-automated blood cell counters, but it is not suitable for some automated systems. It should be stable for 3 weeks if kept at 4^0C.

Method

- Collect a unit of venous blood into CPD anticoagulant. Carry out the subsequent procedures not later than 1 day after collection.
- Filter the blood through a blood transfusion recipient set into a 500ml glass bottle.
- Add 1ml of fresh 40% formaldehyde. Mix well by inverting and then leave on a roller mixer for 1h. Leave at 4^0C for 7 days, mixing by inverting a few times each day. At the end of this period of storage, mix well on a roller mixer for 20 min and then, with constant mixing by hand, disperse in 2ml volumes in to sterile containers.

6.6 Preparation of surrogate leucocytes

Chicken and Turkey red blood cells are used for this purpose as they are nucleated and when fixed their size is within the human leucocyte range as recognized on electronic cell counters.

However, such material may not be suitable for the newer counting systems which are based on technologies other then impedance cell sizing.

For use as which blood cell control, 25ml of blood collected into any anticoagulant will suffice; after processing an appropriate amount is added to preserve whole blood. Sterility must be maintained through out the procedure.

Method

- Centrifuge blood at c 2000g for 20min and remover the plasma aseptically.

- Add an equal volume of 0.15 mol/l phosphate buffer, pH 7.4; mix and transfer to a sterile centrifuge bottle, re-centrifuge and discard the supernatant and buffy coat.

- Repeat the wash and centrifugation twice. To the washed cells, add 10 times their volume of gluteraldehyde fixative (0.25% in 0.15 mol/l phosphate buffer, pH 7.4) leave overnight at 40C.

- On the next day shake vigorously to ensure complete suspension. Mix on a mechanical mixer for 1 h. To check that fixation has been complete, centrifuge 2-3 ml of the suspension, discard the supernatant and add water to the deposit. If lysis occurs the stock gluteraldehyde requires replacement.

- When fixation is complete (i.e. after18 h exposure) centrifuge the suspension at c 2000g for 10min and discard the supernatant. Add an equal volume of water in the fixed cell deposit; re-suspend and mix by stirring and shaking; re-centrifuge at c 2000g for 10 min and discard the supernatant; repeat twice.

- Re-suspend the fixed cells to c 30% concentration in 9 g/l NaCl. Mix well with vigorous shaking. Add antibiotic, cap tightly, seal with a plastic seal and store at 40C.

Before use, stand at room temperature for 10-20 min; then re-suspend by vigorous shaking by hand or on a vortex mixer until no clumps remain at the base of the container and then mix on a rotary mixer for at least 20 min before opening the vial.

For the use as a surrogate, after re-suspension as described above, transfer an appropriate amount to a volume of preserved blood from which the leucocytes have been depleted by passing through a leukocyte filter. Establish the count by 5-10 replicate measurements on these vials and check inter-sample homogeneity by count

on three random vials from the batch. The CV should be not more then 5%.

An occasional batch may be unsatisfactory and should be discarded.

6.7 Quality control preparation for platelet counts.

Reagents

Alsever's solution –

(A) Trisodium citrate, 16g; Nacl, 8.2g to 1 liter with water

(B) Dextrose 41 g to 1 liter with water.

Store at 4^0C. Immediately before use mix equal volumes at A and B; filter through 0.2µm microspore filter.

EDTA solution 100g/l of k2 EDTA in the Alsever's solution; stable for 6 month at 4^0C.

Method

- Collect a unit of blood into ACD or CPD anticoagulant. Centrifuge for 10min at 200g and collect the platelet suspension into a plastic container.

- Add 1ml of EDTA solution, mix well and leave at 370C for 2h to allow the platelets to disaggregate.

- Add 200 ml of gluteraldeheyde fixative (0.25% in 0.15 mol/l phosphate buffer, pH 7.4) shake vigorously by hand to ensure complete platelet

distribution and leave for 48hr. at room tempered with occasional shaking.

- Centrifuge for 30 min at 3500g. Wash the deposit twice in Alsever's solution and finally re-suspend in 15-20 ml of Alsever's solution.

- Carry out a rough of platelet count to determine the approximate concentration and add on appropriate amount of the suspension to preserved blood. Mix well for 20min and with continues mixing dispense into sterile containers. Cap and seal. At 40C the preparation should have a shelf life of 3-4 months. Before use re-suspend by thorough hand shaking followed by mechanical mixing for c 15 min.

A simpler method for preserving platelets by adding prostaglandin E1 to blood in ACD provides a control preparation with stability of about 14 days.

6.8 Preparation and calibration of pooled normal plasma (PNP)

Donors

Minimum of 20 normal healthy individuals not taking medications which interfere with clotting factors and coagulation reaction. It is acceptable to include women taking oral contraceptives. An approximately equal number of males and females are desirable. The age range should be 20-50 years

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Anticoagulant

3.2% tri sodium citrate.

Collection

Donors are bled between 9.00-11.00 a.m. using plastic disposable syringes and 21-G needles.

Method

- Collect 9 ml blood and mix with 1 ml anticoagulation in plastic containers.
- Store sample on melting ice during preparation of pool.
- Centrifuge at 40C for 15 min at 2500g.
- Record individual sample for PT/ APTT/ TT. Samples falling out side the range to be discarded.
- Pool plasma in plastic non-contact container. Check PT/ APTT from pool plasma to establish the range for the lab.
- Dispense 0.5ml aliquots of pooled plasma using a plastic pipette into 1.5ml plastic vials.
- Snap freeze on day ice/solid co2 if available. Alternatively place immediately on an open shelf at -700C.
- store at -700C for up to 6 months

Complete above procedures within 4 hours.

A pooled normal plasma (PNP) prepared in this way can be assumed to have a concentration of 100u/dl or 1u/ml of most clotting factors, where 1 unit is defined as that amount of clotting factors contained in 1ml of PNP. This is acceptable for factors ii, v, vii, x, xi and xii. In

practice this is also the case for factors ix. Since these pools may contain variable amounts of these factors it is not acceptable to assume that they contain 100u/dl of factors vii & VWF. It is essential that PNP for factor viii and VWF and preferably factor ix to be calibrated with reference preparations, (available now world Health organization) before being used as standards in assays of these clotting factors.

Protocol for calibration of a local PNP

- Obtain calibrated standard e.g. WHO international standard (IS) is (minimum 2 vials). Perform the test on 2 different days. On each day use 1 vial of IS and 4 aliquots of local PNP.
- On day 1 test the samples in the following order. IS; local; local; IS and repeat this using test dilutions of each plasma.
- on day 2 test the sample in this order local; local; lS ; lS; local; local and repeat this using fresh dilutions of each plasma.
- Calculate potency of each aliquot of local standard against average of results with the two lS.
- The mean result of 4 aliquots x 2 dilutions x 2 (n=16) is assigned to the local standard as it's potency.

6.9 Reference:

1 Dacie JV,Lewis SM.*Practical haematology*,Edinburgh:Churchill Livingstone,1994.