BUFFERED 3.2% CITRATE SOLUTION (PROFACT)

(Courtesy: Tulip Group of Companies)
Ready to use 3.2% buffered citrate solution for coagulation assays and ESR by Westergren method.

Summary

Accurate coagulation testing is dependent on numerous preanalytical variables, which may affect the results of routine coagulation assays. To improve the precision and accuracy of laboratory testing, it is important to identify these variables and control their potential effect on results.

Preanalytical variables pertinent to routine coagulation testing can be classified into three major categories: specimen collection, specimen processing and specimen storage and transport. 3.2% citrate is also the anticoagulant of choice for performing ESR by Westergren method.

Reagent

Laboratory reagent: Ready-to-use solution.

Profact is a unique ready-to-use 3.2% buffered trisodium citrate solution formulated for collection of blood for routine coagulation assays. Profact can be used for sample preparation in the following clot based assays such as PT, APTT, TT, quantitative estimation of fibrinogen, test for factor deficiency, test for lupus anticoagulants, protein C and protein S tests. Profact can also be used for collection of blood to perform ESR by Westergren method.

Principle

About 3.2% trisodium citrate is the anticoagulant of choice for coagulation studies. When anticoagulated blood is centrifuged for preparing PPP for routine coagulation assays, the centrifugation process leads to release of carbon dioxide (CO_2). The end result being shift in pH, which has an adverse impact on the results of clot-based assays. Profact incorporates 3.2% trisodium citrate in a unique protective solution, which arrests shift in pH due to the release of carbon dioxide during centrifugation. Also labile factor V and VIII are well preserved and the results of clotbased assays are more accurate.

Also Profact incorporating 3.2% citrate is the anti-coagulant of choice for ESR by Westergren method.

Storage and Stability

- Store the reagent at 2 to 8°C
- Stability of unopened vial: 12 months from the date of manufacturing.
- Stability of opened vial: 90 days from the date of opening, provided it is not contaminated.

Material Required But Not Provided

Sterile and clean 0.5/1 mL micropipettes, micropipette tips or glass blow out pipettes, ESR tube.

Sample Collection and Preparation

For Coagulation Assays

Though no special preparation of the patient is required prior to sample collection by approved techniques, it is preferable that patients are not heavily exercised before blood collection. Fasting or only light non-fatty meals prior to blood collection provides sample with a desirable low opacity.

Withdraw blood without undue venous or frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. The venipuncture must be a 'clean' one and if there is any difficulty, take a new syringe and try another vein. Transfer the blood into tubes containing Profact, after detaching the needle from the syringe. Do not delay mixing blood with Profact. Avoid foam formation during mixing. Mix exactly nine parts of freshly collected blood with one part of Profact. For occasional patients with hematocrit less than 20% or greater than 50%, this ratio must be readjusted to ensure valid results. Centrifuge immediately for 15 minutes at 1500 to 3000 rpm (approximately 1500 g) on a laboratory centrifuge and transfer the plasma into a clean test tube. It should be ensured that the plasma is free from platelets (PPP). Cap the test tubes to prevent deterioration of samples. Plasma must be tested preferably immediately. However, if the specimen is held at 22 to 24°C then they may be tested within 2 hours and if the specimen is held at 2 to 4°C then they may be tested within 3 hours. Also plasma samples obtained after collection with Profact may be stored at –20°C for 2 to 3 weeks before testing.

For ESR by Westergren Method

For performing the test, venous blood is mixed accurately in the proportion of 1 part of Profact and 4 parts of whole blood.

The sedimentation rate is reduced in stored blood, hence, the test should be carried out within 4 hours of collecting the blood, and a delay up to 6 hours is permissible provided that the blood is kept at 4°C.

Precautions

1. Take every possible aseptic precaution to minimize contamination while drawing the reagent.

2. Avoid dipping contaminated pipettes/micropipette tips in the reagent vial. Ideally pour the required quantity for the day's work into another sterile clean vial.
3. Recap and replace the reagent vial immediately back at 2 to 8°C.

Remarks

1. Since most of the routine coagulation assays use PPP, each laboratory must calibrate the necessary force and time required during centrifugation to yield PPP.
2. Incorrect mixture of blood and Profact is a potential source of error both in coagulation assays and ESR estimation.
3. If the reagent vial develops turbidity, do not use the reagent as this would lead to erroneous results.

Calibration of Instruments/Equipments

- Water baths or heating blocks calibrated and preset at 37 ± 0.5°C are an important requirement to achieve accuracy and reproducibility.
  - The whole process of the coagulation tests is based on a series of enzymatic reactions, which are dependent on pH, ionic strength and the temperature of the reaction process. A correct temperature at 37 ± 0.5°C is critical as most of the reagent systems are standardized at this temperature. Day-to-day shift in reaction temperature of equipment will introduce uncontrolled variation into test conditions. Therefore, temperature of all equipments must be calibrated daily and diligently to avoid erroneous results and ensure accuracy and reproducibility.
  - Sample/reagent dispensing mechanisms must be accurate and precise.
  - Well-calibrated dispensing mechanisms are required for all coagulation- based tests to accurately dispense samples as well as reagents. Any shift in ratio or individual volumes of the sample and/or reagent can lead to shortening or prolongation of results.
  - Straight 0.1 and 0.2 mL glass pipettes are usually satisfactory, provided they are scrupulously clean and dry.
  - Automatic micropipettes, which are able to deliver the required volumes, are replacing the glass pipettes, provided these pipettes are calibrated frequently. The use of clean disposable tips places this system at an advantage over the older mechanisms.

Storage of Reagents

- Usually reagent manufacturers recommend aspiration of adequate reagent for the days use in a thoroughly clean and dry tube instead of intermittent aspiration from reagent vials at the time of test.
  - Most coagulation reagents are extremely delicate reagents. For them to maintain their sensitivity and performance the reagent formulations must maintain reagent integrity over the usage period. Repeated intrusions into the reagent vial exponentially increases the chances of reagent contamination and destruction of reagent formulations and integrity. Undried and/or contaminated pipettes, tips, glassware are usually the main culprits. Such contaminated reagents perform suboptimally.
  - The reagent vials must be immediately stored back to the recommended storage temperatures after the aspiration of the day's requirement separately so that the remaining reagent remains at optimal temperature for future use. Keeping unused reagents at higher ambient temperatures during the day causes steady deterioration of the reagent due to thermal stress.
- The recommended storage temperature for reagents should be strictly complied to:
  - Most of the liquid stable or reconstituted reagents such as PT and APTT are colloidal suspensions of lipoproteins and/or phospholipids. Subjecting them to elevated temperatures through repeated freeze-thaw cycles stresses the colloidal system. Especially detrimental are the effects of freezing (below 2°C). After freezing the reagent colloidal suspension undergoes an irreversible change and precipitates out or present itself as a particulate mass. Such reagents give erroneous results.
- Bringing reagents/samples to room temperature should be a two-step process:
  - When enough reagents are aspirated out for the days testing as recommended the reagent and samples stored at 2 to 8°C should be first allowed to attain room temperature (25 to 30°C) and then they should be subsequently brought to the optimal test temperature of 37 ± 0.5°C.
  - When reagent samples from 2 to 8°C are directly brought to 37°C the required time of 3 to 5 minutes may not be sufficient for the reagent samples to attain a homogeneous temperature of 37°C within the recommended time. This affects the reaction kinetics leading to erroneous results.