PART 1 General Quality Planning in the Hemostasis Laboratory

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TOPICS COVERED

- ✓ Control of Potentially Hazardous Substances to Health
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- ✓ Safety Officers
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- ✓ Safety Measures–Universal Precautions
- ✓ Laboratory General Equipment
- ✓ Metrology
- ✓ Evaluation and Use of Coagulometers
- ✓ Reagents

Control of Potentially Hazardous Substances to Health: The EN ISO 15189 standard/norm specifies the quality and competence requirements specific to medical biology analysis laboratories. ISO 15189 is intended to be used in all disciplines practiced by medical laboratories. Its application is therefore fundamental for laboratories because their services must meet the needs of both patients and clinicians responsible for the care provided to their patients. Those services include processing requirements, patient preparation and identification, and sample collection, transportation, storage, pre-processing, and analysis, followed by validation of results, their interpretation, reporting, and advice, while ensuring staff safety and respect for ethics.

Laboratory Safety: Laboratories that handle chemicals and biological samples are potentially hazardous places. In recent years, there has been an increasing appreciation of the importance of safe working practices in industry, for both health and environmental reasons. This awareness has led to greater stress on issues such as safety documentation, staff training, and risk assessment. Employers have a responsibility to provide the necessary protective clothing and equipment, and are required to provide training in safe working practices. Implementing such safe working practices should greatly reduce the probability of serious injury to yourself, your colleagues, and members of the public.

Safety Officers: It is important to appoint a safety officer or officers for each department. These people will take on the responsibilities of introducing and maintaining safety procedures. Nevertheless, safety is the responsibility of all staff in the laboratory.

Safety Manual: There should be a comprehensive safety manual that covers all aspects of safe working practices for the whole department. All staff members must read the manual and sign a declaration to indicate that they have understood it. Copies should be kept with the safety officers and also made available in places that are easily accessible to all staff members, either in print, or more preferably, in electronic form, to ensure the latest version is available.

Safety Measures–Universal Precautions: The system of universal precautions requires that any danger of infection from any source will be avoided or minimized by good working practices. All blood samples, blood products (including plasma-based reagents and kits), and other human body materials should be regarded as posing a possible danger of infection. The fullest possible protective measures should always be taken when working with any material. No other classification of risk should be made. All body fluids and materials other than blood, whether collected or brought into the unit for testing or any other purpose, should be handled with the same care as that given to blood.

The laboratory: The laboratory should always be clean and tidy. Paperwork should be kept separate from laboratory testing areas. Try not to use the laboratory for storage of bulk items. Try to ensure that everyone participates in keeping the laboratory orderly.

Protective clothing: Everyone who enters the laboratory, including visitors, should wear a laboratory coat. They should immediately replace the coat if it becomes contaminated.

Disposable gloves: Even though many people do not like to wear gloves, it is recommended to wear latex or polyacrylamide disposable gloves, as every sample handled in the laboratory is potentially hazardous. Gloves should always be worn when handling toxic material. Gloves and coats will obviously not protect against a needle stick-type accident, but they will prevent, for example, any cuts or abrasions on your skin being in contact with HIV positive serum or plasma. It is mandatory to always replace gloves immediately if they are broken or punctured.

Eye washing: Wash your eyes immediately with lots of cold running water if contact with a possible infectious material may have occurred, as many infections can be easily acquired by contact with the mucous membranes of the eyes.

Sharps: Sharps, in the form of needles and broken glass, present a great danger. Use a sharp box capable of containing sharps without being punctured. There have been cases of workers becoming infected as a result of needle stick injuries.

Aerosols: Avoid all practices in the open laboratory that may cause splashing or the release of airborne droplets or dust. Operations that cause aerosols must always be carried out in a suitable fume cupboard, and safety glasses must be worn. All spills should be cleaned up immediately, using bleach or a neutralizing agent as necessary.

Toxic and flammable substances: Toxic or flammable materials must always be contained within a fume cupboard or suitable safe box.

Electrical equipment: Take special care with any equipment that uses liquids, such as electrophoresis tanks and water baths. Always leave installation, servicing, and repairs to qualified personnel.

Personal possessions and behavior: Never take personal items, such as pens, bags, and combs, into the laboratory. Avoid bringing your hands into contact with your face or mucosae (eyes, nose, and mouth) while in the laboratory, but if you must do this, always wash your hands first. Always wash hands thoroughly before leaving the laboratory. Never mouth a pipette. Food, cigarettes, and cosmetics must never be brought into the laboratory. This implies that eating, drinking, and smoking must be avoided in the laboratory.

Accidents: All accidents should be reported immediately and should be recorded in an accident book kept by the unit Safety Officer. This is particularly important in relation to needle stick injuries. In these situations, follow local hospital systems for recording and reporting, along with any locally recommended or mandated actions.

Control of Potentially Hazardous Substances to Health: Laboratories must comply with the local regulation, which often issues useful guide in identifying risks and hazards, such as the Control of Substances Hazardous to Health (COSHH) in UK laboratories.

Hazard and risk: The hazard presented by a substance is its potential to cause harm. The risk from that substance is the likelihood of its harming someone under the actual conditions of use.

Identification of hazards: The identification of hazards is an essential prerequisite of risk assessment. The time spent identifying the hazards will vary according to the substance.

Risk assessment: Consider the following facts:

- Hazards
- Conditions of use
- Amounts to be used
- Likely routes or sites of exposure (inhalation, ingestion, skin, or eyes)

The outcome of the risk assessment will determine:

- Storage conditions
- Handling procedures
- Disposal procedures
- Requirement of monitoring and health surveillance
- Emergency procedures

Risk assessment must be reviewed annually and updated if necessary. An example of how to record information for risk assessments, using the COSHH procedure used in UK laboratories, is shown in Table 1. The purpose of such forms is to identify the hazards and control measures associated with equipment used in a particular procedure. Only staff documented as competent should perform any procedure, and they should perform that procedure only after reviewing the health and safety documentation related to that particular test.

Table 1. Control of potentially hazardous substances to health (COSHH) for prothrombin time and APTT-based clotting factor (F) assays

COSHH Ref. No. Assays 1		**Lab. Ref. One-Stage clotting assays for FII, FV, FVII, FVIII, FIX, FX, FXI and FXII**
Title of Procedure/Experiment:		
Substance	**Approximate quantity**	**Hazard identified**
Glyoxaline (imidazole) buffer, contains (see**)	<5 ml	Harmful if ingested.
**Imidazole	3.4 g/l	Corrosive: causes burns. Harmful if inhaled, ingested, or absorbed through skin. Irritating to eyes.
**Sodium chloride	5.85 g/l	Irritating to eyes and lungs. Avoid skin contact.
Factor-deficient plasma	1 ml	Risk of infection
Thromboplastin	2 ml	Low risk
APTT Reagent	2 ml	Low risk
0.025M calcium chloride	5 ml	Low risk
Owren's buffer	<500 ml	Contains barbitone. Harmful if swallowed. May cause sensitization by contact to skin or inhalation.
Coagulation analyzer wash solution 1	<50 ml	Causes burns: harmful to eyes, skin, etc. Do not mix with other disinfectants. Corrosive. Contact with combustible materials may cause fire. Contact with acid liberates toxic gas. Reacts violently with ammonium salts; organic solvent - explosive risk.
Coagulation analyzer wash solution 2	<50 ml	Contains 0.16% hydrochloric acid and detergent. Irritant: may harm eyes and skin.
Standard/control/patient plasma	<1000 µl	Risk of infection.

Laboratory General Equipment: Any laboratory involved in the diagnosis and treatment monitoring of bleeding disorders employing some or all the techniques described in this manual will require a minimum of basic equipment.

General equipment: The basic equipment requirements are:

1) A 4°C refrigerator for reagent storage. Reagents should normally be maintained at 2–8°C unless otherwise stated by the manufacturer. A good-quality domestic-grade unit may be adequate.
2) A deep freezer able to maintain at least -20°C (preferably -35°C). A lower temperature, such as -70°C, is useful for more prolonged storage, as clotting factors are stable at this temperature for at least 6 months. Freezers with an auto defrost cycle are completely unsuitable.
3) Regulated water bath(s) capable of maintaining temperatures of 37 ± 0.5°C. Temperature is normally better maintained in a water bath than in dry hot blocks, which may or may not be suitable, depending on the unit.
4) A pH meter.
5) A light source.
6) Stopwatch (es).
7) Calibrated automatic pipettes capable of accurate and precise delivery of sample and reagent volume in the range of 0 µl–200 µl and up to 1000 µl. It is important to check the accuracy of these pipettes regularly.
8) A calibrated pipette for delivery of liquid volumes up to 5 ml.
9) A centrifuge capable of generating at least 1700g, and preferably 2200 to 2500g. For most coagulation analyses, centrifugation at room temperature (20–25°C) is acceptable, even though centrifugation at 4°C is recommended in some techniques.
10) A calibrated analytical weighing scale/balance capable of accurate measurement of grams to three decimal places.

Additional equipment is required for some procedures, including:

11) A coagulation analyzer (coagulometer).
12) A microplate reader for enzyme-linked immunosorbent assays (ELISAs).
13) A platelet aggregometer. Equipment specified on particular method sheets.

Air conditioning in each room is a great advantage in countries where temperatures are high.

There should be an adequate supply of consumables. Reusing laboratory test tubes and pipette tips after washing should be avoided, since residual material can adversely affect results, causing wastage of reagents and time. The same applies to collection tubes, which are designed for single use and must not be reused even after extensive washing.

Metrology: To aid quality management, pipette volume and balance calibrations should be checked on a regular basis, such as every 3–6 months. Apparatus significantly out of calibration should be immediately removed from use until recalibration has been done. All pipettes should carry a unique identifier.

Method for checking pipette calibration: Pipettes may be for a single volume, for two or three volumes, or have a continuous range of volumes.

- Pipettes with one or two fixed settings are checked at each setting.
- Pipettes with three fixed settings are checked at minimum and maximum setting.
- Pipettes with a continuous range of volume settings: check the maximum setting as well as a volume of around 25% of the maximum setting. That is:
  - 10 ml pipette – 10 ml and 2.5 ml
  - 5 ml pipette – 5 ml and 1.25 ml
  - 1 ml pipette – 1 ml (1000 µl) and 0.25 ml (250 µl)

- 0.2 ml pipette – 0.2 ml (200 µl) and 0.05 ml (50 µl)
- 0.1 ml pipette – 0.1 ml (100 µl) and 0.025 ml (25 µl)
- 50 µl pipette – 50 µl and 15 µl

Check calibration by weighing five replicate volumes of distilled water (at room temperature) on a balance. Each weight is recorded in grams (with three decimal places). For practical purposes, 1.000 ml distilled water weighs 1.000 g.

Results and any action taken should be recorded. Pipettes should preferably be accurate within significantly less than 10% (see examples below). When a pipette is shown to be inaccurate because the mean pipetted volume differs by more than 10% from stated volume, it must be taken out of use immediately and not used until re-calibrated following manufacturer's instructions.

Note: If a pipette is inaccurate beyond the following limits (mean weight), it must be taken out of use immediately.

10 ml pipette
10 ml: 9.000 – 11.000 g
2.5 ml: 2.250 – 2.750 g

5 ml pipette
5 ml: 4.500 – 5.500 g
1.25 ml: 1.125 – 1.375 g

1 ml pipette
1 ml: 0.900 – 1.100 g
0.25 ml: 0.225 – 0.275 g

0.2 ml pipette
0.2 ml: 0.180 – 0.220 g
0.05 ml: 0.045 – 0.055 g

0.1 ml pipette
0.1 ml: 0.090 – 0.110 g
025 ml: 0.225 – 0.0275 g

50 µl pipette
50 µl: 0.045 – 0.055 g
15 µl: 0.013 – 0.165 g

For additional information, see ISO 8655-2-2002 document, available in different languages.

Method for checking balances: To ensure their accuracy, calibrated weights are weighed at six-month intervals, and the values recorded.

1) Zero the balance.
2) Weigh three calibrated weights, one at a time. Record the weights to three decimal places (e.g. 1.003 g).
3) If any weights are outside the stated limits (by >2%), remove them from use until the problem is fixed.

Method for checking temperature of refrigerated enclosures: Internal temperature in the refrigerators must be kept at +4°C (usually in the range from +2°C to +7°C) using a temperature probe, and should ideally be recorded constantly, either using a local printed disk or electronically. The same applies to freezers, which must keep at temperatures of -20°C, -35°C, or even below -70°C.

Evaluation and Use of Coagulometers: Automation in coagulation laboratories is now in widespread use in most parts of the world. It has contributed to improvements in standardization and facilitating tests that demand specific training and special working conditions, so that laboratories may improve their efficiency and repertoire. Automation in hemostasis is relatively recent. Manual methods based on visual detection of the fibrin clot and using incubators at 37°C were once the only techniques for coagulation studies. Then, in the 1970s, new semi-automatic equipment appeared based on photometric or mechanical principles to detect fibrin clots. More recently, fully automated instruments have become common in modern laboratories. New equipment connected to laboratory information systems, usually including specific data processing systems, can perform clotting, chromogenic, and immunological tests.

Two main methodologies are available today, which are based on mechanical and optical detection systems. Mechanical systems only allow performing clotting assays, whereas optical systems allow performing chronometric, chromogenic, and immunological assays based on photo-optical, nephelometric, chromogenic, and immunological principles. In addition, fluorescence- and chemiluminescence-based analyzers are becoming available on the market, allowing specific assays with a wide range of measurements to be performed.

Mechanical principle: Electromagnetic methods are based on the detection of an increase in plasma viscosity when fibrin is formed. Two variations to this principle are applied to laboratory equipment today.

The first uses an electromagnetic field applied to test cuvettes that detect movement within a stainless steel sphere placed in the plasma sample. The steel sphere follows a pendulum movement, swinging from one side to the other in a plasma reagent solution with a constant movement. As the fibrin begins to form, viscosity increases, and the sphere's movement is delayed. When the sphere's oscillation movement reaches a predetermined level, the chronometer stops, indicating the time of plasma coagulation.

A second mechanical detection method also uses a stainless steel sphere, located this time in a single point slot. A magnetic sensor detects the sphere's position, and as it rotates, the sphere maintains its inclination while the liquid sample remains fluid. When fibrin is formed, the clot captures the sphere, moving it from its original position. As it moves outside the sensor's range, the circuit is interrupted and the chronometer stops.

Optical or spectrophotometric principles:

Photo-optical principle: Optical systems are based on the notion that clot formation induces change in the plasma's optical density. As the clot is formed, there are changes in the optical characteristics from the initial reading of the plasma/reagents. These changes are monitored and used to derive the time taken for a particular degree of change to occur.

Nephelometric principle: The nephelometric principle is employed by some systems. In coagulation assays, a monochromatic laser light source is transmitted, for example, by fiber optics. The light dispersion readings are made possible by a sensor that may be installed at 90 or 180 degrees from the light path, depending on the system, which then measures scattered light at an angle or records the change in light transmission. When the light reaches insoluble complexes such as fibrin fibers, it disperses in forward scattered angles (180 degrees) and lateral scattered angles (90 degrees). The chronometer stops when the amount of scattered light or transmitted light reaches a specific predetermined level. The difference between light scattered or transmitted before and after the clot formation is normally proportional to the amount of fibrin formed.

Chromogenic principle: This is based on the use of a color-specific generating substance known as chromophore, of which para-nitroaniline (pNA) is the most common. It has a maximum absorbance at 405 nm. The principle of chromogenic testing resides in adherence of pNA to synthetic substrates. pNA is attached to a series of amino acids that mimics the target sequence of the activated coagulation factor we want to determine. The coagulation protein cleaves the chromogenic substrate at a specific site between a defined amino acid sequence and releases the pNA. The intensity of the yellow color is proportional to the amount of pNA released. This is measured by photo detection at 405 nm wavelength. As more pNA is cleaved and freed, the absorbance capacity of the sample increases, which leads to greater change in the solution's optical density. The first coagulation equipment could only provide a single definition parameter, such as a mechanical or photo-optical one. The photo-optical tools were initially designed for reading at a single wavelength (for example, 500 nm or 600 nm) that could only be used for the detection of clot formation. More recently, some coagulometers can read at two or more wavelengths, often including 405 nm, thereby increasing the capacity for newer reactions (chromogenic substrate methods). In the 1990s,

a number of manufacturers successfully included multiple detection methods, which now give a single laboratory the possibility of using the same equipment for different methodologies.

Immunological principle: Latex microparticles coated with a specific antibody are generally used against the analyte (antigen) being measured. A beam of monochromatic light goes through a latex microparticle suspension. When the wavelength is greater than the suspension particle diameter, the particles absorb a small amount of light. Yet, when the specific antigen-coated latex microparticles come in contact with the antigen present in the solution, they adhere to the antibody, forming links between the particles, which produces agglutination. When the particles' diameter approaches the wavelength of the monochromatic light beam, a greater amount of light is absorbed. This increase in light absorbance is proportional to the agglutination, which, in turn, is proportional to the amount of the antigen present in the sample. This type of technology is available in more sophisticated coagulation analyzers introduced in the market in the 1990s. Usually time-consuming standard immunological assays can be performed in minutes when using any of these automated tools.

Table 2. Advantages and disadvantages of detection methods in defining parameters

Method	Advantages	Disadvantages
Mechanical	No interference due to physical characteristics such as lipemia or icterus May use small sample volumes	Impossible to observe graphics of clot formation May present problems of endpoint detection in some samples with low fibrinogen
Photo-optic	Possibility of graphics on clot formation Optical checks for hemolysis/lipemia/icterus on some optical systems May use small sample volumes	Interference due to lipemia, hemolysis, hyperbilirubinemia, or protein increase on some systems Some systems may present difficulties with clot detection when using some completely transparent reagents Very short coagulation periods may go undetected owing to delay prior to initiation of monitoring
Nephelometric	Can measure antigen-antibody reactions in proteins present in very small amounts	Limits number of available tests Cost of reagents
Chromogenic	Fully specific assays may be easier Additional parameters not suitable for measurement by clot detection may be possible Increases the repertoire of possible tests Possible improvements in precision compared to clot-based analyses	Limited by the instrument's wavelength Requires large test volumes for positive cost-benefit ratio Cost of instruments and reagents
Immunological	Can automate time-consuming, manual methods Increases the number of possible tests	Limited number of tests available Cost of instruments Cost of reagents

Advantages of automation in the coagulation laboratory:

1) Improves the capacity and flexibility of professional time spent (Rodak, 1995).
2) Improves the performance of the tests. In the past, manual coagulation tests were inaccurate, with variation coefficients greater than 20%; the semi-automatic equipment provided greater accuracy in coagulation testing. However, with manual dispatch of samples and reagents, testing had to be done in duplicate. With fully automated equipment, accuracy improved, attaining

variation coefficients of less than 5%, and even 1% for some tests. This has led authors to introduce the notion of single tests and the possibility of reducing reagent costs and cuvettes by half.

3) Reduces cost in samples and reagents, by allowing the use of lower volumes of plasma and reagents (at least half).
4) Facilitates data storage and recovery systems by means of computer programs.
5) Allows automatic replay of results when mistakes are made in the first run.
6) Offers the possibility of running different tests using a single sample.
7) Permits sampling from a closed tube (so-called "cap-piercing"), which improves safety and efficiency in coagulation tests. This reduces, largely, the possibility of exposing the operator to sprays or patient sample spills, or mistakes in labelling. Anecdotally, one manufacturer offered a patented screening system that automatically separates plasma from erythrocytes before tests without previous centrifugation.
8) Provides capacity to dilute samples, calibrators, and controls. The equipment can be programmed for additional dilutions if the initial results escape the method's linearity. It can also automatically carry out other tests without the operator's intervention if clinically indicated or because of initial run results.
9) Most analyzers include alarm systems that warn the operator of readings in excess of pre-established limits, which may identify equipment problems (e.g. small amount of reagent, temperature failure, too small sample volume, and quality control errors), as well as pre-analytical errors (under-filled tubes, hemolysis, icterus, lipemia, and presence of clots).

The different methodological types available have advantages and disadvantages that should be known and understood to guarantee precision and validity of test results. It is important to consider that laboratories are responsible for trustworthy results. A laboratory's main concern is to select the coagulation equipment that will generate appropriate results in spite of budget restraints. Such instruments demand regular technical maintenance, permanent knowledge, and system control, since a mistake or failure may decisively influence a number of results. Control systems that guarantee analytical confidence are therefore compulsory.

Many laboratories may be fortunate enough to be able to evaluate equipment before purchasing. If this is not possible, it is very important to obtain adequate information and advice from a reference laboratory in addition to the review of the literature.

When evaluating new equipment before purchase, first compare analyzers according to criteria such as:

- equipment and maintenance costs
- inactivity period and reliability
- repair response time
- ease of use
- availability of adequate maintenance within an appropriate timeframe
- validation process and throughput
- cost of disposable elements
- flexibility in using reagents from other manufacturers
- possibility of adding new tests protocols
- ability and cost of connection with the laboratory information system
- training courses and continuous training support

The sensitivity of different types of equipment to multiple parameters will differ depending on how the machines are calibrated and how endpoints are detected. Laboratories have different needs, and it is advisable to rank priorities. For example, see Table 3.

Table 3. Characteristics of specialized equipment (adapted from Rodak, 1995)

Characteristics	Description
Random access	With patients' sample, various different tests are possible in any order and at the same time.
Sample primary tube	Plasma sample is directly taken by aspiration in an opened collection tube placed in the analyzer.
Penetrating plug and closed sampling tube	The analyzer vacuums the plasma sample within the collection tube with the rubber plug in place.
Barcode	Allows identification of reagent, patient samples, or both by means of a barcode. This reduces manual data entry.
Bidirectional inter-phase	The analyzer queries a centralized computer to determine the requested number of tests. The operator does not need to manually program the information in the equipment.
Sample indicator	Warns the operator of problems with sample integrity.
Liquid level sensor	Warns the operator of insufficient sample or reagent volume for adequate testing, or if the equipment did not vacuum enough from sample to perform the requested test.
Integrated quality control programs	Instrument's computer program stores and organizes quality control data. It may include the complete application of Westgard rules to indicate off-limit results.
STAT capacities	Allows the operator to cancel the test verification sequence in order to place a new STAT sample in the verification isle.
Refrigeration capacity of integrated samples	Preserves the integrity of samples, reagent, or both during the verification process.
Storage capacity of integrated samples	Indicates the amount of patient sample that can be loaded in the analyzer at any given time.
Reflex testing capacity	Makes it possible to program the equipment to repeat or add tests under specific parameters set by the operator.
Patient data storage	Analyzer capacity to store test results that can be recalled at any given moment. May store clot formation curves.
Reagent volume monitoring	Warns the operator of insufficient reagent for programmed tests.
Processing	Number of tests that can be processed within a given period (generally classified as number of tests per hour).
Clot formation curve	Allow the operator to visualize the clot formation within the cuvette. Helps detect certain unruly conditions or morbid states, or the location and solution of deviant test result failures.
Pre-analytical checks	Detection of under-filled tubes, hemolysis, icterus, lipemia, clot.

Technology is on the rise and growing daily demands generate the need for instruments of this nature in the laboratory. They will constitute a great step forward in the lab field, given the possibility of undertaking tests in a reliable, accurate, and precise manner, and delivering results more quickly (shorter turn-around time) and under better control. The advantages of automation are numerous. Technology is continuously advancing to meet new developments in the field and to reduce turnaround times, allowing tests to be reliable, accurate, and precise, while maintaining quality.

Reagents: In addition to specific reagents dedicated to specific assays, which will be detailed in the corresponding chapters, some reagents are widely used in the hemostasis laboratory (e.g. calcium chloride solution, various buffers). They can be purchased from reagent manufacturers or locally prepared from bulk reagents or concentrated solutions.

25 mM calcium chloride solution: For example, if a molar solution is purchased, to obtain a 25mM solution, dilute 25 ml 1M solution to 1 liter in volumetric flask with distilled water.

Buffers:

- Owren's barbiturate buffer pH 7.35

Weigh 5.875 g sodium diethylbarbiturate (barbitone sodium) and 7.335 g sodium chloride.

Place in a volumetric flask and dissolve in approximately 780 ml distilled water.

Add 215 ml 0.1M hydrochloric acid.

Adjust volume to 1 liter with distilled water.

Check pH and adjust to pH 7.35, if necessary.

- Owren's buffered saline

200 ml Owren's barbiturate buffer (see above).

Add 800 ml normal saline (0.9% sodium chloride).

- Imidazole (glyoxaline) buffer

Weigh 2.72 g imidazole (glyoxaline) and 4.68 g sodium chloride.

Place in volumetric flask and dissolve in approximately 650 ml distilled water.

Add 148.8 ml 0.1M HCl and adjust pH to 7.3.

Adjust volume to 1 liter with distilled water, if necessary.

Reagents for coagulation screening tests: In the initial stages of investigation and diagnosis of bleeding disorders, selection and application of suitable screening test reagents, particularly for prothrombin time (PT) and activated partial thromboplastin time (APTT) tests, are of great importance. Many different reagents are available throughout the world. Where a wide choice is available, selection should take into account the variation in sensitivity. In screening for a bleeding disorder by PT and APTT, the following sources of information in relation to the likely performance of a particular reagent can be considered:

- Comparative data in relation to other reagents from external quality assessment (EQA) schemes, such as the International EQA scheme
- Published data
- Local testing of plasma from patients with known defects
- Manufacturers' data sheets

Local production of PT and APTT reagents may be financially attractive, but can cause standardization difficulties, and therefore must be avoided. It should also be noted that some manufacturers offer different reagents. In addition, the composition of reagents bearing the same name may be altered from time to time. This means that recommendations for the use of a particular source of material cannot be given.

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