Everyday Practice

Setting up a coagulation laboratory at a district hospital

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INTRODUCTION
A district-level hospital acts as an essential link between primary and tertiary healthcare. A reliable diagnostic facility is essential for providing quality health services. A good district-level laboratory could reduce the load at tertiary healthcare facilities in India. Diagnostic facilities for haemostatic disorders, whether hereditary or acquired bleeding problems, are poorly developed in the country, particularly at the district level. According to the National Rural Health Mission (NRHM) norms,1 district hospitals should be at least 100-bedded with a gynaecologist, an anaesthetist, a paediatrician, a surgeon and an orthopaedic surgeon. Transfusion services and some component preparation facilities will also be available in district hospitals. However, only a handful of coagulation tests will be sufficient to diagnose and monitor most haemostatic disorders of both hereditary and acquired origin.

ROLE OF A COAGULATION LABORATORY AT THE DISTRICT LEVEL
A coagulation laboratory at the district level is useful for the diagnosis and follow-up of a number of disorders.

• Thrombocytopenia (both true and spurious) is common. Viral infections such as dengue-induced thrombocytopenia require daily monitoring of platelet counts and hence a reliable platelet count is an important test.

• The preliminary diagnosis of inherited coagulation defects such as haemophilia A, B and von Willebrand disease can also be done.

• Disseminated intravascular coagulation (DIC) is a complication of various medical, obstetric and surgical conditions. It is important to detect DIC early to enable appropriate and necessary interventions to be done as soon as possible. Also, daily monitoring is required for appropriate clinical management to decrease the associated mortality and morbidity.

• The presence of coagulation factor inhibitor, lupus anticoagulant (LA) and monitoring of patients on anticoagulants (warfarin) can also be done.

However, such a laboratory need not perform the complete gamut of coagulation tests. The diagnosis of platelet function disorders, von Willebrand disease and its classification, assays of various coagulation factors, factors causing thrombophilia, combined deficiencies, molecular tests and carrier detection of haemophilia A and B should remain out of the scope of a district hospital. Collection of blood samples for some of these tests also needs special attention. Hence, patients suspected to have these conditions should be referred to tertiary healthcare centres.

TESTS TO BE PERFORMED AT THE DISTRICT LEVEL
A complete blood count including platelet count is important in patients with bleeding disorders (Table I). This should be supported by a peripheral blood examination especially for the assessment of the platelet count and for the presence of schistocytes in patients with suspected DIC. Manual counting can also be done in a modified Neubauer chamber using 1% ammonium oxalate as a diluent.2

Bleeding time (Ivy method, with disposable lancet), prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time are baseline screening tests for coagulation defects. Clot solubility test (FXIII screening) should also be available. Mixing and correction tests of PT/APTT with serum, adsorbed plasma and aged plasma can also be done. Screening tests for inhibitors and LA should also be done.

D-dimer and fibrinogen assay are required in patients suspected of DIC. Also monitoring of patients on anticoagulants is essential. Unfractionated heparin and warfarin can be monitored by APTT and PT-INR, respectively.

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Reagents required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete blood count</td>
<td>Automated cell counter</td>
<td>As per the manufacturer’s requirements</td>
</tr>
<tr>
<td>Peripheral smear examination</td>
<td>Microscopic examination</td>
<td>Giemsa/Wright stain</td>
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<tr>
<td>Prothrombin time (PT) and international normalized ratio (INR)</td>
<td>Manual/semi-automated coagulometer</td>
<td>Tissue thromboplastin and calcium/commercial reagent</td>
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<tr>
<td>Activated partial thromboplastin time (APTT)</td>
<td>Manual/semi-automated coagulometer</td>
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<tr>
<td>Thrombin time</td>
<td>Manual/semi-automated coagulometer</td>
<td>Thrombin</td>
</tr>
<tr>
<td>Clot solubility test</td>
<td>Manual</td>
<td>1% chloroacetic acid</td>
</tr>
<tr>
<td>Correction test of PT/APTT</td>
<td>Manual</td>
<td>Fresh serum, adsorbed plasma</td>
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<td>Screening test for inhibitor</td>
<td>Manual</td>
<td>Normal platelet-poor plasma</td>
</tr>
<tr>
<td>Fibrinogen assay</td>
<td>Manual/semi-automated coagulometer</td>
<td>Thrombin solution, commercial reagent</td>
</tr>
<tr>
<td>D-dimer/fibrin degradation products</td>
<td>Manual (agglutination on cards)</td>
<td>Commercial reagent</td>
</tr>
<tr>
<td>Bleeding time</td>
<td>Manual</td>
<td>Lancet/template</td>
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EQUIPMENT
An automated cell counter for a complete haemogram is one of the essential requirements of a haematology laboratory (Table II). A low-end cell counter is needed with a three-part differential matching with a throughput according to the patient load of the hospital. A provision for an annual maintenance contract should be included at the time of purchase. Annual calibration of all equipment and participation in a proficiency testing programme should be an integral part of the laboratory. Standard operating procedures (SOPs) for preventive maintenance, daily/weekly checklist, daily internal quality control and Levey–Jennings chart should be meticulously used to maintain the quality of tests done.

For coagulation tests, a semi-automated coagulometer based on mechanical/photo-optical method is adequate. However, these tests can be done with a manual coagulation test set-up using a water bath, multiple stop watches, thermometer, centrifuge calibrated regularly and a refrigerator. A manual test set-up can function as a back up for the semi-automated coagulometer.

REAGENTS
Most of the reagents required can be prepared in the laboratory and are cost-effective in running a coagulation laboratory. However, stringent quality assurance measures need to be followed. Most reagents used for coagulation tests are very labile and need storage conditions to be controlled strictly. All reagents prepared in the laboratory must be labelled with the date of preparation and expiry, as well as instruction for storage. Commercial unopened/opened vials have different expiry periods and hence any vial once opened, must be labelled with the date of opening and the expected date of expiry.

An in-house normal control can be prepared from platelet-poor plasma (PPP) of 20 healthy adults (men and non-pregnant women). PT test is done on each sample and mean normal PT (required for INR) is calculated. Then all the plasma is pooled and aliquoted at -20 °C and used as daily PT, APTT normal control. Abnormal plasma of known factor defects can be stored in aliquots for use as abnormal controls.

Platelet-poor plasma (PPP). Anticoagulated blood (in 3.2% citrate) is centrifuged at 2000 g for 15 minutes and the platelet count should be <10 000/cmm.

Aged plasma. PPP of a healthy person (0.9 ml) is mixed with 0.1 ml of potassium oxalate (14 g/L) and incubated for 2–3 days at 37 °C. The aged plasma is deficient in factors V and VIII, and is used in mixing tests.

Adsorbed plasma. 1 g of moist Al(OH)₃ gel is mixed with 4 ml of water to make a smooth suspension. 0.1 ml of this suspension is mixed with 0.9 ml normal PPP, incubated for 2 minutes and then centrifuged. The supernatant plasma is deficient in factors II, VII, IX and X, and is used in mixing tests.

Tissue thromboplastin (Rabbit brain extract). The reagent for PT can be prepared as described by Dacie and Lewis.

Commercial PT reagent. Where possible, a thromboplastin that is insensitive to heparin in the therapeutic range should be selected. The American College of Chest Physicians recommends thromboplastins with ISI (International Sensitivity Index) of <1.20.

Caprinus substitute (Rabbit brain extract). The reagent for APTT test can be prepared as per Dacie. Alternatively, a commercial APTT reagent can be used.

PERSONNEL
No laboratory can function without a competent and dedicated team. A trained technical person with at least a diploma in medical laboratory technology is required to perform tests for coagulation. Such a person should receive training at a tertiary care hospital, where these tests are done frequently.

GOOD LABORATORY PRACTICES (GLP)
SOPs should be prepared for these tests. These should be modified and updated when there is a change in kits/reagents/procedure.

There should be defined guidelines and criteria for specimen collection and rejection of incorrectly obtained samples. Guidelines for selection and validation of equipment and reagent kits should be made. Calibration and preventive maintenance procedures of all equipment should be as per the instrument manual.

The reference ranges of tests being done should be determined. The control samples should be run every day. Manual tests should be run in duplicate. The turnaround time (TAT) of the coagulation tests from time of sample collection to release of report should be assessed and strictly adhered to.

QUALITY ASSURANCE
This is an integral part of setting up of a coagulation laboratory because coagulation tests are extremely sensitive to sample collection procedures, quantity of anticoagulant, storage of sample/reagents, quality of reagents, etc. Quality assurance parameters, which begin from patient preparation before sample collection till release of report (with interpretation by the doctor), need to be strictly adhered to in order to achieve reproducible and accurate test report and diagnosis.

The coagulation laboratory should participate in an external quality assurance programme (EQAS) in coagulation. Coagulation laboratories should promote active interaction with clinicians for appropriate and effective clinical management.

INFORMATIVE REQUISITION FORM
This should include a brief clinical history particularly related to bleeding, clotting and history of transfusions and drugs (especially anticoagulants). Any previous investigation record, particularly liver function tests and coagulation profile, should be included.

BLOOD COLLECTION
The anticoagulant recommended is 3.2% trisodium citrate in a ratio of 9:1 and the sample should be collected in plastic tubes (vacutainer) using a larger (21G) needle. Samples from a vascular access device should be avoided. Special care must be taken to avoid contamination with heparin.

Tests should be done within 4 hours of collection of sample, which can be kept at room temperature (<25 °C) except for PT.
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For PT, the sample should not be kept in a refrigerator and test can be done with the sample at room temperature up to 24 hours. A system of critical alerts also needs to be developed. A considerably prolonged test needing immediate action must be informed to the clinician immediately.

CONCLUSION

A coagulation laboratory with a limited number of tests with a high-quality assurance programme will go a long way in assisting the diagnosis of bleeding disorders such as haemophilia and acquired bleeding conditions such as DIC as well as in the management of patients on warfarin or heparin at district hospitals.

REFERENCES


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