Assays for Monitoring Haemostasis

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Overview of Presentation

- Haemostasis – sample collection and transport
- Laboratory issues
  - Sample processing
  - Pre-analytical checks for haemostasis

- Routine Assays
  - PT – reagents and ISI calibration
  - APTT – reagents and validation
  - Fibrinogen
  - Thrombin time
Collection for Haemostasis Testing

- Collection is a critical step as the coagulation factors can be easily activated or denatured.
- Patient should be calm and at rest.
- Venous blood samples, atraumatic collection, avoid tissue thromboplastin release.
- Release of tourniquet to prevent haemoconcentration.
- Haemolysis – difficult and syringe collects.
- Collection from heparinised lines.
- Avoid drip arms.
- Well mixed, immediately, invert at least 4 times
Collection for Haemostasis Testing

- 3.2% trisodium citrate (109mM) – reference ranges established based on tube brand, reagents, analysers.
- Lithium heparin and EDTA interfere with assays.
- Order of draw of collection tubes important.
- In date collection tubes – vacuum not compromised
- Correct ratio blood to anticoagulant 9:1
- Under filled/overfilled tubes are not acceptable.
  - CLSI guidelines >10% under or over not acceptable
  - Hct > 0.55 may need to adjust citrate volume in tube.
Transport of Samples

- Transport of specimens should be at room temperature.
- Delays in testing then specimens should be double centrifuged, plasma removed and frozen.
- Centrifuge to produce platelet poor plasma (<10)
- 3000g for 10 minutes (refer to local policy)
  - INR, D Dimer – 24 hours
  - Coag Profile, Factor assays, Thrombophilia assays – 4 hours
  - APTT/Heparin Assays (patients on UFH) – 4 hours
Pre-analytical Checks

- Under filled/over filled specimens checked for clots prior to testing
  - Reject clotted samples
  - Reject samples under filled by 10% or more
- Haemolysis check
  - Comply with organisational guidelines
- Intravascular haemolysis tested.
- Optical end point analysers – affected by lipaemia and bilirubin.
  - Clarify lipaemic samples by ultracentrifugation
Haemostasis Testing

- Investigate a cause of bleeding
- Determine the cause of previous abnormal results
- Monitor patients on anticoagulants

- Evaluate the risk factors for a hypercoagulable state
- Assess platelet function
Prothrombin Time

- Thromboplastin reagent (Tissue factor/phospholipid and calcium)
- Warfarin therapy
- Liver disease, particularly obstructive
- Vitamin K deficiency
- DIC
- Factor deficiency – extrinsic pathway
PT Reagent – Thromboplastin

- PT varies in factor sensitivity
  - most sensitive to FVII and least to FII and fibrinogen
- PT reagents all contain thromboplastin
  - tissue factor plus phospholipids and calcium

- Reagents differ in phospholipid concentration, source and type of tissue factor.
  - Biological – human, bovine, rabbit brain or lung or human placenta
  - Recombinant – DNA techniques

- Lyophilised or liquid reagents
- Instrument and reagent specific ISI provided
- Heparin neutraliser
- Minimal sensitivity to LA
Sensitivity PT reagents factor deficiencies varies.

ISI is used to assess responsiveness to Vit K dep. factors. compared to IRP.

- ISI – International Sensitivity Index used in the calculation of INR


- INR = (plasma PT/MNPT) _ISI_
- MNPT – mean normal PT with test system (reagent/analyser combination, geometric mean)
ISI Calibration

- Reagent and lot number changes
- QC or QAP issues
- Major instrument repairs/changes
- Each analyser should have individual ISI/MNPT

Various methods to do this (CLSI H54)
- Local procedures
- Commercial calibration plasma set
- ISI provided by manufacturer & establish local MNPT
- Calibrated back to a central lab analyser
Investigation prolonged PT

- PT mixing studies with normal pooled plasma (1:1) to differentiate factor deficiency from inhibitors.
- Rule out other causes prior to investigation with factor assays.

- Echis time – snake venom Echis carinatus
  - Acts directly on prothrombin (FII) to form thrombin, is unaffected by the descarboxy forms of FII.
  - Echis time normal indicates warfarin/ Vitamin K deficiency.
  - Echis time prolonged indicates factor deficiency (FII, liver function impairment).
Activated Partial Thromboplastin Time – APTT

- Phospholipid (no TF) + Contact Activator
- Calcium

- HMWK, PK, XII, XI, VIII, IX def
- X, V, II, fibrinogen def
- Heparins, Hirudins, DTI
- Inhibitors
  - Factor VIII, IX, V
  - Lupus Anticoagulant
- Sample quality issues
  - activated, aged, diluted volume, contaminated, pH changes (acidosis)
Mixture of procoagulant phospholipids and contact activator
- Contact system – HMWK, Prekallikrein, FXII, FXI 
  activators such as celite, kaolin, silica or ellagic acid
- Phospholipid provides a surface for interaction of coagulation factors

Test plasma incubated with APTT reagent and calcium added to start clotting.

Reagent /instrument combinations show varying sensitivities to factor deficiency and heparin.
- Ideally a reagent should show abnormal APTT results Factors VIII, IX, XI below 30% activity
Commercial APTT reagents vary in terms sensitivities to heparin, factor deficiencies and LA.

Labs to evaluate when selecting or validating APTT reagent for use.
- Heparin sensitivity – manage patients on UFH
- Factor sensitivity – screening reagent
- Lupus anticoagulant sensitivity – LA testing
Heparin Sensitivity – APTT

- Heparin sensitivity – established locally when introducing a new APTT reagent or change of lot number.
  - Therapeutic range valid for patients receiving UFH therapy to minimise bleeding and thrombotic risk

- Validation of heparin sensitivity
  - Perform anti Xa assay UFH (U/mL) and APTT on samples from patients receiving UFH. APTT range corresponding to 0.3 – 0.7 anti Xa U/mL
  - Comparison study – established APTT reagent with known therapeutic range, select new reagent with similar sensitivity, compare APTT on both using stored samples from patients receiving UFH.
Evaluation of Factor sensitivity

- Perform APTT and Factor levels on a normal plasma diluted with factor deficient plasma across several dilutions from 10 – 100%.

  - Ideal reagent should have abnormal APTT when factor levels < 30% for FVIII, IX and XI.

Studies show variable results depending on the normal plasma and factor deficient plasma used.

- Study performed on six commercial APTT reagents to determine best reagent for the laboratory and to check our reference intervals for APTT.
Factor Sensitivity

APTT Reagent vs Factor Levels

APTT (secs) RR 25 – 37 secs

APTT FVIII
APTT FIX
APTT FXI
Assess patient’s anticoagulant status ie receiving heparin or a DTI

Sample collection from a heparinised line
- Perform diagnostic heparin neutralisation test using polybrene/protamine
- APTT or TT based test

Mixing studies with normal pooled plasma (1:1)
- Correction indicates Factor deficiency
- Non-correction indicated Inhibitor
Fibrinogen

- Used in conjunction with the PT and APTT as a screening profile.
- Main routine assays
  - Clauss fibrinogen assay (modified TT)
  - Derived fibrinogen assay (PT based – OD change)
- Other assays
  - Clottable protein
  - Immunological assays – dysfibrinogenaemia
  - Genetic testing
- Clinical utility
  - dysfibrinogenaemia, hypofibrinogenaemia
  - DIC, liver failure, primary fibrinolysis
  - Guiding transfusion therapy with cryoprecipitate
Conversion of fibrinogen to fibrin.

Reagents are either bovine or human sourced, differ in thrombin concentration.

Sensitive to amount and function of fibrinogen in the plasma – Hypo and dysfibrinogenaemias

UFH, DTI, Fibrin(ogen)SP, paraproteins.
Importance of correct collection, processing and transport of coagulation specimens.

Pre-analytical issues and checks

Routine assays
- PT – reagents and ISI calibration
- APTT – reagents and validation
- Fibrinogen
- Thrombin Time


References


- Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline. CLSI Vol 25. No23. 2005
