Lecture 6 Outline

Laboratory Evaluation

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| Test Name | What is being measured? | | Principle/Procedure | | | | Reporting  What is reported and how? | | Normal Range | Comments/Other Information |
| PT (Prothrmombin Time) | Extrinsic System | | When factor VII is activated, formation of fibrin will occur at a normal rate only if the factors involved in the extrinsic and common pathways are present in normal concentrations.  To plasma, add PT reagen, which contain tissue thromboplastin (which is TF and a source of phospholipid) and CaCl2. Time for formation of a clot.  Our reagent also has heparin inhibitor. | | | |  | |  | \*Principal only works if the factors are present and working correctly.  Why is Platelet Poor Plasma (PPP) used?  Coumadin is prescribed for what conditions? What factors does it inhibit?  What is INR?  What is the formula?  What is ISI?  The the ISI, the sensitive the reagent. |
| Test Name | | What is being measured? | | | Principle/Procedure | | Reporting  What is reported and how? | | Normal Range | Comments/Other Information |
| APTT (PTT) (Activated Partial Thromboplastin Time) | | Intrinsic System | | | When factor XII is activated, the formation of fibrin will occur at a normal rate only if the factors involved in the intrinsic pathway and common pathway are present in normal concentrations.  PTT reagent is added to plasma. PTT reagent contains a partial thromboplastin (i.e. phospholipid) plus a negatively charged particulate activator. Following incubation, CaCl2 is added and the sample is timed for the formation of a clot. | |  | |  | Activates which factor?  Monitors (drug) therapy? |
| ACT (Activated Clotting Time) | | Intrinsic System | | | 1. Add a particulate activator to non-anticoagulated blood which activates contact system and determine the time until a clot forms. 2. Similar to APTT | |  | |  | Serum or plasma?  What is Protamine Sulfate and what is it used for? |
| Test Name | What is being measured? | | | Principle/Procedure | | Reporting  What is reported and how? | | Normal Range | | Comments/Other Information |
| TT (Thrombin Time) | Level of fibrinogen (<80-100), function of fibrinogen, and presence of circulating anticoagulants such as heparin and FDPs | | | 1. Thrombin time is time it takes for thrombin to convert fibrinogen to a fibrin clot. Works by splitting off FP A and B from fibrinogen. 2. Thrombin added to the sample bypasses earlier part of cascade. 3. Concentration of thrombin in reagent is very dilute; differs from fibrinogen test. 4. If there are other thing which occur at this part of the cascade, since thrombin is weak, these other things can affect the result of the TT (i.e. if there is anything that interferes with the enzymatic or polymerization steps, the TT will be prolonged. | |  | |  | |  |
| Reptilase Time | Similar to TT, except not affected by heparin | | | 1. Similar to TT, except reagent is snake venom (reptilase), not thrombin 2. Reptilase hydrolyzes FP A from (intact) fibrinogen, not FP A and FP B (as in TT); resulting fibrin monomers can then polymerize end-to-end to form fibrin clot | |  | |  | | Activates fibrinogen to \_\_\_\_\_\_\_\_\_\_\_, which will affect all processes using this pathway. |
| Fibrinogen (Clauss technique) | Fibrinogen | | | 1. Clot-based method of Clauss 2. Prepare a standard curve with a known calibrator, making dilutions in the linear range of fibrinogen. 3. Mix each dilution with an excess of thrombin (i.e. fibrinogen reagent) and see how long it takes for clot formation. 4. Plot fibrinogen concentration (mg/dl) against clotting time (sec) on log/log paper. 5. Prepare a dilution of patient plasma. Mix with fibrinogen reagent and see how long it takes for a clot to form. 6. Compare clotting time with standard curve; extrapolate to fine fibrinogen concentration. | |  | |  | | Concentration of thrombin is so strong that nothing will affect the test except the patient’s ability to convert fibrinogen. |

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| Hepzyme | Any heparin present is being removed | 1. Commercial hepzyme reagent contains an enzyme specific for heparin. 2. One ml patient plasma is added to a vial of Hepzyme reagent. 3. During the 15 minute dissolution period, any heparin present will be inactivated by this enzyme. 4. The PTT is then retested to see if it normalizes. |  |  |  |
| Mixing Studies (Dilution Studies) | Intrinsic and/or extrinsic factors | Mix 1 part abnormal patient plasma with 1 part normal plasma. Perform a PT or PTT. A normal PT or PTT suggests that the patient has a factor deficiency. |  |  |  |
| Factor Assays | Specific clotting factors | Assay uses plasma which is deficient in a specific factor (has normal activity of all clotting factors except one in which it is deficient). When the factor-deficient plasma is mixed 1:1 with various dilutions of patient plasma, the PT or PTT of the mixture will be dependent on the amount of the (missing) factor present in the patient plasma. The factor activity of the patient plasma is determined from a standard curve, prepared from the PT or PTT values of 1:1 mixtures of factor-deficient plasma with a serially diluted reference plasma with known factor activity. Comparison of the patient’s clotting time to the standard curve will give the percent factor activity present in the patient’s plasma. |  |  |  |

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| XIII screen | Fibrin or fibrinogen degradation products | 1. XIII stabilizes the fibrin clot by converting hydrogen bonds to covalent bonds and making the clot resistant to proteases. 2. In the absence of XIII, the hydrogen-bonded fibrin polymers are soluble in 5 M urea or 1% monochloroacetic acid. 3. Incubate patient plasma with thrombin solution for 30 minutes. 4. Remove clot and rinse with saline; add 5 M urea and incubate at RT for 24 hours. 5. Observe for presence of formed clot. |  |  |  |
| FDP/FSP | D-dimer unit, indicative of cross-linked fibrin formation | 1. Reagent contains antibodies to fragments D and E which are absorbed to a suspension of latex particles. 2. When patient serum is added which contains FDP in a concentration greatert than a predetermined amount, the latex particles will clump together and give macroscopic agglutination. 3. Do test with 2 dilutions, so can semi-quantitate the test.    1. 1st dilution is highest dilution for which a N person will be negative (1:5)    2. N FSP <8 ug/ml; test sensitivity to 2 ug/ml    3. By diluting sample to 1:5, pass NR so if 1:5 is positive report >10 ug/ml |  |  |  |

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| D-Dimer | D-Dimer unit, indicative of cross-linked fibrin formation | 1. Latex particles in the D-Dimer test are coated with two types of anti-human D-Dimer monoclonal antibodies 2. When patient plasma is mixed with a latex particle suspension, D-Dimers present will bind with the antibodies and agglutinate. This induces an increase in the turbidity of the reaction medium. This increase in turbidity is reflected by an increase in absorbance, the latter being measured photometrically. The increase in absorbance is a function of the D-Dimer level present in the sample. 3. Level of D-Dimer is determined by comparison to a standard curve. |  |  | What is a DVT?  D-Dimer is considered a good negative predictor for DVT.  If D-Dimer is positive, clotting has occurred in circulation.  In primary fibrinolysis, FSP will be positive, but D-Dimer is negative. |