

Survival Guide to Coagulation v2026-01-15

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General

-After Hours Requests for Special Coagulation Testing

- As of early 2017, Special Coagulation technologists are scheduled during weekdays (ie: no techs are scheduled evenings/nights, weekends, or statutory holidays).
- No special coagulation techs are officially on call. However routine Heme/Coag (407-3726) at the UAH has a list of techs that can be called off-shift for urgent requests from a hematopathologist. The routine techs can try calling these individuals to see if they are available but they are not obligated to come in (not paid to be on-call).
- If no one is able to come in then the pathologist will be called back to let them know it's not possible. They would then tell the clinician it cannot be done until the next business day.
- It is not necessary to contact the manager as they would always approve the request if it's approved by a pathologist.
- Managers will be notified at the time of payroll by the tech III/II.
- Reasons for After Hours Special Coagulation Testing:
 - Factor Level in patients being managed peri-operatively.
 - Acquired Hemophilia A/Specific Factor Inhibitors and Bleeding may require a PTT Inhibitor Screen urgently in some circumstances. See: [PTT and PT Inhibitor Screen](#)

- Extra samples:

- With EPIC go-live, COAGX and COAGZ are no longer available.
- A HOLD SPECIMEN FOR UAH SPECIAL COAGULATION was planned, but never built.
- Consider order a "Miscellaneous" test and include in the comments:
 - The type of tubes to collect: eg. Collect 2 sodium citrate tubes.
 - How to prepare aliquots: eg. Spin down to make Platelet Poor Plasma and freeze in 0.5 mL aliquots.
 - Where to send samples: eg. Please send frozen aliquots to UAH Special Coagulation Laboratory.
 - To who's attention: eg. ATTN: Dr. Artur Szkotak

- Usual duties of the Pathologist on service for the Coagulation Lab (in addition to providing consultation for clinicians and technical staff where necessary):

- SIGN OFF on coagulation results that require a pathologists signature before send out WITHIN 7 DAYS of running:
 - Results that have been generated but not signed out within 7 days get removed from a variety of logs and lists.
 - LIS has advised us that results not signed out with in 7 days may get lost or missed.
- HIT Assays – positive and select equivocal HIT assays need to be verbally communicated with the ordering Physician and documented in Epic Comm log. Also notify the BB.
- Platelet Aggregation Interpretation – platelet aggregations are typically performed every Thursday and require Pathologist interpretation for sign-out.
- Platelet Electron Microscopy triage and sign-out.
- Notify Blood Bank of newly diagnosed HITs and Bleeding Disorders.
- PT/PTT Inhibitor Screens – require pathologist interpretation for sign out, during regular hours.
- Bethesda Assay – requires pathologist confirmation of results and communication with clinicians to ensure management/referral is appropriate.
- Lupus Anticoagulant – the pathologist must provide an interpretation of the results prior to being sent.
- Factor levels – results below 20% in patients not previously known to have a deficiency (eg. known Hemophiliacs) require pathologist investigation.
- In general, to interpret for or warn clinicians where interference in a test may confound their own interpretation. For example in the context of a Lupus Anticoagulant on a therapeutic anticoagulant.

- Booked Tests at 780-407-7484 ("Short Cut" phone 780-407-7865 ext 2); section verified/updated 2025-08-18:

- The pre-booked special coagulation tests are DDAVP Trials, Platelet Aggregations, Electron Microscopy and Platelet Mixing Studies.
 - These must initially be booked by the ordering Physician or their designate (eg. nurse, unit clerk, etc.).
 - However, patients can call directly to change their booking date/time or rebook a missed appointment.
- Orphan Codes (not universally available in Epic, but working on them):
- *= outside normal reference range
 - CLPI= "Consult laboratory physician for interpretation if required. Call University Hospital switchboard (780-407-8822) and ask for hematopathologist on-call."
 - COM1/2... – see below for comment
 - DUPR=Duplicate Request
 - G054 = "Insufficient specimen for repeat analysis."
 - HE28= "Stated reference range/ranges have been derived from normal adults (18 years and above) and may not apply to pediatric patients."
 - HE31= "Platelet Free Plasma"
 - HE46="Assay performed after in vitro removal of heparin."
 - RISL = "Referred-in specimen. Not known if plasma has been prepared platelet free. Presence of platelets may cause false negative results."
 - INRHI = "Patient's PT INR is elevated. Potential causes include Coumadin, vitamin K deficiency, liver dysfunction, infancy and coagulopathy, which are all known to be associated with low Protein C and Protein S levels."
 - PTIE= "Patient's PT INR is elevated. The patient is presumably on Coumadin therapy, which is known to decrease Protein C and Protein S levels."
 - REP= "Results confirmed by repeat analysis."
 - DTIXA= "CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban."
 - APCPL= "This assay may produce falsely normal results in patients recently transfused with plasma containing blood products."

Heparin Levels

- On-site availability within North Sector APL: UAH, RAH, GNH, MIS, SGH, GPRH
- Reagent for Heparin Levels: Biophen Anti-Xa reagent
 - Calibrated using enoxaparin to make a hybrid calibration curve for UFH and LMWH.
 - CAUTION: a previous version of the Survival Guide to Coagulation (v2025-01-28) incorrectly indicated that the Biophen Anti-Xa reagent had been switched to Werfen Anti-Xa reagent. While initially planned, this switch was never implemented due to increased costs.
- Heparin Levels and Prothrombin Complex Concentrates (added 2015-06):
 - PCC (Octaplex and Beriplex) affects measured Anti-Xa levels in spiking experiments of Normal Pooled Plasma
 - This effect is entirely mediated by the Heparin in the PCC, and disappears if Hepzymed.
 - The effect on Heparin levels can be substantial – giving Anti-Xa results in the therapeutic range for UFH.
 - The effect of PCC would be expected to be transient given the short half-life of IV UFH.
 - CAUTION: Patients on Direct Xa Inhibitor drugs treated with PCC may have transient spikes in their measured Heparin Level due to the heparin present in the PCC.
- Interference and making dilutions:
 - Normally the patient plasma sample is NOT diluted prior to assay.
 - Assay measures a change in OD, so low to moderate levels of interference do not affect results; they may affect absolute OD values but not delta OD.
 - Interference (eg. high plasma Hb or bilirubin) would either be detected by the tech visually, or as a test failure due to an absolute OD that is off scale.
 - If dilution is necessary, then this is done using Normal Pooled Plasma and would generally require referral to the special coagulation bench.

- Dilutions reduce the amount of Heparin present, but this is corrected for by multiplying the result with the dilution factor prior to reporting the result.
- Indications for Dilution include Hemolyzed or Icteric samples:
 - Hemolysis while on Heparin is particularly common in Pediatric samples from children (or adults) on ECMO or other mechanical heart support.
 - When severe enough it will impact Heparin Levels as this is a chromogenic assay.
 - Reducing interference is achieved by diluting the patient sample with Normal Pooled Plasma.
- Another Indication for Dilutions is very high Heparin Levels that exceed the upper limit of the analytic range:
 - Most commonly this would be in Cardiopulmonary Bypass (CPB) patients.
 - A clotted circuit while the patient is on Cardiopulmonary Bypass means certain death and as a consequence OR Physicians/Surgeons would rather keep patients over-anticoagulated rather than under anticoagulated (bleeding being less life threatening than clotting).
 - Most of the time the OR is satisfied monitoring Point of Care ACTs for Heparin affect:
 - Traditionally ACT levels >480s were targeted while on CPB on the basis of fear of clotting rather than any evidence.
 - Studies at the UAH by Dr. S. Nahirniak and Dr. M. Buss suggest that target levels of >400s are equally safe in adults (and likely children).
 - Occasionally patients are labeled “Heparin Resistant” when ACTs remain below 400s despite very high doses of Heparin.
 - Unfortunately, ACTs are a very poor whole blood test whose ability to measure the degree of anticoagulation is at best suspect.
 - Thus, often patients with so called “Heparin Resistance” are well anticoagulated (or even over anticoagulated) but simply have inadequate ACTs
 - From doing levels on these patients we know that they can range from 5-15 U/ml (using previous Biophen Heparin assay), and published literature suggests that ACTs of 480 sec correspond to Heparin levels of 4-5 U/ml {*Arch.Pathol.Lab.Med.* 1998; 122: 782–798}. However, at present we do NOT know what the target heparin level should be.
 - Given that the upper limit of our assay is 2.0 U/ml – most if not all of these patients would require a dilution in order for a result to be obtained. If you do a Heparin Level on CPB patients I suggest asking the routine bench Tech to do a 1/10 dilution first as this would change your effective analytic to 0.50-20.0 U/ml.
 - If a patient’s ACTs are below 400s despite high Heparin doses and reasonably normal AT (>0.3 U/ml) it may be necessary to perform a diluted (1/10) Heparin Level to convince the Anaesthetist to ignore the ACT.
- Making dilutions has a number of very IMPORTANT affects on the results:
 - The greater the dilution factor, the lower the precision of the results.
 - The lower limit of detection increases by the dilution factor, for example:
 - Normally the lower limit of detection is 0.10 U/ml
 - With a Dilution Factor of 2 (1:1) mix the lower limit of detection is 0.20 U/ml.
 - With a Dilution Factor of 3 (1:2) mix the lower limit of detection is 0.30 U/ml.
 - With a Dilution Factor of 4 (1:3) mix the lower limit of detection is 0.40

- The dilutions are made with normal pooled plasma meaning that exogenous Antithrombin is being supplanted and may give falsely high Heparin Level results:
 - If the patient's Antithrombin level is near 1.0 U/ml (within the normal range) then dilution with normal pooled plasma has little or no affect on the measured Heparin Level.
 - If the patient's Antithrombin level is significantly below 1.0 U/ml, then making dilutions will give falsely high Heparin Levels. One way to correct for this is to try to keep the patient's Antithrombin level close to 1.0 U/ml with factor concentrate or plasma infusions.
 - The lower the patient's Antithrombin the lower their "true" Heparin level compared to the measured Heparin Level.
 - The higher the dilution factor the lower the patient's "true" Heparin Level compared to the measured Heparin Level.
 - Note: patients with abnormally high Antithrombin levels who require dilutions be made for Heparin level measurement will get falsely low Heparin level results – but practically speaking this situation rarely (if ever) occurs.
- On Stago analyzers, previously all Heparin Levels with an AT level below the normal reference range had the HEPU comment appended:
 - "Patient's antithrombin (AT) level is low. Due to test limitations, heparin assay was performed with normal plasma containing AT. Exogenous AT may falsely elevate the heparin level. Interpret with caution."
 - Note that the dilution factor is not reported but if you need to know the dilution factor you can always call the routine coagulation bench; the dilution factor will be recorded on the analyzer on which it was run.

Apixaban (Eliquis®) and Rivaroxaban (Xarelto®)

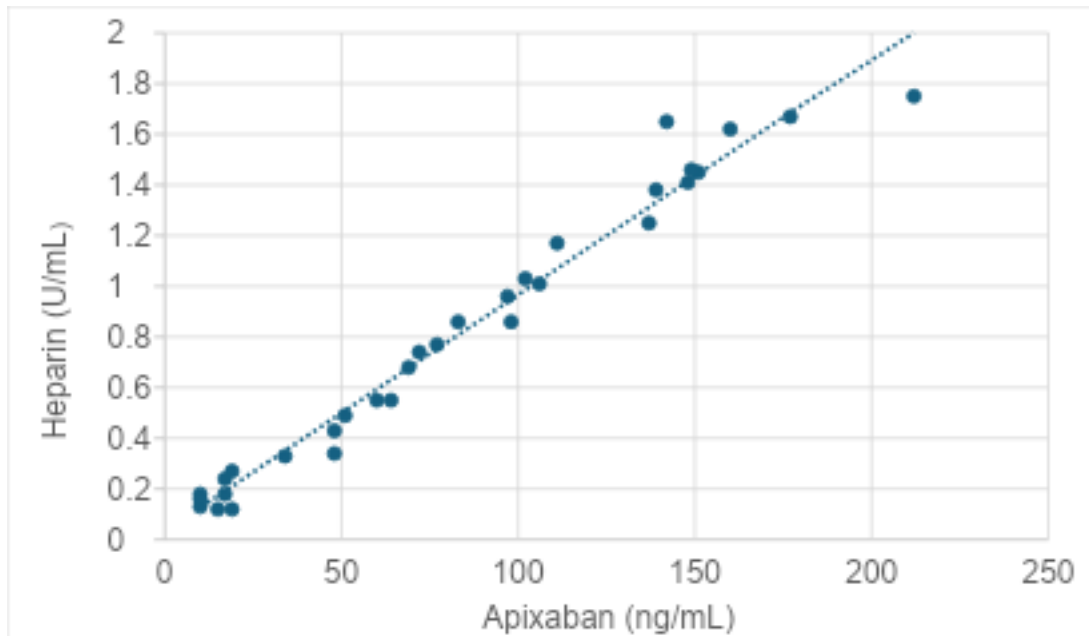
- Levels can be measured using Anti-Xa assays using Werfen reagent:

- Identical assays for Apixaban and Rivaroxaban (same dilutions, reagent and QC), just differ in their Calibration.
- Note that though these assays are similar in principle to the Heparin Anti-Xa assay, we use a different reagent supplier for the Heparin Anti-Xa assay (Biophen) which is substantially more cost effective. The Biophen reagent did not work as well for measuring Rivaroxaban and Apixaban levels, so we had to use the more expensive Werfen reagent.
- Testing went Live at UAH on 2025-04-02 and will be run once daily. With go live we will continue to run reflex Heparin Anti-Xa levels (performed immediately 24/7) in the EDM Zone. This is just in case the patient was bleeding to assess for drug clearance, rather than for the purpose of dose adjustment because:
 - The Heparin Anti-Xa assay is available at multiple sites in the Zone.
 - This allows us to batch Apixaban/Rivaroxaban level testing.

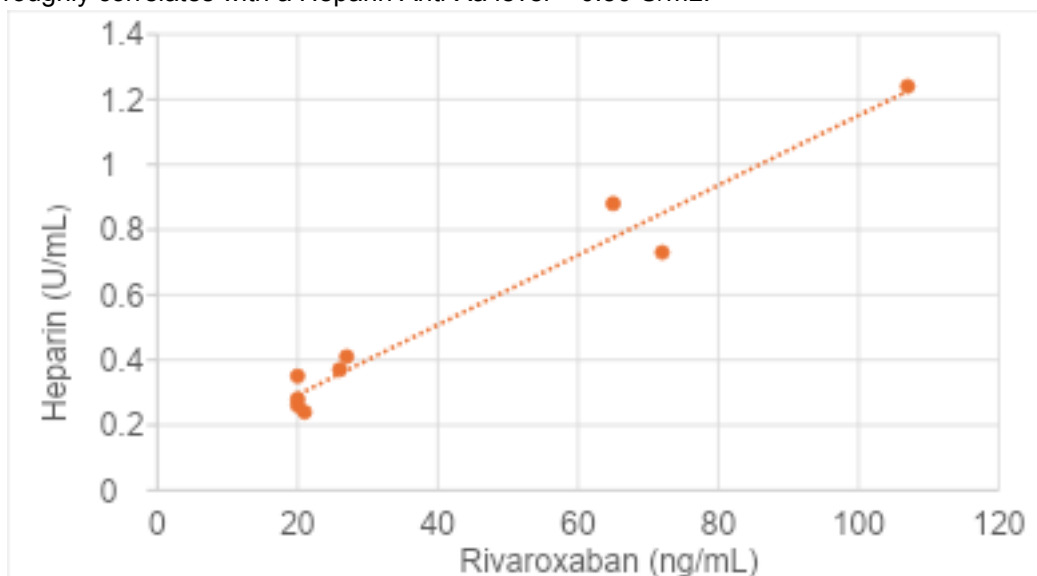
- Codes:

- DTIXA= "CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban."
- DTICA = "Test cannot be performed on anti-coagulated patients, except for those on warfarin or heparin, which can be neutralized in vitro. Please call Client Response (780-407-7484) if the patient was not on anticoagulants at the time of collection."

- A comparison of real patient samples over the linear range of the Heparin Anti-Xa Level (the Apixaban has a wider range due to the use of 2 different dilutions) shows:



-
- Apixaban present at a concentration of 50 ng/mL is expected to yield a Heparin Anti-Xa Level of 0.50 U/ml.
- Data obtained using the Werfen Anti-Xa Apixaban assay in Calgary and the Biophen Anti-Xa Heparin assay in Edmonton.
- A similar comparison can be performed for Rivaroxaban but this medication is used less frequently, and there is fewer data points to analyze. Nevertheless, 50 ng/mL of Rivaroxaban also roughly correlates with a Heparin Anti-Xa level = 0.50 U/mL:



Antithrombin Levels

- HemosIL Liquid Antithrombin (ACL TOP) assay:

- Incubation of plasma with Bovine FXa reagent & excess heparin
- Quantification of residual FXa activity with a synthetic chromogenic substrate. Paranitroaniline (pNA) release measured at 405 nm is inversely proportional to AT level.
- - Interferences on ACL TOP:
- Direct FXa Inhibitors {theoretical}
- Not affected by:

- Heparin (UF or LMW heparin) up to 4 U/mL {product monograph}
- Heparin Cofactor II up to 4 U/mL (product monograph)
- Free Hemoglobin up to 5 g/L {product monograph}
- Triglycerides up to 26 mmol/L on ACL TOP {product monograph}
- Bilirubin up to 684 umol/L on ACL TOP {product monograph}
- Direct Thrombin Inhibitors {theoretical}
- Lupus Anticoagulant

Danaparoid (Orgaran®)

- The Drug:

- Classed as a Heparinoid and contains: Heparan Sulfate, Dermatan Sulfate and Chondroitin Sulfate.
- In 2002 was withdrawn from the US but continues to be marketed in Canada and Europe.
- Like Heparin, acts via ATIII and Heparin Cofactor II
- Much more Anti-Xa activity than Anti-IIa activity compared to LMWH (22:1 vs 3:1).
- T_{1/2} of Danaparoid is 25 hours with normal renal function and is primarily renally excreted.
- Monitoring can only be done using Anti-Xa levels (PTT is unaffected), but needs own Standard Curve.

- Previously used in Hemodialysis patients instead of heparin:

- May be due to chronic toxicities of heparin, such as Alopecia or Osteoporosis.
- May be due to HIT, particularly in children where it is the agent of choice.
- Levels are mainly done pre-hemodialysis, to ensure that the drug is not accumulating – dose is adjusted based on the level.
- As of Apr. 30, 2010 the regional Nephrology service has transitioned away from Danaparoid as a Heparin alternative to Citrate; the latter can be measured using PTT.

- ~10% of HIT+ pts will also crossreact with Danaparoid

- However, this is almost never clinically significant
- Unfortunately, the drug is greatly under-utilized due to this stigma, but actually causes less bleeding than Lepirudin.

Dabigatran (Pradaxa®)

- Codes:

- DTIXA= “CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban.”
- DTICA = “Test cannot be performed on anti-coagulated patients, except for those on warfarin or heparin, which can be neutralized in vitro. Please call Client Response (780-407-7484) if the patient was not on anticoagulants at the time of collection.”

- Oral Direct Thrombin Inhibitor

- Pharmacokinetics {*J.Thromb.Haemost.* 2011. 9(S1):12-19; *Clin.Pharmacokinet.* 2010, 1:49(4):259-68}:

- Dabigatran etexilate is the orally bioavailable prodrug form (MW=627 Da)
- Oral bioavailability is quite low at 6.5%; can be taken with or without food.
- Metabolized by circulating esterases to the active Dabigatran (MW=471 Da)
- T_{max}=1-3 hours (Time to max concentration)
- T-1/2
 - 80% renal excretion
 - 9-17 hours (sources vary quite widely but 12 hours seems the most closely agreed upon number) when CrCl >30 ml/min
 - T-1/2 is >24 hours if CrCl is <30 ml/min
- No significant Cytochrome interactions
- p-glycoprotein inhibitors, especially the “__conazoles”, such as ketoconazole and voriconazole. Others p-glycoprotein inhibitors include ritonavir, amiodarone and verapamil. These would increase active drug levels and promote bleeding.
- There has been a suggestion that antacids (including PPI's) may inhibit drug absorption.

Emicizumab (Hemlibra®)

- Emicizumab details

- Treatment for Hemophilia A
- Humanized bispecific monoclonal antibody that binds FIXa and FX, mediating the activation of the latter.
- Administered subcutaneously:
 - Weekly loading dose x4 weeks
 - Thereafter maintenance dosing is given every 1-4 weeks

- Effect on coagulation tests:

- Shortens or normalizes the PTT even in patients with severe FVIII deficiency.
- Clot based FVIII levels are factitiously high.
- Does not affect the chromogenic FVIII assay (ie. results are still reliable in the presence of emicizumab)

Hepzyme, Hepzorb, Heparin Inhibitors and Thrombin Time

- In Epic, hepzyme testing can be specifically requested for routine coagulation tests with the following names:

- Heparin Neutralization Fibrinogen
 - Fibrinogen Hepzyme cancelled if:
 - 1. PTT or TT is normal
 - 2. If PTT or TT are abnormal, fibrinogen hepzyme will still be cancelled if fibrinogen is >4.0 g/L with the following comment/explanation:
 - Heparin Neutralization not performed. Heparin interference in the Fibrinogen assay produces falsely low results, so Heparin Neutralization is only performed if the original result is <4 g/L. The Fibrinogen assay is also quite insensitive to Heparin interference, only occurring at very high concentrations, such as those encountered in Cardiopulmonary Bypass.
- Heparin Neutralization PTT
 - PTT Hepzyme is cancelled if original PTT is normal.
- Heparin Neutralization INR
 - INR Hepzyme is cancelled if original INR is normal.
- Heparin Neutralization Thrombin Time

- All Hepzyme results receive the following comment:

- Neutralization removes 2 U/ml of Heparin. Higher concentrations of Heparin, such as those encountered in Cardiopulmonary Bypass, may not be completely removed by Neutralization.

- Clinician ordering of any heparin neutralization in Epic requires an answer to a HARD STOP question regarding indication with the following available options:

- A) Prior to transthoracic line removal
- B) Liver transplant patient to exclude heparin contamination as cause of elevated INR
- C) Rule out bleeding diathesis (DIC, septic coagulopathy, known bleed) and PTT >50s.
- D) Heparinized or pediatric hematology/oncology patient to assess underlying coagulation status.
- E) Other (if selected, the ordering provider must page the hematopathologist on call for approval)

- Hepzyme® (lyophilized bacterial Heparinase I with stabilizers) neutralizes heparin or from plasma/serum or solution:

- 0.5 to 1.0 ml of citrated Platelet Poor Plasma is added to a Dade® Hepzyme® vial, closed with a rubber stopper, and inverted gently 5-10 times.
- The sample volume is an absolute minimum of 0.5 ml and all the lyophilized material must be dissolved.
- Sample is incubated for 15 min at Room Temp.
- Remains in the sample after incubation, thus any subsequently added Heparin would also be destroyed.

- Hepzyme® is not routinely performed on all samples where it could be of benefit (eg. Lupus Anticoagulant) because:

- It adds an additional step which includes a timed incubation – any such additional sample manipulation has the potential to affect assay performance, eg. via clotting factor degradation.
 - It is relatively expensive.
 - Some assays already contain a Heparin Inhibitor.
 - As a compromise we perform a Thrombin Time prior to doing some tests (eg. Lupus Anticoagulant and PT/PTT Inhibitor Screen), which gives us the option of doing a Hepzyme if prolonged.
- Hepzyme® can be requested by pathologists or clinicians on the routine coagulation bench (ie. 24/7) for a variety of reasons:
- Samples drawn from heparinized line and thus contaminated by heparin can be hepzymed to get a PTT result. This mainly happens in PICU/NICU and in 2005 we had an agreement with them that has largely been ignored:
 - A) All hepzyme requests had to be specific orders from a physician (now mostly coming from nurses)
 - B) Hepzyme protocol was restricted to only patients who were not on therapeutic heparin, including low molecular weight heparin (incidents of nurses ordering hepzyme on patients on heparin treatment have occurred)
 - C) Hepzyme stamp or sticker stating "Perform PTT and/or PT pre- and post-hepzyme, and report both results. Results will be interpreted by the attending physician." along with the name of the ordering physician and the signature of the nurse
 - D) Hepzyming of a specimen adds 20-30 minutes to the turn around of any results.
 - To assess coag parameters in a patient receiving Heparin therapy. Occasionally, it is necessary to remove therapeutic Heparin to assess other coagulation parameters. This should only be done in consultation with a Pathologist
 - To assess whether the patient has received Heparin.
 - To assess whether the patient has received a Direct Thrombin Inhibitor or Direct Xa Inhibitor.
- Hepzyme® Performance and Limitations:
- While the majority of validation performed by the manufacturer was on UFH, the product monograph does indicate that LMWH will also be neutralized (11 patients tested).
 - Will NOT remove Fondaparinux, which is a pentasaccharide heparinoid.
 - Will NOT remove Dabigatran or Rivaroxaban.
 - At least 2 U/ml of UFH is removed by Hepzyme, which is sufficient for most indications with the exception of patients on Cardiopulmonary Bypass.
 - Hepzyme can be repeated on the sample ONCE to remove at least 4 U/ml of UFH.
 - If this is necessary I would suggest running 1 ml in the first Hepzyme vial, using 0.5 ml for testing, followed by repeat Hepzyming of the remaining 0.5 ml in a second vial and repeating/comparing the same coag testing.
 - Some Hepzyme will remain in the sample even after it has been removed from the vial, so any test that requires heparin to be added back to the sample (eg. HIT Lumi-aggregometry) cannot be performed on a Hepzymed sample.
 - Hepzyme degrades heparins to oligosaccharides that are 1000 Daltons on average and these may have some very mild degree of persistent anticoagulant effect; the product monograph states:
 - "Plasma samples from 157 patients [on UFH or spiked with UFH]...treatment with Dade® Hepzyme® showed a reversal of the heparin effect with aPTT results returning to within the aPTT reagent's normal range or within 15% of the patient's baseline before treatment with heparin."
 - While nearly all of the validation of Hepzyme performed by Dade/Siemens was on UFH, the product monograph does indicate LMWH is also neutralized (11 patients studied).
- Hepzorb is a suspension of Styrofoam like beads that bind heparin from sample rather destroying it:
- Advantage over Hepzyme is that the Hepzorb is centrifuged out of the sample and assays that require Heparin be added back can be performed (only used in the HIT assay at present).
 - Disadvantage from Hepzyme is increased processing involved.
- Heparin Inhibitor(s)
- Polybrene is the most common and is a cationic substance that neutralizes anionic Heparin

- Present in some reagents such as the DRVVT to prevent heparin interference in the assay.
 - Most assays that contain it have enough to neutralize up to 1 U/ml Heparin.
- Thrombin Time:
- Our STA Thrombin assay contains 1.5 U/ml of Thrombin and is very sensitive to UFH and Direct Thrombin Inhibitors (DTI). Therapeutic quantities of these agents will usually cause TTs to be off-scale (>120 sec).
 - Conversely the Thrombin Time is only mildly affected by hypofibrinogenemia. Producing results in the 20-35 sec range with Fibrinogen levels of 0.4-1.5 g/L {*Clin. Chem.* 20003, 49:169-172}.
 - The main indication for testing is for Dysfibrinogenemia which can prolong or shorten the TT.
 - Other factors that may prolong TT:
 - Thrombolytic therapy
 - Fibrinogen Degradation Products (FDPs)
 - High Paraprotein levels
 - Hypoalbuminemia (<2 g/dL) {*Arch.Pathol.Lab.Med.* 2002; 126: 1387-1390}
 - Warfarin therapy through an unclear mechanism {*Arch.Pathol.Lab.Med.* 2002; 126: 1387-1390}.
 - Radiocontrast Agents
 - Other factors that may shorten TT:
 - Dextran and Hydroxyethyl Starch {*Intensive Care Med.* 1985; 11: 300-303}.
 - Can be performed on the routine coagulation bench 24/7 but only at the UAH (this may be expanded to most sites in the Edmonton Zone in the spring of 2012).
 - May take time as reagent would have to be reconstituted (30 min) and QC run first.

Partial Thromboplastin Time, LAC-insensitive

- Testing implemented at UAH & RAH 2025-01-15

- We also discontinued 1:1 PT and PTT Mixing Studies at the same time.

- Interpretation:

- Lupus Anticoagulants (LACs) cause a prolongation in PTT but do not, by themselves, increase bleeding risk. Nevertheless, it is often desirable to identify the presence of a LAC because it can have other clinically important consequences. For this reason the regular PTT is relatively sensitive to the presence of LACs. However, the presence of a LAC can mask other clotting problems (eg. clotting factor deficiency, anticoagulant medications, etc). By running a LAC-insensitive PTT we can assess whether the underlying coagulation pathway is normal. If the regular PTT is prolonged but the "PTT, LAC-insensitive" is normal it generally means a LAC is the cause of the prolongation, and coagulation is otherwise intact. If the regular PTT and "PTT, LAC-insensitive" are both prolonged the results are essentially uninterpretable. It would be tempting to interpret a marked regular PTT prolongation along with a milder "PTT, LAC-insensitive" prolongation as indicating the presence of both a Lupus Anticoagulant plus something else, but that would not be valid. The reason is that the two reagents also differ in other ways; not just with regard to their sensitivity to the presence of a LAC. This means that other (non-LAC) coagulation disturbances might have markedly different effects on the two tests.
- Note that the use of "PTT, LAC-insensitive" is never definitively diagnostic for a LAC; dedicated Lupus Anticoagulant testing by the special coagulation laboratory is still required for a formal diagnosis. Nevertheless, the "PTT, LAC-insensitive" is a quick way to evaluate bleeding risk when there is insufficient time to complete formal investigations.

Lupus Anticoagulant

- NEW ACL TOP Lupus anticoagulant process effective September 2023 associated with change to ACL TOP instrument:

- Anti-Xa heparin level now performed on every "abnormal" DVVT/SCT sequence, which facilitates identification of Xabans and heparins.
- ACL TOP Lupus Anticoagulant testing will follow the recommended Devereese et al (2020) ISTH guideline where "patient sample's normalized ratios are calculated by the clotting time of the Normal Pool Plasma (NPP) measured on the same run which helps to compensate for the day-to-day variation" (p. 2835).

- TOP instruments are susceptible to spectral interference. There may be comments or complete failure to generate a result(s).
- A guide with examples was circulated in Dec 2023. Please note that the ranges in the guide may no longer be relevant. Refer to the most updated survival guide for interpretative ranges.



NEW ACL TOP
Lupus anticoagulan

○

- Ratios are now calculated as follows:

$$\text{DVVT Screen Normalized Ratio (DVVT}_{\text{NR}}) = \frac{\text{sample dRVVT Screen (sec)}}{\text{NPP dRVVT Screen (sec)}}$$

$$\text{DVVC Confirm Normalized Ratio (DVVC}_{\text{NR}}) = \frac{\text{sample dRVVT Confirm (sec)}}{\text{NPP dRVVT Confirm (sec)}}$$

$$\text{DVVC Total Ratio (DVVC TR)} = \text{DVVT}_{\text{NR}} / \text{DVVC}_{\text{NR}}$$

$$\text{DVVM Mix Normalized Ratio (DVVM}_{\text{NR}}) = \frac{\text{sample dRVVM Mix (sec)}}{\text{NPP dRVVM Mix (sec)}}$$

SCT process is generally unchanged (same reagent)

- Converted to a total ratio based calculation as well:

$$\text{SCT Screen Normalized Ratio (SCRN}_{\text{NR}}) = \frac{\text{sample SCT Screen (sec)}}{\text{NPP SCT Screen (sec)}}$$

$$\text{SCT Confirm Normalized Ratio (CONF}_{\text{NR}}) = \frac{\text{sample SCT Confirm (sec)}}{\text{NPP SCT Confirm (sec)}}$$

$$\text{SCT Total Ratio (SCT TR)} = \text{SCRN}_{\text{NR}} / \text{CONF}_{\text{NR}}$$

- Ranges for Dilute Viper Venom Confirmation and Silica Clotting Time (SCT) Ratio:

- DVVC before Feb. 2, 2026:
 - Borderline Positive Range: upper limit of normal to 1.75
 - Positive: > 1.75
- DVVC after Feb. 2, 2026:
 - Borderline Positive Range: upper limit of normal to 1.90
 - Positive: > 1.90
- SCT (both before and after Feb. 2, 2026):
 - Borderline Positive Range: upper limit of normal to 1.48
 - Positive: > 1.48
- Note: Borderline Positive Ranges reflect a 2-6 SD range (based on Gestalt).

- Heparin interference

- The DVVT_{NR} was demonstrated to be resistant to interference by UNFH & LMWH (Tinzaparin) up to a maximum concentration of **0.80 U/mL**.
- The DVVC_{NR} was demonstrated to be resistant to interference by UNFH & LMWH (Tinzaparin) up to a maximum concentration of **1.00 U/mL**.

- INTERPRETATION GUIDE

Interp	DVVC	SCT Ratio	TT	Anti-Xa	DOAC/Hep
LANEG	NEG	NEG	N/A	N/A	N/A
LAPOS	EITHER (or both) POS		Normal	<0.1	No
LABOR	BOR/BOR BOR/NEG Or NEG/BOR		Normal	<0.1	No
LADI	EITHER (or BOTH) POS/BOR		High	<0.1	DTI
***	***		High	>0.1	Heparin
LADI*	EITHER (or BOTH) POS/BOR		Normal	>0.1	Xa inhibitor
LAONE**	FAIL		NEG	N/A	N/A
	NEG		FAIL	N/A	N/A

*Direct Xa Inhibitors are much more likely to cause false positives than false negatives.
We don't have enough data with Direct Thrombin Inhibitors.

** .LAONE: Negative for a Lupus anticoagulant by one test method only. The other assay was not interpretable due to the presence of spectral interfering substances in the plasma. Recollection is suggested as the recommendation is that TWO assays be performed in order to reliably exclude a Lupus Anticoagulant (Brandt et al. Thromb.Haemos. 74:1597-1603). Note: testing during warfarin therapy is known to reduce Lupus Anticoagulant assay sensitivity.

***Heparin is the usual cause of both an elevated TT and Anti-Xa.

- UFH typically causes a marked TT prolongation with Anti-Xa levels <0.70 U/mL (higher levels can be seen in supratherapeutic states).
- LMWH typically causes a much milder TT prolongation, typically with higher Anti-Xa levels. Note that it is primarily the HMW multimers in heparin that inhibit FIIa. LMWH not only has fewer HMW multimers than UFH, but these are cleared faster from the circulation than LMW multimers such that TT prolongation with LMWH is usually quite mild compared to that with UFH.
- In theory, the reagent contains a heparin neutralizer so it should not affect results BUT sometimes we do see interference in the form of: elevated DVVT, normal DVVC, high DVVM, normal SCT ratio. In this case, please use the LAHEP comment:
 - **LAHEP** = Contradictory results: Lupus Anticoagulant activity cannot be demonstrated, yet the dilute viper venom mixing study does not correct. This may be secondary to residual Heparin activity in the sample. Repeat testing is suggested once heparin therapy is discontinued.
- If the DVVC and/or SCT ratio is high than results can be reported with LAPOS (or LABOR) as usual.
- If the DVVC, DVVM and SCT ratio are all normal than results can be reported with LANEG as usual.

- Main Coded Comments (built as smartphrases in Epic):

- **LANEG** = Negative for a Lupus Anticoagulant by both the Dilute Russell's Viper Venom Time (DVVT) and the Silica Clotting Time (SCT). This fulfills recommendations that two sensitive clot based assays be performed in order to reliably exclude a Lupus Anticoagulant (Brandt et al. Thromb.Haemos. 74:1597-1603). Note: testing during warfarin therapy is known to reduce Lupus Anticoagulant assay sensitivity.
- **LABOR** = Borderline positive. This result could indicate a weak Lupus Anticoagulant or a false positive. Recommend repeat testing in 1-4 weeks and only consider as a true positive if results are consistent.

- **LAPOS** = Positive for a Lupus Anticoagulant. The revised Sapporo Criteria indicate that the Lupus Anticoagulant assay should be positive on two separate occasions at least 12 weeks apart to be clinically significant (*J.Thromb.Haemost.* 4:295-306).
 - **LAONE**= Negative for a Lupus anticoagulant by one test method only. The other assay was not interpretable due to the presence of spectral interfering substances in the plasma. Recollection is suggested as the recommendation is that TWO assays be performed in order to reliably exclude a Lupus Anticoagulant (Brandt et al. *Thromb.Haemos.* 74:1597-1603).
- Additional coded comments
- Direct Thrombin Inhibitors (DTI) and Direct Xa Inhibitors (DXI) should be suspected when there is an elevated DVVT, normal DVVC, high DVVM, and normal SCT ratio. With DXI the TT is normal and the anti-Xa is elevated, with DTI the TT is high and does not correct with heparin, the anti-Xa is normal except in cases of extreme overdose. Unpublished data from other centers suggests that Rivaroxaban causes false positive DRVVT-based LAC results. Suggested comment in either case:
 - **LADI** = The patient appears to be on either a Direct Thrombin Inhibitor, Direct Xa Inhibitor or a Low Molecular Weight Heparin (LMWH) medication, which confounds test interpretation. If not, consider the possibility of an acquired common pathway inhibitor such as Fibrinogen Degradation Products (FDPs), Dysfibrinogenemia or an antibody to FV, FX or FII. For questions or additional testing options please page the Hematopathologist on-call at the University of Alberta Hospital (780-407-8822).
 - **DTIXA**= CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban.
- Low DVVC and/or SLRAT:
- Ratios of ≤ 0.6 tend to occur in the context of low factor levels such as in Vitamin K deficiency/antagonism and liver dysfunction.
 - When it occurs, the sensitivity of the assay for the Lupus Anticoagulant is likely quite poor.
- INR35 = "The Lupus Anticoagulant assay will not be performed when the INR is >3.5 . The sensitivity of the assay progressively decreases as the INR rises, such that beyond an INR of 3.5 it is essentially of no value."
- The Verbatim Anti-Phospholipid Syndrome definition by the revised Sapporo Criteria/Sydney Criteria (*J.Thromb.Haemost.* 4:295–306, 2006):
- At least one Clinical Criteria
 - Vascular thrombosis – One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.
 - Pregnancy morbidity
 - One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or
 - One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe preeclampsia defined according to standard definitions, or (ii) recognized features of placental insufficiency, or
 - Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.
 - At least one Laboratory Criteria

- Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies).
- Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.
- Anti-β₂ glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures.
- Caveats (summarized, non-verbatim):
 - The thrombotic event and testing be with in 5 years of each other to be valid.
 - Stroke and TIA can be used to meet the Thrombosis criteria.
 - Heart Valve vegetations are a common manifestation in APLS but are not specific enough for the syndrome to be diagnostic.
 - Antiphospholipid Associated Nephropathy can be used to meet the Thrombosis criteria provided that other causes are excluded.
 - Superficial venous thrombosis does not count as a thrombotic event.
 - Thrombocytopenia cannot be used to meet the Thrombosis criteria:
 - It is known that ITP (<100) concomitant with LA/APA is associated with higher risk of Thrombosis than either of these factors alone.
 - However, the finding is not specific enough to be a diagnostic criterion.
 - It is recommended that these patients be labelled Anti-Phospholipid Associated Thrombocytopenia.

- Other Inhibitors

- FVIII Inhibitors (Hemophilia A with Inhibitors and Acquired Hemophilia):
 - A FVIII Inhibitor would not be picked up by the DVVT as this is upstream of the DVVT activation point.
 - A FVIII Inhibitor could potentially give a false positive SCT ratio:
 - During validation 3 patients with known FVIII Inhibitors (2 Hemophilia A with Inhibitors, and 1 Acquired Hemophilia) were tested and did not give a false positive. However, this does not rule out the possibility.
 - Suspect this if the DVVT based assay is negative for an inhibitor (Lupus Anticoagulant or otherwise) and the SCT ratio is only mildly increased BUT both the SCT Screen (SLCT) and SCT Confirm (SLPL) are markedly increased.
- Acquired common pathway inhibitors/antibodies (increased fibrinogen degradation products, dysfibrinogenemia, Anti-FV, Anti-FX, or Anti-FII) and the Lupus Anticoagulant Cofactor Affect:
 - Possible presentations:
 - Normal DVVC but with a DVVM that does not correct. Most often this will have been caused by a DTI or DXI medication but if the patient is definitively not on either consider the possibility of one of these.
 - Common pathway inhibitors are also usually associated with prolonged PTT/PT and positive mixing studies/inhibitor screens.
 - Strong Anticardiolipin Antibody but Normal DVVT and/or SCT ratio may suggest the Lupus Anticoagulant Cofactor Affect.
 - Testing for common pathway inhibitors is via the respective factor levels.
 - The mechanism of the Lupus Anticoagulant Cofactor Affect:
 - LA mainly binds β₂-GPI (the LA Cofactor) rather the PL directly.
 - If β₂-GPI is depleted in the patient sample for some reason the LA cannot bind to the PL without this bridge/cofactor.
 - Mixing with Normal Pooled Plasma replenishes the β₂-GPI bridging the interaction resulting in a drop in PL, which causes prolongation in clotting time.

- How to test for the LA Cofactor Affect
 - The clot based LA test is negative, ie. DVVC is within normal limits.
 - Mix patient plasma with Normal Pooled Plasma then repeat the DRVVT and DRVVT(PL) on the mixed sample.
 - If the regular DVVC ratio is within normal limits but the DVVC performed on a 1:1 mixed sample is increased you have a LA Cofactor Affect.
- LA affects on other Coagulation Assays:
 - Coagulation Assays affected by a LA:
 - PTT
 - PT-INR (but much less frequently than PTT – indicate higher titer)
 - DRVVT
 - Factor Levels (except possibly FVII and Fibrinogen).
 - Protein C and Protein S levels
 - Coagulation Assays NOT affected by a LA:
 - Fibrinogen assay
 - Thrombin Time
 - Heparin Level
 - ATIII Level
 - FVII, FX, FII, and FV levels (usually) – because these are PT based and PT is less sensitive to LA.
 - APC-Resistance (Acticlot assay)
- β_2 -Glycoprotein I:
 - Testing can be sent to CLS if requested but since diagnosis requires only 1 of Lupus Anticoagulant, Anti-Cardiolipin or Anti- β_2 -Glycoprotein I for a diagnosis it is not necessary unless the first two are negative or borderline/indeterminate.
 - Cancel requests for Anti- β_2 -Glycoprotein I with the following comment if Lupus Anticoagulant or Anti-Cardiolipin are clearly positive:
 - B2CAN- B2 Glycoprotein I Antibody testing is performed at an external reference laboratory. Testing was cancelled because either the Lupus Anticoagulant or Anticardiolipin assay was positive on the current (or a recent) sample.
 - Hopefully the direct β_2 -Glycoprotein I IgG assay will be added to the special coagulation test menu in the future.

Anti-Cardiolipin/Anti-Phospholipid Antibody

CAUTION: SECTION NEEDS REVIEW/UPDATING

- Anti-Cardiolipin (aCL)

- Note: Cardiolipin is so named because it was originally extracted from bovine heart; its proper chemical name is diphosphatidyl glycerol.
- aka Antiphospholipid Antibody (APA) assay – Note: while Cardiolipin is a specific type of negatively charged phospholipid, there are many others that this assay does not test for and so the name APA is somewhat misleading.
- First developed in the mid-1980s as a radioimmunoassay and eventually modified into an aCL-ELISA.
- Assay ingredients have been modified over the years but now are specifically:
 - The antigen bound to the plate is still Cardiolipin
 - Bovine AND human serum is added to the assay supplying bovine AND human β_2 -GPI, which will bind as a cofactor.
 - Note: some assays use only human or only bovine β_2 -GPI, but the UAH assay uses both.
 - Enzyme linked Anti-IgG is used to detect Anti-Cardiolipin binding.
 - Note: some centers also use Anti-IgM, but the UAH only uses Anti-IgG.
- Sensitivity
 - No aCL or other APA assay has perfect sensitivity
 - Sensitivity of the aCL assay is better than the Lupus Anticoagulant assay.

- Detection of IgG, IgM and IgA antibodies gives the best sensitivity:
 - However, revised Sapporo\Sydney criteria only recognize IgG and IgM isotypes in diagnostic criteria.
 - Use of IgM improves sensitivity only marginally but markedly reduces specificity and thus is avoided by most labs.
- Specificity
 - Use of IgG only (rather than IgM) improves specificity for AntiPhospholipid Syndrome, vs nuisance aCL.
 - Even with IgG use only, the aCL assay is less specific than the Lupus Anticoagulant assay for clinical Antiphospholipid Syndrome.
 - Will also be positive in Syphilis for which the assay was originally developed.
 - Also positive in a variety of other infectious (esp. HCV and HIV) and drug induced conditions (Possibly cocaine/levamisole – significance unknown).
- Calibrators
 - Polyclonal APA IgG, IgM and IgA calibrator antibodies have been used since the 1980's to standardize results all over the world (though inter-laboratory variation in results remains a problem).
 - These calibrators are real patient sera with defined titers quantitated in units called GPL, MPL and APL for IgG, IgM and IgA respectively.
 - High positive levels are ≥ 80 GPL
 - Medium positive levels are >20 and <80 GPL
 - Low/indeterminate values are those that are higher than the cutoff of the assay for the specific isotype (≥ 15 for GPL) and lower than 20 GPL/MPL. The Sydney Criteria/Revised Sapporo Criteria recommend repeating low/indeterminate results in 12 weeks.
- β_2 -Glycoprotein I Antibody (Anti- β_2 -GpI)
 - IgG now included in Antiphospholipid Antibody Syndrome Panel in Epic.
 - In 3-10% of cases of Anti-Phospholipid Syndrome the only laboratory abnormality is anti- β_2 -GpI, and it is primarily for this reason that it is recommended in addition to aCL and LA testing (Blood. 113:985-994, 2009; *Semin. Thromb. Hemost.* 34(4): 313-328, 2008; *J. Thromb. Haemost.* 4:295-306, 2006).

PTT and PT Inhibitor Screen

- Criteria for suspected Acquired Hemophilia A/Specific Factor Inhibitor necessitating urgent (after hours)

PTT Inhibitor Screen:

- Bleeding or suspected bleeding
- Isolated unexplained prolonged PTT - usually pronounced
- PTT-LACi is not compatible with Lupus Anticoagulant
- Lacking historic PTT results:
 - If historic PTT results were normal then it tends to confirm a new Inhibitor and urgent treatment (ie. bypassing agents) for same. Urgent testing should not typically be needed.
 - If historic PTT results were similar to current then it tends to suggest heritable factor deficiency, and suggest urgent replacement therapy is needed. Urgent testing should not typically be needed.

- Cancellation Codes:

- NEDI – No definite evidence of an inhibitor detected.
- PTTN – patient's PTT result is Normal
- PTNN – patient's PT INR result is Normal
- PTTL – elevation in the patient's PTT is too minimal to allow accurate detection of inhibitor by screening.

- Comment Codes:

- ININD = Negative for an immediate and a delayed acting inhibitor.
- IPIUD = Positive for an immediate acting inhibitor. Cannot assess for a delayed acting inhibitor due to degradation of clotting factor activity with incubation.

- INIUD = Negative for an immediate acting inhibitor. Cannot assess for a delayed acting inhibitor due to degradation of clotting factor activity with incubation.
 - INIPD = Positive for a delayed acting inhibitor. There is no evidence of an immediate acting inhibitor.
 - IPIND = Positive for an immediate acting inhibitor. Negative for a delayed acting inhibitor.
 - IPIPD = Positive for both an immediate and a delayed acting inhibitor
- PTT and PT Inhibitor Screens:
- Performed on the special coagulation bench, thus only available during the day (includes weekends) and only at the UAH site.
- Heparin and Hepzyme
- A Thrombin Time is (normal range: 15.2-18.8) is always performed on the sample to exclude Heparin effect.
 - If the Thrombin Time is prolonged then the sample is Hepzymed and PTT remeasured:
 - If the PTT does not change post-hepzyme then Inhibitor Screen is run on the neat (not hepzymed) sample.
 - If the PTT normalizes (must be in the normal reference range or within 3 seconds of the upper limit) the Inhibitor Screen is cancelled.
 - If PTT partially corrects with hepzyming but is still high (>3 sec beyond upper limit of normal) then the Inhibitor Screen can be done on the Hepzymed sample:
 - The assay does not contain a Heparin Inhibitor (Polybrene) so in this circumstance you need to run on the Hepzymed sample.
 - If the assay was run on a Hepzymed sample then it should be noted in the final report. Because Hepzyming adds a 15 min incubation step to the assay and additional manipulation it carries with it the potential to affect the results.
- Normal Pooled Plasma + FVIII Deficient Plasma Control
- Often is slightly out of the 5-6 second window we need for significance
 - Mechanisms that may be contributing to this include:
 - Our PTT reagent is quite sensitive to FVIII levels, such that even 50% is not quite adequate to give a normal result (often runs in the 42-43 sec window).
 - The FVIII deficient plasma we use is manufactured from Normal Pooled Plasma by immunodepletion rather than the alternative “naturally” FVIII deficient patient plasmas that are commercially available (from George King Bio-Medical) but are significantly more expensive.
 - Our Normal Pooled Plasma does not appear to have adequate buffering capacity resulting in a pH rise over the incubation period which results in some factor deactivation.
- Interpretation:
- In the absence of an Immediate Acting PTT Inhibitor the immediate 1:1 PTT should correct to within 5-6 seconds of the Normal Pooled Plasma (2-3 seconds for the PT Inhibitor). A difference of 7-8 seconds (1:1 immediate PTT mix) should be interpreted as Equivocal. A difference of >8 seconds (1:1 immediate PTT mix) should be interpreted as positive for an inhibitor.
 - If the “separate” plasma 1 hour incubation prolongs by >3-4 seconds (compared to the immediate mix) then the presence or absence of a delayed inhibitor cannot be included or excluded.
 - In the absence of a Delayed Acting PTT Inhibitor the PTT should correct to within 3-4 seconds of the Immediate 1:1 Mix.
 - PT Mixing Studies are of limited value and even factor deficiencies (eg. due to Vitamin K deficiency) can sometimes result in an apparent inhibitory effect. If an inhibitor is detected follow-up is recommended by the pathologist. In particular we have seen cases where Vitamin K administration completely resolves the PT prolongation and apparent inhibitor effect.

High HCT Coagulation

- Effective 2024-04-10 there is a new provincial process for performing coagulation testing when HCT >0.55.
- For a list of designated sites and what to do in case collection is requested at a “non-designated” site see 2024-04-05 Operational Update:

- [New Provincial Process for High HCT Coagulation \(PT/INR, PTT, Thrombin Time and Fibrinogen\) Testing \(albertahealthservices.ca\)](https://albertahealthservices.ca)
 - When assessing clinical need consider patient location, ability to travel (eg. inpatient vs outpatient, distance to nearest designated site) and whether testing is indicated.
- For clinician instructions on how to order testing, see 2024-04-15 Lab Bulletin:
- [Provincial High HCT Coagulation \(PT/INR, PTT, Thrombin Time and Fibrinogen\) Testing Process \(albertahealthservices.ca\)](https://albertahealthservices.ca)
- Relevant SmartTexts translations in Connect Care:
- LAB HIGH HCT COAG NEED = Interpret with CAUTION. When a patient's Hematocrit is >0.55 , clotting times (PT/INR, PTT and TT) can be falsely prolonged while Fibrinogen levels can be falsely decreased. In theory, Anti-Xa levels, Antithrombin levels and D-Dimer results should not be affected but this has not been validated locally. If patient's HCT is expected to be persistently >0.55 , a special (citrate adjusted) collection is needed. Please order "High HCT Coagulation". Note: see Test Directory for a list of sites able to perform a special collection for this testing.
 - LAB HIGH HCT COAG DONE = Test successfully done on a special (citrate adjusted) collection, performed to compensate for a high Hematocrit (>0.55).
 - LAB HIGH HCT COAG FAIL = The results provided are derived from a regular (citrate unadjusted) collection. A citrate adjusted tube was collected (to compensate for Hematocrit >0.55) but testing on this sample failed. Repeat "High HCT Coagulation" testing would be recommended. {+} LAB HIGH HCT COAG NEED
 - LAB HIGH HCT COAG NOT = Patient HCT is <0.56 . Testing successfully performed on a regular (citrate unadjusted) tube. A special collection is/was not necessary.
- We have performed some experiments, adding extra exogenous sodium citrate to patient blood samples to mimic high HCT levels:
- D-Dimer, Antithrombin and Heparin levels do not appear to be affected by HCT up to 0.70.
 - The literature also supports these tests not being affected by high HCT levels.

Factor Levels

- Terminology:

- One-Stage Clotting Assay (OSA or OSCA) referred to in the literature is the most widely used clot-based assay for Factor Levels and is often just called the clot based assay. Most UAH factor levels are performed using an OSA.
- Chromogenic Substrate Assay (CSA) referred to in the literature is also relatively widely used.
- Two-Stage Clotting Assay is now rarely done around the world and is not used locally. Note that the CSA evolved from the Two-Stage Clotting Assay and is sometimes also referred to as a Two-Stage Assay – which can lead to confusion. However, the original Two-Stage Assay relied on clot formation as the detectable end-point, while the CSA relies on a color change as the detectable end-point.

- Coded Comments (built in Epic as Smartphrases):

- NLFAC = The presence of a dilutional effect on the measured factor level is consistent with an immediate acting PTT Inhibitor – such as a Lupus Anticoagulant and some anticoagulant medications. Since the factor level reaches a plateau within (or above) the normal range this patient does NOT have a deficiency.
- LLFAC = "The presence of a dilutional effect on the measured factor level is consistent with an immediate acting Inhibitor – such as a Lupus Anticoagulant. The true factor level cannot be determined with certainty but is at least as high as that reported at the greatest dilution."
- DOFAC = The presence of a dilutional effect is consistent with clotting factor inhibiting medication. Since the factor level reaches a plateau within (or above) the normal range, this patient does NOT have a deficiency.
- HE144 = No significant increase in factor activity seen with increasing dilution of patient's plasma to 1/80 dilution.
- NPTTF = "Normal PTT, without an excessively high FVIII level that could mask factor deficiency. Sample will be held for one week. Please contact Client Response for further information."

- Heritable FVII Deficiency:

- FVII deficiency is the most common of the rare factor deficiencies (ie. non-Hemophilia A, B or C).

- However, FVII is also both Vitamin K dependent and has the shortest half-life of all the factors. This makes it highly susceptible to perturbations in sick patients. As a consequence a number of local patients have been tentatively diagnosed and treated as FVII deficiency while acutely ill which has resulted in some adverse outcomes – most of these patients were subsequently shown to be normal.
 - In order to prevent over-diagnosis of FVII deficiency, the following comment will be auto-appended to all low results:
 - F7LOW = A single low value is not diagnostic of heritable deficiency as FVII levels can be low due to vitamin K deficiency, certain anticoagulant medications, liver dysfunction or consumptive coagulopathy.
 - In the context of an acutely ill patient it may be of value to perform a FVII:Protein C ratio as these factors have similar half lives. A ratio near ~1 argues against heritable FVII deficiency. In future, we hope to establish a normal reference range for the FVII:Protein C ratio.
- In order to address over-investigation of patients with normal PT and/or PTT results the following guidelines are proposed with regard to Factor Levels (subject to the Hematopathologist's discretion):
- FXII levels should be cancelled if the PTT is normal.
 - FX levels may be performed if there is a diagnosis of Amyloidosis, which can cause a specific deficiency of this factor.
 - FII, FV and FVII are more complex as specific levels may necessary for prognostic reasons, particularly in those with Liver dysfunction, Vitamin K deficiency, Antiphospholipid Syndrome, and DIC. These must be evaluated on a case by case basis.
- Cord Blood Factor Levels:
- Often there is a suspicion of Hemophilia even pre-partum, for example with a family history of hemophilia.
 - The Hemophilia clinic likes to do FVIII levels (though not usually FIX) on cord samples under some circumstances but agrees that the results are not reliable, because:
 - High hct level is likely – ideally, collect citrate adjusted tubes per the High HCT Coagulation process.
 - The cord (and their associated placenta) may sit on a bench for variable time periods before being collected.
 - The effect of Wharton's Jelly is unknown (at least to me).
 - FIX levels may be low at birth due to Vitamin K deficiency.
 - Acute Phase Reaction (eg. trauma of birth) will presumably raise FVIII levels.
 - Generally the Hemophilia Clinic does not consider factor levels as diagnostic until 3 months of age {evidence?}.
 - How to address a low cord FVIII Level:
 - A ratio of FVIII to FV can be of value as they have approximately the same half-life and shouldn't be affected by Vitamin K deficiency.
 - Venipuncture recollection of the neonate is always recommended.
 - Levels should be repeated at 3 months of age for confirmation.
- Clot-based FIX assays in the context of rFIXFc (Alprolix®) from Biogen Idec
- It is a form of recombinant FIX Fc fusion protein which extends the half-life of FIX by ~3.5 fold (from ~24 hours to ~86 hours).
 - It is known that rFIXFc factor levels tend to be underestimated by Kaolin based PTT reagents {Sommer et al. Thromb.Haemost. 2014; 112:932-940}.
 - In a Proficiency Testing exercise at the UAH the CK-Prest kaolin-based FIX assay yielded:
 - 0.80 U/ml rFIXFc standard was measured as 0.50 U/ml at UAH.
 - 0.20 U/ml rFIXFc standard was measured as 0.13 U/ml at UAH.
 - 0.05 U/ml rFIXFc standard was measured as 0.035 U/ml at UAH.
 - The Proficiency Testing exercise problem is clearly reagent dependent as there were 2 other labs in the study using the same reagent-analyzer combination who showed virtually identical results. Labs performing Silica based FIX levels (eg. Synthasil) obtained accurate results.
 - According to this Proficiency Testing exercise UAH results demonstrate a -50% bias (using the UAH result as the denominator). Therefore, a rough rule of thumb is to multiply the UAH FIX result 1.5X to obtain the "true" FIX level in a patient on Alprolix.

- This formula is conditional on the patient not having any native circulating FIX present. Therefore, works best in a patient with severe Hemophilia B, with no other recent FIX therapy (eg. Benefix®).
- Of note, it also appears that not all silica based FIX levels are created equal, with regard to Alprolix sensitivity. Calgary uses APTT-SP reagent (from IL) on TOP instrumentation which employs “micronized silica” activator and they obtained virtually the same results that we did:
 - 0.80 U/ml rFIXFc standard was measured as 0.53 U/ml at CLS.
 - 0.20 U/ml rFIXFc standard was measured as 0.13 U/ml at CLS.
 - 0.05 U/ml rFIXFc standard was measured as 0.030 U/ml at CLS.
- McMaster uses the Siemens Actin FS (Ellagic acid based) reagent on a Stago STA-R analyzer, and they demonstrated excellent accuracy:
 - 0.80 U/ml rFIXFc standard was measured as 0.80 U/ml at McMaster.
 - 0.20 U/ml rFIXFc standard was measured as 0.22 U/ml at McMaster.
 - 0.05 U/ml rFIXFc standard was measured as 0.04 U/ml at McMaster.
- Cryocheck Chromogenic FVIII assay from Precision Biologic at UAH:
 - Utility:
 - A subset of mild hemophilia A patients demonstrate higher FVIII activity in one-stage clot vs. two-stage or chromogenic FVIII activity assays where results may be significantly lower. The bleeding phenotype in these patients is more closely related to the lower FVIII activity. Note: the chromogenic assay is nearly the same as the two-stage clot assay, and has essentially replaced it. {[George King Bio-Medical, Inc.](#)}
 - Used for monitoring FVIII levels in patients on emicizumab.
 - Less prone to interference from Lupus Anticoagulant.
 - Two stage assay (reagents contain a heparin inhibitor):
 - Stage 1: Citrated test plasma is incubated with reagent containing Phospholipid, Ca^{2+} , human Thrombin, human FIXa and bovine FX for a defined period. The Thrombin activates any FVIII to FVIIIa, which combines with FIXa to activate FX. The proportion of FXa generated is proportional to the initial amount of FVIII in the test plasma. A fibrin polymerization inhibitor is also present to prevent clotting.
 - Stage 2: FXa activity is measured by chromogenic substrate (very similar to that used in Anti-Xa assays) and pNA generation at 405 nm is measured. OD is converted to a FVIII level using the calibration curve. This reagent includes a Thrombin inhibitor.
 - Interferences/Limitations:
 - Porcine FVIII (rpFVIII, pig, Obizur) levels are underestimated by the Chromogenic Assay (~20-50% of true levels). The drug mfg recommends use of clot-based assays (OSA) for assessment instead, but we have never validated those locally for this purpose. As of 2025-11-04 Dr. Linda Sun indicates it is only available by special access, is challenging to use, and better options are now available anyway.
 - DOACs (both DTI & DXI)
 - Performance in the context of vWD Type 2M is unknown.
 - Not affected by the following interferences {product insert unless otherwise specified}:
 - Hemoglobin ≤ 5 g/L but found to be <40 g/L in local validation.
 - Triglycerides <25.86 mmol/L {local validation}
 - Bilirubin (unconjugated, indirect) ≤ 435 umol/L but found to be <513 umol/L in local validation
 - Bilirubin (conjugated, direct) ≤ 34 umol/L
 - von Willebrand factor ≤ 20 µg/mL
 - Unfractionated heparin ≤ 2 IU/mL but found to be <10 U/ml in local validation
 - Low molecular weight heparin ≤ 2 IU/mL
 - Hepzyme {local validation}
 - Fondaparinux ≤ 1.25 mg/L
 - Lupus Anticoagulant ≤ 1.8 dRVVT ratio
 - Emicizumab does not interfere {verified locally at therapeutic concentrations} due to the assay's use of bovine FX (emicizumab targets human FX).

- Mim8 (aka Denecimig) is a new product and another FVIIIa-mimetic biphasic Antibody (similar to Emicizumab) set to enter the market after 2025. Prompting Precision Biologic to modify their assay:
 - Old formula used hybrid (Human-Bovine) FIX (as of 2025-10-27 is still in use at UAH), which CANNOT be used in the presence of Mim8
 - New formula uses Bovine FIX and can be used in the presence of Emicizumab or Mim8.
- Chromogenic FIX assay at UAH Special Coagulation laboratory.
 - Utility:
 - Reserved for monitoring extended half-life (EHL) factor IX products (in Canada these include rFIX-FC (Alprolix, Biogen) and rFIX-GP (Rebinyon, Novo Nordisk). Special coag will maintain a list of patients receiving product.
 - Clinical hematologists commencing patient on EHL FIX are asked to call the special coagulation to have their patient added to the list.
 - Testing is performed when a full batch is received, or every two weeks, whichever comes first. Earlier turnaround is possible at the hematopathologist's discretion.
 - Assay:
 - Incubate citrated plasma sample with reagent containing FXIa, FVIIIa, FX, Ca^{2+} , and phospholipids. Note: also contains FIIa and a fibrin polymerization inhibitor.
 - Incubate with the FXa chromogenic substrate. Includes a FXIa inhibitor.
 - Read OD of pNA released at 405 nm. OD correlates directly with FIX level on calibration curve.
 - Not affected by the following interferences:
 - Free Hemoglobin <10 g/L {local validation}
 - Triglycerides <51.7 mmol/L {local validation}
 - Bilirubin <2,052 $\mu\text{mol/L}$ {local validation}
 - Dabigatran <500 ng/mL {product insert but on a non-ACL TOP analyzer}
 - Apixaban 50 ng/mL {product insert but on a non-ACL TOP analyzer}
 - Unfractionated heparin <10 U/mL in local validation
 - LMWH ≤ 2 IU/mL {product insert but on a non-ACL TOP analyzer}
 - Hepzyme {local validation}
 - Lupus Anticoagulant {local validation}

FXIII Screen and Assay

- Since 2020 we have been performing a Factor XIII activity assay at UAH.
 - Berichrom "Factor XIII Chromogenic"
 - Ammonia-Release methodology
 - The transglutaminase activity of FXIII releases ammonia. Ammonia then reduces NADH; it is this reaction that is quantitated spectrophotometrically.
 - Blanking procedure is important to maximizing lower limit of detection of the assay (as normal plasma contains substances that will reduce NADH or produce ammonia)
- Historically, UAH performed a clot solubility assay, but this was discontinued due to poor sensitivity, specificity and proficiency testing results:
 - The solubility assay was usually considered positive if the FXIII level is <1% according to NASCOLA.
 - The assay used at UAH until 2018 was completely homebrewed – that is to say there was no manufacturer specific kit in use. The test was cheap and easy:
 - Clot is placed into a tube with a strong protein denaturing agent:
 - 5 M Urea requires a 24 hour incubation
 - 1% Monochloroacetate requires 1 hour incubation (used at UAH)
 - The tube is shaken and assessed for clot lysis/dissolution after 24 (or 1) hour(s).

- Interpretation:

- Normal results will be verified by the special coagulation technologists.
- Abnormal results require interpretation by a hematopathologist.
- Some patients develop significant bleeding with levels of 0.05-0.4 U/ml.
- Comments:
 - Mild reduction in factor XIII activity. Mild reductions are most commonly acquired due to liver disease, acute illness, consumption, or, less commonly, an acquired factor XIII inhibitor. Mild reductions may also be inherited in carrier states or severe deficiencies on replacement therapy. Clinical correlation is required.
- I would recommend reviewing the patient context and only including the comment about an acquired inhibitor if the scenario is *not* suggestive of another etiology. We do not have access to a test for FXIII inhibitor testing.

- Factor XIII Antigen Testing:

- ISTH SSC recommends factor XIII activity as the initial screening test with assessment of antigen levels as a follow-up for reduced activities.
- Factor FXIII-A antigen deficiency is the most common heritable deficiency and testing is available in Calgary; ELISA based with a lower limit of detection, which is of value in severe deficiency.
- All factor XIII activities <0.4 U/mL will be sent to Calgary for FXIII-A subunit quantification.

Inhibitor (Bethesda and Nijmegen) Assays

-EPIC Update: A numerical result must be entered for all Bethesda results.

- For clearly positive results, enter the appropriate numerical value, no comment necessary.
- For results in the equivocal range (between 0.42 and 1.0 BU/mL), it is likely most appropriate to enter the numerical result with the following chartable comment (**.BETHEQUIV**): Equivocal result. Inhibitor levels between 0.42 to 1.0 have poor specificity and may represent a false positive result. Correlation with the clinical circumstances, other laboratory results, and treatment response (if applicable) is required. A PTT inhibitor screen may be of value.
- For undetectable results, enter 0.00 as the result (otherwise it flags as an abnormal), and put the following chartable comment (**.BETHNEG**): No detectable inhibitor (<0.42 BU/mL)

- We have decided to cancel Bethesda Assays when the FVIII level is >0.20 U/ml (a somewhat arbitrarily chosen threshold for sake of consistency):

- CANBU = Inhibitors are effectively undetectable when the underlying factor level is greater than 0.20 U/ml.
- However, there are some patients where testing even above 0.20 U/ml may be appropriate. This is may be especially true in those with acquired hemophilia where inhibitor kinetics often produce a residual baseline of factor which can be substantial.

- Chromogenic FVIII Inhibitor Assay

- Performed identically to the clot based assay but using the Chromogenic FVIII assay.
- Done on patients who cannot be tested with a clot-based assay due to therapy (eg. emicizumab) because the Chromogenic Assay is insensitive to these therapies.
- Techs know to use the Chromogenic FVIII assay because the clinicians have ordered a Chromogenic FVIII Activity.
- Note: patients on [Emicizumab](#) often have normal PTT, despite having low (or even undetectable) FVIII levels.

- Basic principle of inhibitor quantitation and interpretation:

- In coagulation as in blood banking and elsewhere antibody titers are determined on the basis of dilutions.
- The difference in coagulation is that the endpoint is not a simple positive vs negative as it is in eg. blood banking (agglutination vs no agglutination) because factor activity is a continuous variable.

- Thus, in coagulation titers are generally defined as the dilution at which 50% of factor activity is inhibited.
- This definition can be applied to coagulation factor inhibitors (eg. FVIII Inhibitor), lupus anticoagulants (though we don't usually bother), vWF inhibitors etc.
- Ideally an inhibitor would be empirically quantitated by performing every possible dilution and determining the exact dilution at which 50% inhibition occurs, but this is impractical:
 - Quantitation is usually done with doubling dilutions (eg. 1:1, 1:2, 1:4, 1:8, 1:16, etc.) followed by extrapolation of the 50% point of inhibition.
 - eg. you find that you have 40% factor inhibition at a 1:4 dilution and 60% factor inhibition at a 1:8 dilution
 - You could make dilutions of 1:5, 1:6 and 1:7 and repeat factor quantitation, but it's much easier to just average the two for an answer of 1:6 dilutions or 6 BU/ml.
 - However, this begs the question of which dilutions you should use – all of them or only some? Expert consensus has been that you can only use points that fall within the 25% to 75% range of factor inhibition. {Verbruggen, et al. Thromb. Haemost. 73:247-251, 1995}. While the 25% and 75% limits are somewhat arbitrary they are necessary as we know that at extremes linearity is lost.
 - Another benefit of this rule is that we can quantitate inhibitors below 1 U/ml – if you could only measure inhibitor titer by directly demonstrating 50% inhibition, the Limit of Detection would be 1 U/ml.
 - Using the 25/75 rule the Limit of Detection becomes 0.42 U/ml. This is simply due to the fact that at the lowest possible dilution of 1:1 the inhibitor must reduce factor activity by at least 25% in order to get into the range of quantitation.
 - Theoretically, dilutions lower than 1:1 (eg. 1:0.5, 1:0.25, etc.) could be performed but then you run into other major analytic problems ie. variations in the levels of the other clotting factors that cause factitious results.
 - A few important notes about the 0.42 U/ml analytic limit:
 - The limit applies to any test where the lowest allowable dilution is 1:1 but extrapolation is allowed in the 25%-75% inhibition range.
 - This limit therefore applies to both the Bethesda and Nijmegen assays.
 - This limit is analytic NOT clinical: it defines what we can measure not what matters.
 - A patient may have a inhibitor titer of <0.42 U/ml that causes clinical bleeding symptoms.
 - Conversely a normal control can have an inhibitor titer of 0.7 U/ml.
 - The clinical importance of an inhibitor will depend on the quality of the assay performed as well as the normal reference range.

- Assays:

- The Bethesda Assay (BU/ml):
 - 1 BU/ml is the dilution at which 50% of commercial (1 U/ml) factor activity is inhibited, as measured at 37 deg C with a 2 hour incubation.
 - Compared to a control of normal pooled plasma mixed 1:1 with Imidazole Buffer (pH=7.4).
- The Nijmegen Assay – pronounced nī'mā'gən (niymaygan)
 - 1 NU/ml is the dilution at which 50% of commercial (1 U/ml) factor activity is inhibited, as measured at 37 deg C, pH=7.4 and with a 2 hour incubation.
 - Differs from Bethesda Assay:
 - Uses buffered Normal Plasma (to pH=7.4 with 0.1 M imidazole) to mix with the patient sample.

- Uses the same buffered Normal Plasma to mix with FVIII immunodepleted Plasma for the control.
 - Has been shown to have greater specificity than the Bethesda assay when the inhibitor is at low levels {Verbruggen, *et al. Thromb. Haemost.* 73:247-251, 1995}:
 - The Bethesda assay may give false positives for inhibitors up to 1.0 BU/ml – apparently due to increases in pH that occur after 2 hours of incubation, which impairs subsequent FVIII:C activity.
 - The Nijmegen assay correctly identified all true negatives as 0.0 NU/ml.
 - The UAH FVIII Inhibitor assay:
 - Our assay essentially works like a standard Bethesda Assay:
 - A buffered Normal Pooled Plasma (from Precision Biologic) is used – but it doesn't seem to have enough buffering capacity:
 - We have tested pH and FVIII stability with 2 hours incubation and have shown that this buffering capacity is not adequate.
 - Another potential contributor to the problem is related to the nature of the immunodepletion process. This can also result in residual immunodepletory activity remaining in the plasma which can cause mild apparent inhibitor effects (typically also only seen in the 1:1 mix with incubation) in the absence of pH perturbations.
 - We have looked into other sources of Buffered Normal Pooled Plasma and it is just not available in North America.
 - We've considered making our own Buffered Normal Pooled Plasma but this is impractical because:
 - If we make our own from donors routinely, standards necessitate that we measure ALL the clotting factor levels with each lot that we make.
 - We can obtain unbuffered commercial Normal Pooled Plasma but then this necessitates thawing the plasma at receipt, buffering and re-freezing which compromises product quality and stability.
 - Buffered saline (rather than FVIII Immunodepleted Plasma) is used for the control like in the Bethesda assay.
 - The authors of the Nijmegen assay assert that the importance of using Buffered Normal Pooled Plasma + FVIII Depleted Plasma as a control prevents FVIII degradation due to a lack of stabilizing proteins such as vWF that would otherwise be missing in simple buffered saline.
 - However, our laboratory routinely obtains control mix FVIII levels of 0.5 U/ml (ie. perfect yields) when mixing with buffered saline and we propose that mixing with FVIII Depleted Plasma is unnecessary.
 - Furthermore, our lab has tried using FVIII Depleted Plasma for the 1:1 mix only to find that the pH does rise with 2 hour incubation and FVIII levels are <0.5 U/ml – consistent with the Buffered Normal Pooled Plasma NOT having sufficient buffering activity.
- Our data suggest that the "Nijmegen Assay" performed in other centers around North America may not be very robust:
- The only major commercial source of Buffered Normal Pooled Plasma is from Precision Biologic – the same supplier we use that, that does not appear to have adequate buffering capacity.
 - This may be explained by the fact that Precision Biologic validates the pH and buffering capacity of the concentrated buffering solution that it adds to its plasma preparations but does NOT bother to measure the pH or buffering capacity with incubation after they've been mixed. We filed a complaint with the company on Oct. 1, 2010.
 - Many centers use the Precision Biologic Buffered Plasma suggesting that they should have the same lack of buffering that we observe, unless they make their own.

- However, (and I'm speculating here) they may be able to get away with it because the use of the FVIII immunodepleted plasma for control rather than buffered saline means they get the same degree of degradation in their denominator as in their numerator.
- Though there is no good evidence to support the practice of calculating the affect of residual FVIII (>0.05) in the patient plasma we do perform this as per standard practice {Verbruggen, *et al. Thromb. Haemost.* 73:247-251, 1995}.
- See "Inhibitor Titer Quant.xls" for excel spreadsheet.
- Sensitivity compared to PTT Inhibitor Screen:
 - The Bethesda assay is more sensitive for delayed specific factor inhibitors (Hemophilia A with Inhibitor) then the PTT Inhibitor Screen because the incubation is longer.
 - The PTT Inhibitor Screen isn't usually done with a 2 hour incubation because even the tubes held "separate" and mixed after incubation tend to prolong due to loss of labile factors.
 - After a specific patient incident in October of 2009 in which the PTT Inhibitor Screen was negative but the Bethesda assay demonstrated a 3 BU/ml Inhibitor the Lab has agreed to perform the Bethesda assay despite a negative PTT Inhibitor Screen in patients with Hemophilia A when requested by the Clinician.
- Specificity:
 - The analytic limit of the assay is 0.42 U/ml. Though this has nothing to do with specificity (see above for explanation), it does mean that any value <0.42 U/ml must be negative.
 - Based on our experience, levels between 0.42-1.0 U/ml have poor specificity – ie. they may represent false positives, due to factor degradation with incubation especially due to changes in pH (see discussion above).
 - Thus, I recommend reporting levels between 0.42-1.0 U/ml as either equivocal or negative, depending on clinical circumstances.
 - Ways to improve specificity that are being examined:
 - Create a "normal" reference range using Hemophilia patients "known" not to have an inhibitor.
 - Find a better Buffered Normal Pooled Plasma or work with Precision Biologic to improve their product.

HIT Assays

- **PLEASE NOTIFY BLOOD BANK OF ANY PATIENT THAT HAS CONFIRMED HIT:** Contact blood bank and ask to have the patient's Wellsky registry file updated (780-407-8802) with the following comment:
 - **BBHIT** = HIT positive. Contact pathologist before issuing any clotting factor concentrates, PCC or FEIBA.
- Ticagrelor (and Cangrelor):
 - 2024-08-27: Newly published data clearly shows that reversible P2Y12 inhibitors causes false negatives in functional HIT assays. ELISA based assays are unaffected. A comment to this effect will be appended to all **negative** Lumi-aggregometry results.
 - Investigators cannot exclude that prasugrel and clopidogrel (irreversible P2Y12 inhibitors) also show some inhibition at peak plasma concentrations, but this is thought to be less of an issue.
 - Inhibition of functional assays can be ameliorated by {2020 Greinacher} the following; though none of this has been attempted at the UAH:
 - Isolating the IgG fraction from the patient serum
 - Pre-incubating the serum with the ticagrelor antidote (PB2452)
 - Pre-incubating the serum with 20 mg of charcoal powder.
 - Cangrelor, has a very short half-life which can be overcome by stopping the drug up to 30 minutes before the blood sample for HIT testing is obtained.
- The SRA is only orderable in Epic (this is the outcome of provincial standardization). The process for handling recollections where an SRA may be indicated (see subsequent discussion for scenarios that may require an SRA):
 - Upon recommendation by Hemepath, the Special Coag technologist should be asked to place the order into Epic for BOTH an SRA and a HIT Assay (ie local lumi test).
 - Pathologist should preferably leave a message at 407-8487. The answering machine is checked daily.

- If a Secure Chat is used instead then the pathologist must ensure the tech receives it and follows up. This may not happen at all if they have left for the day and are not on working the next few days.
 - Pathologist to call the clinician and let them know lab will place the order for SRA and repeat HIT.
 - If it's after hours then the lab tech won't place the order until the next morning.
 - The clinician must then signs the orders if they approve. This is necessary to ensure results are sent to them.
 - **Clinicians must NOT place the HIT order themselves** as the conflict may lead to incorrect tubes being collected.
 - A repeat HIT should always be requested at the same time for correlative purposes.
- The tubes required for SRA plus HIT are:
- Two red tops (serum)
 - One blue top (Na citrate) tube
 - One gold top (SST gel no additive).
- Resulting of Lumi-Aggregometry Results is done in Beaker, with the former HITP, HITNE, and "see interpretation" results available as options. Additional comments for equivocal results will have to be entered as a comment for the time being.

- If ELISA is NEGATIVE (Lumi-aggregometry not performed):
 - **IgG HIT by ELISA:** Negative + auto-appended HITN1-HITN2 = "All HIT assays are subject to a small but significant risk of falsely negative results if the pre-test probability is low at the time of testing. This can occur if the assay is performed before the HIT Antibody titer is sufficiently high enough to produce the typical clinical features of HIT. To avoid false negatives all clinicians are urged to withhold HIT testing until the patient's pretest probability is "intermediate" or "high" as measured by the 4T score developed specifically for this purpose (Lo *et al. J.Thromb.Haemost.* 2006, 4:759-765)."
 - HIT by Lumi-Aggregometry: hidden
 - Interpretation: hidden
- If ELISA is NEGATIVE and Lumi-aggregometry is EQUIVOCAL:
 - IgG HIT by ELISA: Negative.
 - HIT by Lumi-Aggregometry: "See Interpretation"
 - **Interpretation:** Negative for immune mediated HIT. Lumi-aggregometry is equivocal but ELISA is negative. Though ELISA is not specific for HIT it is very sensitive.
- If Lumi-aggregometry is POSITIVE (regardless of ELISA results):
 - **IgG HIT by ELISA:** Negative, Equivocal, Positive or not reported.
 - HIT by Lumi-Aggregometry: Positive
 - **Interpretation:** HITP = "Positive for immune mediated HIT. This diagnosis necessitates immediate discontinuation of all sources of heparin, both unfractionated and low molecular weight formulations, including line flushes. HIT generally requires immediate treatment with non-heparin parenteral anticoagulation by a physician familiar with the management of this disorder. Patients should be advised to avoid all heparin contact in the future, unless under the close supervision of a physician aware of the previous diagnosis of HIT. The use of a Medic-Alert bracelet is recommended."
- If ELISA is EQUIVOCAL and Lumi-aggregometry is NEGATIVE:
 - IgG HIT by ELISA: Equivocal.
 - HIT by Lumi-Aggregometry: "See Interpretation"
 - **Interpretation:** HITNE = "Negative for immune mediated Heparin Induced Thrombocytopenia (HIT). The ELISA based IgG assay is only a screening test for HIT and confirmatory testing by Lumi-aggregometry is negative."
- If ELISA is EQUIVOCAL or POSITIVE and Lumi-aggregometry is EQUIVOCAL:
 - **IgG HIT by ELISA:** Equivocal or Positive.
 - HIT by Lumi-Aggregometry: "See Interpretation"
 - **Interpretation:** "Lumi-aggregometry testing is Equivocal. Immune-mediated HIT is neither ruled-out nor confirmed. Recollection for Serotonin Release Assay (SRA) testing

and repeat local HIT assay testing is required (as of July 2018 the SRA can no longer be performed on the original specimen). The SRA is the Gold Standard test but because it is performed externally, it takes approximately a week. Results were discussed with....

- If ELISA is POSITIVE and Lumi-aggregometry is NEGATIVE:
 - IgG HIT by ELISA: Positive
 - HIT by Lumi-Aggregometry: "See Interpretation"
 - **Interpretation:** HITNE = "Negative for immune mediated Heparin Induced Thrombocytopenia (HIT). The ELISA based IgG assay is only a screening test for HIT and confirmatory testing by Lumi-aggregometry is negative."
 - Note: If ELISA OD is >2.0 request repeat HIT assay and SRA for confirmation. It is not generally necessary to append a comment stating that SRA was sent for confirmation. However, if the pathologist feels that the risk is very high (eg. very high OD, very high 4T score or very anxious clinician) and wants the clinician to be aware that additional testing is pending, the following alternate interpretation may be used:
 - **Interpretation:** Over-all equivocal results. Lumi-aggregometry testing is negative, but ELISA based testing is strongly positive. Though ELISA is usually used as a screening test and Lumi-aggregometry as the definitive confirmatory test, this patient's ELISA result is so strongly positive that it casts doubt on the Lumi-aggregometry result. Immune-mediated HIT is neither ruled-out nor confirmed. Recollection for Serotonin Release Assay (SRA) and repeat local HIT assay testing is strongly recommended (as of July 2018 the SRA can no longer be performed on the original specimen). The SRA is the Gold Standard test but because it is performed externally, it takes approximately a week. Results discussed with...
- If the SRA is POSITIVE then the report is modified as:
 - A copy of the results should be given to Amy Bajer as part of ongoing Proficiency Testing.
 - If previous Interpretation was reported as equivocal and SRA is positive then add an addendum to the interpretation:
 - ADDENDUM ISSUED DD/MMM AT HH:MM: The Serotonin Release Assay (SRA) which is the Gold Standard test for Heparin Induced Thrombocytopenia is reported as POSITIVE by the reference laboratory. The SRA result supersedes all other results. The full SRA report has been forwarded to the ordering Physician. Testing was performed at the Platelet Immunology Laboratory, McMaster University, 1280 Main Street W. HSC 3H42, Hamilton, ON L8S 4K1.
- If previous Interpretation was reported as negative but SRA is positive then it should be amended:
 - **Interpretation:** The Serotonin Release Assay (SRA) which is the Gold Standard test for Heparin Induced Thrombocytopenia is reported as POSITIVE by the reference laboratory. The SRA result supersedes all other results. The full SRA report has been forwarded to the ordering Physician. Testing was performed at the Platelet Immunology Laboratory, McMaster University, 1280 Main Street W. HSC 3H42, Hamilton, ON L8S 4K1.
CORRECTED ON DD/MMM AT hhmm. PREVIOUSLY REPORTED AS: Negative for immune mediated Heparin Induced Thrombocytopenia (HIT). The ELISA based IgG assay is only a screening test for HIT and confirmatory testing by Lumi-aggregometry is negative.
- If the SRA is NEGATIVE:
 - No need to change interpretation if the original report did not mention the SRA would be performed. A copy of the results should be given to Amy Bajer as part of ongoing Proficiency Testing.
 - If the original interpretation did mention that an SRA would be sent than an addendum should be appended to the interpretation:

- ADDENDUM ISSUED DD/MMM AT hhmm: The Serotonin Release Assay (SRA) which is the Gold Standard test for Heparin Induced Thrombocytopenia is reported as NEGATIVE by the reference laboratory. The full SRA report has been forwarded to the ordering Physician. Testing was performed at the Platelet Immunology Laboratory, McMaster University, 1280 Main Street W. HSC 3H42, Hamilton, ON L8S 4K1.

- An IMPORTANT note on HIT assay sensitivity:

- Sensitivity (and specificity) is always determined relative to a Gold Standard, which in the case of HIT is the Serotonin Release Assay (SRA). As a consequence the SRA is presumed to be 100% sensitive.
- This 100% sensitivity of the SRA is a phenomenon of the methodology of Evidence-Based Medicine and is NOT reflective of its true sensitivity.
- Similarly the sensitivity of all other HIT assays are relative to the SRA and not the true incidence of HIT – thus the ELISA sensitivity of 100% means it is approximately the same as the SRA, NOT that it will give a positive result in all cases of HIT.
- Though no one knows the true sensitivity rates, expert estimates are on the order of 97% for the SRA.
- An important determinant of the true sensitivity of a HIT assay is how long after starting heparin the test is performed:
 - If you diagnose HIT on a patient at 5 days after the start of Heparin but retrieve an old sample from just 1 day after the start of Heparin, chances are that sample will give a negative result by any HIT assay – simply because the antibody titer has not yet reached a detectable level.
 - Generally, it is believed that once the antibody titer is high enough to produce clinical manifestations such as a platelet count drop or thrombosis then it should give a positive result by HIT assay.
 - However, one can conceive of numerous situations in which an apparent clinical sign of HIT triggers an assay whose result is falsely negative because it was run too early. For Example:
 - Patient X develops a low platelet count on day 5 of heparin therapy for an unrelated reason (eg. bleeding, surgery, myelosuppression, etc.)
 - A HIT assay is performed and the result is negative.
 - At day 10 there is a further drop in platelets due to True HIT antibody formation.
 - The concern is that the HIT assay will not be repeated because it has already been performed.
- Lumi-aggregometry's exact sensitivity is not precisely known because there aren't many labs that perform the test:
 - The UAH has been performing Lumi-aggregometry for HIT since at least 1995, when local experts published their results on the method (*Br.J.Haematol.* 91:173-177).
 - Sensitivity was always presumed to be equivalent to the SRA based on the published data as well as many years of local experience.
 - SRA testing was sporadically requested over these years in select cases and there was never a discordant result with Lumi-aggregometry until May 2012.
 - In May 2012 a patient with very high ELISA OD=2.7 gave a negative result by Lumi-aggregometry but a relatively strong positive result by SRA. Repeated subsequent collections on this patient also failed to produce a positive Lumi-aggregometry result with 2/3 platelet donors (all 3 were from our lab's pedigree of reactive donors) – though one donor did give a weakly positive result with all patient sera tested. Mixing the reactive donor's platelets with other donor's platelets resulted in loss of positivity; precluding the use of a mixed platelet donor pool. We are quite certain the patient was truly immune HIT because she was erroneously re-exposed to Heparin and rapidly developed a 50% drop in her platelet count; fortunately she did not appear to develop thrombosis.

- From Mar, 2012 to June, 2013, the Lab sent all ELISA positive (but not equivocal), Lumi-aggregometry negative results for confirmatory SRA testing. There were 28 patients (including our index case above), 3 of which were positive by SRA ie. discordant. In all of these cases the ELISA OD was >2.0. Therefore, since that time we will continue to send out for SRA on all samples that are Lumi-aggregometry negative but give an ELISA OD>2.0.
- Avoiding HIT false negative results:
 - Only perform the HIT assay on patients with adequate pre-test probability (HIT 4T score of >3).
 - Repeat HIT assay particularly if there is a change in the patient's status (especially if it changes the HIT score).
 - Do NOT rely on a negative HIT assay in patient's who have a very high pre-test probability (6-8/8). These patients should have alternate testing assays and/or repeat testing – WHILE BEING ALTERNATIVELY ANTICOAGULATED.
- ELISA {Bakchoul *et al. JTH. 7:1260-1265, 2009*}:
 - ELISA-GAM kit (detects IgG, IgA and IgM) against PF4/Heparin or PF4/Polyvinylsulfonate (the latter mimics Heparin) complexes:
 - The ELISA-GAM assay is very sensitive (almost 100%), but not perfect as a very rare HIT Antibody is due to Anti-IL8/Heparin complexes.
 - The ELISA-GAM is less specific for HIT (~80%) than functional assays because the assay detects antibody binding, but says nothing about whether it can activate the platelet.
 - ELISA-IgG kit against PF4/Heparin or PF4/Polyvinylsulfonate (the latter mimics Heparin) complexes:
 - There is growing evidence that only IgG HIT antibodies have the ability to activate platelets.
 - One study has shown that IgG-ELISA has better specificity than ELISA-GAM (90%) while retaining comparable (~100%) sensitivity {Bakchoul *et al. JTH. 7:1260-1265, 2009*}.
- Lumi-Aggregometry
 - Effective October 28, 2019, new lumi-aggregometry instruments have been implemented:
 - HIT is suggested when the ratio of amplitudes of the luminol channel:
 - With 0.5 U/ml UFH >5
 - With 0.1 U/mL UFH >4
 - With LMWH >5
 - Request recollection for SRA and repeat local HIT testing if:
 - Lumi is equivocal
 - Lumi is negative and ELISA OD >2.0, or high clinical suspicion
 - Excellent specificity with a positive result but it is difficult to perform and frequently gives un-interpretable results.
 - Requires washed donor platelets.
 - Sample is no longer Hepzorb prior to testing and a very specific amount of Heparin is added in vitro as the final concentration of Heparin is a crucial parameter.
 - We can use any type of Heparin (Enoxaparin, Dalteparin, Tinzaparin, UFH) in our assay – so we need to know the type the patient was on:
 - If the patient is on a UFH then we run the assay with High Dose and Low Dose UFH, only.
 - If the patient is on LMWH then we run the assay with High Dose and Low Dose UFH, as well as the specific LMWH the patient was on.
 - When we do not know the type of Heparin the Patient is on:
 - We will only test these samples against UFH and assume that it should contain all the other subtypes of Heparin.

- However, we have had one case here where a patient tested NEG by UFH, but POS with LMWH.
- Thus, all negative results where the Heparin type is unknown will receive the following comment: "Heparin type not specified on Requisition. The HIT assay was performed using Unfractionated Heparin (UFH). Please note that on rare occasion some patients treated with Low Molecular Weight Heparin may give falsely negative results under these circumstances."
- UAH HIT assay Algorithm effective July 19, 2016:
 - Routine HIT assays will be performed on Mon., Wed., Fri. and on a STAT basis (the latter with pathologist approval). Note that on Monday samples must be received by 10:30 am in the lab to make it on that day's run.
 - ELISA-IgG assay will be performed routinely:
 - OD=0.4-1.0 is Equivocal (mfg recommends anything >0.4 as positive)
 - OD>1.0 is Positive
 - Lumi-aggregometry will be performed in the case of Equivocal or Positive ELISA-IgG assay:
 - This assay can be done instead of the ELISA-IgG on a STAT basis if the Pathologist requests it.
 - SRA will be sent out in the case of a Positive ELISA but Negative Lumi-aggregometry
- Positive HIT assays require pathologist intervention:
 - Consult the ordering Physician.
 - Determine the likelihood of HIT using the 4T's score.
 - If the patient is on ANY heparin ensure they are stopped. Sources of Heparin include:
 - IV or Subcutaneous Therapeutic or Prophylactic doses.
 - Heparin in lines: flushes or locked lines.
 - TPN – the lipid component contains heparin.
 - Blood Factor Concentrates – such as Prothrombin Complex Concentrates (eg. Octaplex) as well as others. Note: PRBCs, Platelets, Plasma and Cryoppt do not contain Heparin.
 - Recommend alternate IV anticoagulation managed by a Physician experienced with HIT – preferably a Hematologist.
 - For any patient with a positive HIT, confirmed by Lumi-aggregometry, please request a repeat HIT assay:
 - We will not perform the assay but credit it instead.
 - We will use the patient's plasma as a positive control in future testing.
 - Call Blood Bank and ask them to add the BBHIT comment to the patient's BAD file.
- Likelihood of HIT using the Hamilton 4T's Score {*Chest* 2012; 141(2)(Suppl): e495S-e530S}:
 - Note: The day Heparin is first started is Day=0

	Score = 2	Score = 1	Score = 0
Thrombocytopenia Compare the highest platelet count within the sequence of declining platelet counts with the lowest count to determine the % of platelet fall. (Select only 1 option)	☐ > 50% platelet fall AND nadir of ≥ 20 AND no surgery within preceding 3 days	☐ > 50% platelet fall BUT surgery within preceding 3 days OR ☐ any combination of platelet fall and nadir that does not fit criteria for Score 2 or Score 0 (eg, 30-50% platelet fall or nadir 10-19)	☐ < 30% platelet fall ☐ any platelet fall with nadir < 10
Timing (of platelet count fall or thrombosis*) Day 0 = first day of most recent heparin exposure (Select only 1 option)	☐ platelet fall day 5-10 after start of heparin ☐ platelet fall within 1 day of start of heparin AND exposure to heparin within past 5-30 days	☐ consistent with platelet fall days 5-10 but not clear (eg, missing counts) ☐ platelet fall within 1 day of start of heparin AND exposure to heparin in past 31-100 days ☐ platelet fall after day 10	☐ platelet fall $\leq$ day 4 without exposure to heparin in past 100 days
Thrombosis (or other clinical sequelae) (Select only 1 option)	☐ confirmed new thrombosis (venous or arterial) ☐ skin necrosis at injection site ☐ anaphylactoid reaction to IV heparin bolus ☐ adrenal hemorrhage	☐ recurrent venous thrombosis in a patient receiving therapeutic anticoagulants ☐ suspected thrombosis (awaiting confirmation with imaging) ☐ erythematous skin lesions at heparin injection sites	☐ thrombosis suspected
Other cause for Thrombocytopenia** (Select only 1 option)	☐ no alternative explanation for platelet fall is evident	Possible other cause is evident: ☐ sepsis without proven microbial source ☐ thrombocytopenia associated with initiation of ventilator ☐ other	Probable other cause present: ☐ within 72 h of surgery ☐ confirmed bacteremia/fungemia ☐ chemotherapy or radiation within past 20 days ☐ DIC due to non-HIT cause ☐ posttransfusion purpura (PTP) ☐ platelet count < 20 AND given a drug implicated in causing D-ITP (see list) ☐ non-necrotizing skin lesions at LMWH injection site (presumes DTH) ☐ other
Drugs implicated in drug-induced immune thrombocytopenia (D-ITP) Relatively Common: glycoprotein IIb/IIIa antagonists (abciximab, eptifibatide, tirofiban); quinine, quinidine, sulfa antibiotics, carbamazepine, vancomycin Less Common: actinomycin, amitriptyline, amoxicillin/piperacillin/nafcillin, cephalosporins (cefazolin, ceftazidime, ceftriaxone), celecoxib, ciprofloxacin, esomeprazole, fexofenadine, fentanyl, fucidic acid, furosemide, gold salts, levofloxacin, metronidazole, naproxen, oxaliplatin, phenytoin, propranolol, propoxyphene, ranitidine, rifampin, suramin, trimethoprim. Note: This is a partial list.			

FIGURE 1. 4Ts score. *Timing of clinical sequelae, such as thrombocytopenia, thrombosis, or skin lesions. **Two points if necrotizing heparin-induced skin lesions even if thrombocytopenia not present. (Modified with permission from Warkentin and Linkins.⁵⁹)

Fig. 1. DTH=Delayed Type Hypersensitivity.

- Note: there is a related Greifswald 4T's (German) score in existence with slightly different criteria, but the Hamilton version has better Positive Predictive Value.

- High Clinical Risk
 - 4T Score=6-8
 - Must D/C heparin and start alternate anti-coagulant while testing.
- Intermediate Clinical Risk
 - 4T Score=4-5
 - D/C heparin while testing
 - As long as there is no thrombosis (either before or during heparin therapy) and the patient does not require anticoagulation for another reason (such as post-surgical) then it is not necessary to start alternate anticoagulation while awaiting results of the HIT assay.
- Low Clinical Risk
 - 4T Scores ≤ 3
 - Lo *et al.* suggest that patients who fall into the Low Clinical Risk category do not require HIT testing, and propose that a positive result (especially if obtained by ELISA) may lead to unnecessary anticoagulation causing more harm than good. A large prospective study is needed.

- The 2008 Chest Guidelines for HIT assay recommendations {Warkentin *et al.* *Chest*, 133:340S-380S, 2008}:

- “In patients who receive heparin, or in whom heparin treatment is planned (eg. for cardiac or vascular surgery), we recommend against routine HIT antibody testing in the absence of thrombocytopenia, thrombosis, heparin-induced skin lesions, or other signs pointing to a potential diagnosis of HIT (Grade 1C).”
 - “For patients who are receiving heparin or have received heparin within the previous 2 weeks, we recommend investigating for a diagnosis of HIT if the platelet count falls by $>$ or $=$ 50%, and/or a thrombotic event occurs, between days 5 and 14 (inclusive) following initiation of heparin, even if the patient is no longer receiving heparin therapy when thrombosis or thrombocytopenia has occurred (Grade 1C).”
 - “For postoperative cardiac surgery patients, we recommend investigating for HIT antibodies if the platelet count falls by $>$ or $=$ 50%, and/or a thrombotic event occurs, between postoperative days 5 and 14 (inclusive; day of cardiac surgery=day 0) [Grade 1C].”
- Guidelines for Heparin Re-Exposure in Patients with a previous History of HIT; usually in the context of Cardiac procedures or requiring Cardio-Pulmonary Bypass. From The 2009 American Society of Hematology Quick Reference Guide on HIT (available for free at www.hematology.org/practice):

A. Cardiac and vascular surgery

- HIT laboratory testing should be used to determine the safety of exposing a patient with a history of HIT to intraoperative heparin:

Clinical picture	Laboratory profile		Recommended intraoperative anticoagulation ^{1,2}
	Immunologic assay	Functional assay	
Remote HIT	Negative	Negative	1. Use UFH (Grade 1B)
Subacute HIT	Positive	Negative	1. Delay surgery, if possible, until immunologic assay becomes negative (Grade 1B) 2. If surgery cannot be delayed, use UFH (Grade 2C)
Acute HIT	Positive	Positive	1. Delay surgery, if possible, until functional and immunologic assays become negative (Grade 1B) 2. If surgery cannot be delayed, use bivalirudin (Grade 1B)

¹If pre- or post-operative anticoagulation is indicated, a non-heparin anticoagulant should be used. ²American College of Chest Physicians Grading System: 1=strong recommendation; 2=weak recommendation; A=based on high quality evidence; B=based on moderate quality evidence; C=based on low quality evidence UFH, unfractionated heparin.

B. Cardiac catheterization/percutaneous coronary intervention

Clinical picture	Laboratory profile		Recommended intraprocedural anticoagulation ¹
	Immunologic assay	Functional assay	
Remote HIT	Negative	Negative	1. Use a non-heparin anticoagulant [bivalirudin (Grade 1B), argatroban (Grade 1C), lepirudin (Grade 1C), or danaparoid (Grade 1C)] 2. If a non-heparin anticoagulant is not available, use UFH
Subacute HIT	Positive	Negative	1. Use a non-heparin anticoagulant [bivalirudin (Grade 1B), argatroban (Grade 1C), lepirudin (Grade 1C), or danaparoid (Grade 1C)]
Acute HIT	Positive	Positive	1. Use a non-heparin anticoagulant [bivalirudin (Grade 1B), argatroban (Grade 1C), lepirudin (Grade 1C), or danaparoid (Grade 1C)]

¹American College of Chest Physicians Grading System: 1=strong recommendation; 2=weak recommendation; A=based on high quality evidence; B=based on moderate quality evidence; C=based on low quality evidence.

- Repeated HIT testing {Warkentin *et al. J. Thromb. Haemost.* 2011; 9: 2498-2500}

- “A negative result of a HIT antibody test performed because of thrombocytopenia or thrombosis generally rules out HIT.”
- “Routine repeat testing a few days later is not indicated unless a new platelet count decline or thrombosis occurs.”

Platelet Light Transmittance Aggregometry (LTA)

- SmartPhrases (aka dot phrases) for Epic entry (Use **.PLAGGINTERPS** smartphrase to put in a list of all of the options, and delete what you do not need):

- **NOR** = Normal
- **PRP** = This assay is validated for Platelet Rich Plasma (PRP) containing 200-600 x 10⁹/L Platelets, but the patient's PRP contains only _____ x 10⁹/L. The results are reported with reference to published data on reduced platelet count samples (Hayward *et al. Thromb. Haemost.* 100: 134-145, 2008), which is not ideal because of variations in methodology and instrumentation. Interpret with caution.
- **21G** = Needles that are 21 Gauge or less should be used to collect samples for Platelet Aggregation studies. However, the current sample was collected using a _____ Gauge needle; results should be interpreted with caution.
- **VWD** = Results are consistent with von Willebrand Disease (vWD), and there is no evidence of a Type 2b or pseudo-VWD defect.
- **SINGL** = The occurrence of a single agonist abnormality at low concentration, on Light Transmission Aggregometry is a non-specific finding. In a Canadian study, Hayward *et al. (J.Thromb.Haemost.* 7: 676-684, 2009) found that there was no statistically significant increase in a patient's odds of having a platelet function defect under these circumstances.
- **2AGG** = The finding of abnormal aggregation with two (or more) agonists suggests that a platelet function disorder is present. However, the pattern of abnormalities seen is not characteristic of a specific disease entity.
- **BOTHC** = The abnormal aggregation seen in this sample occurs at both high and low concentrations of an agonist. This finding is more likely to represent a true platelet function disorder than abnormal aggregation at a single concentration (Mezzano *et al. Semin. Thromb. Hemost.* 35: 242-254, 2009). Clinical correlation with the bleeding history is essential for diagnosis of a platelet function disorder.
- **ABSSH** = The only detectable abnormality on Light Transmission Aggregometry is the absence of an initial platelet shape change, with all agonists where such a shape change would be expected. The clinical significance of this finding is uncertain.
- **ASP** = Aspirin-like defect, which is usually drug induced, but can be inherited. Drug history should be reviewed and repeat testing when the subject is not taking aspirin or other nonsteroidal anti-inflammatory drugs is recommended (*Am.J.Clin.Pathol.* 134: 955-963, 2010).
- **GLANZ** = Consistent with Glanzmann Thrombasthenia (inherited or acquired). Further testing of Fibrin Receptors (GpIIb/IIIa) is recommended.
- **BSS** = Consistent with Bernard Soulier Syndrome (inherited or acquired). If von Willebrand Factor deficiency has been excluded, then additional studies of the platelet von Willebrand Factor receptor (GpIb-IX-V) are recommended.
- **ADP** = Aggregation is abnormal with all concentrations of ADP, and with significant deaggregation. The possibility of a platelet ADP receptor defect (P2Y₁₂) should be considered. A drug-induced defect should be excluded as the cause.

- **NOD** = The findings are non-diagnostic for a platelet disorder. However, they may provide supportive evidence of a platelet defect in the context of a persuasive bleeding history if clotting factor deficiency, von Willebrand Disease and anatomic abnormalities are excluded.
- **XBS** = The findings exclude Bernard-Soulier Syndrome and Glanzmann Thrombasthenia. However, the sensitivity of aggregation studies for other platelet function disorders is limited and poorly defined. Thus, normal results do not exclude a platelet function disorder.
- **RPTM** = Repeat platelet aggregation studies (*Am.J.Clin.Pathol.* 134: 955-963, 2010) accompanied by electron microscopy may be of value.
- **EMRS** = The results of electron microscopy are reported separately. In Netcare see: "Pathology\UAH Surgical Routine".
- **INTRP** = Interpret with caution: the patient reports taking _____ which has/have been shown to interfere in testing in some laboratories (Kottke-Marchant *et al. Arch.Path.Lab.Med.* 2002, 126: 133-146).
- **NOINT** = Of note, the patient reports taking _____ but this/these are not known to interfere with testing.

- Booking Process (as of Sep. 22, 2011):

- LTA performed Thursdays
- Routine booking of 2 patients per day that LTA is performed
- A third (or fourth) patient can be booked with pathologist consultation, as long as no patient to be run that day is macrothrombocytopenic and therefore requires a modified PRP preparation.

- Assay process (as of Mar. 24, 2010):

- As of Mar. 24, 2011: PRP will no longer be diluted to $250 \times 10^9/L$ but instead will be run at its native Platelet count.
 - However, the assay is only validated for PRP with platelets of $200-600 \times 10^9/L$
 - If the PRP is outside this range then it must be noted in the interpretation section.
 - For example: "This assay is validated for Platelet Rich Plasma (PRP) containing $200-600 \times 10^9/L$ Platelets. The patient's PRP sample contains _____ $\times 10^9/L$; results should be interpreted with caution."
- As of Mar. 24, 2011: Agonists will be run progressively:
 - 5 ug/ml Collagen will only be run if 2 ug/ml Collagen response is reduced
 - 50 uM Adrenaline will only be run if 5 uM Adrenaline response is decreased.
 - 10 uM ADP will only be run if 2 uM ADP response is reduced.
 - U46619 will only be run if the Arachidonic Acid response is reduced.
 - 2 ug/ml Ristocetin will only be run if 1.2 ug/ml Ristocetin response is reduced (note: 0.5 ug/ml Ristocetin is always run).
 - TRAP is run on all samples but will not be reported routinely until we have had more of a chance to evaluate.
- As of Mar. 24, 2011: Normal Reference Ranges for each agonist are available with regard to Maximal Aggregation and Slope – both should be used to determine whether the result is normal or not.
- As of Sep. 2011: for macrothrombocytopenic patients:
 - Large/Giant platelets tend to get removed by the centrifugation process necessary for removal of RBCs and WBCs.
 - Lowering centrifugation to 80g does not remove large platelets, but nor does it effectively remove RBCs.
 - Allowing whole blood to sit for 2 hours on the bench works reasonably well to settle out RBCs but is not very effective for removal of WBCs.
 - Residual RBCs or WBCs in PRP interfere with light transmittance and cause factitiously reduced responses {Dacie and Lewis Practical Haematology. 10th Edn. P. 432}.

- A compromise can be achieved by allowing platelets to settle for 2 hours followed by a low speed (80g) spin; this can be used for testing if the following conditions are met:
 - A normal control is prepared similarly.
 - PRP count for WBCs and RBCs is negligible
- Sample variables potentially affecting Analysis:
 - If underfilled samples are assayed then only severe bleeding disorders (GT and BSS) can be excluded – this should be noted on the final report.
 - Collection {*Brit.J.Haematol.* 2011, 155:30-44}:
 - 21G or larger (ie. smaller Gauge) needles should be used.
 - Butterfly needles are acceptable.
 - The first 3-5 ml of blood should not be used for LTA.
 - Photo-optical detection can be impaired by hemolysis, hyperbilirubinemia or lipemia – though this is usually compensated by the use of a PPP from the same patient.
 - Grossly Hemolyzed samples will contain high levels of ADP/ATP (from RBCs) and must be discarded.
 - Mildly lipemic or icteric samples can be used but this should be noted in the final report.
 - Platelets cannot be cooled below RT at any point, and must be run within 4 hours of collection (otherwise should discard or at least comment).
 - Platelet counts:
 - Studies indicate that PRP platelet counts of $200-600 \times 10^9/L$ do not require adjustment, and that adjustment actually increases the CV of measured parameters {*Brit.J.Haematol.* 2011, 155:30-44}.
 - Lower platelet counts can also be used but this should be commented in the final report.
 - Duration of monitoring according to ISTH criteria:
 - At least 3 min after agonist addition (some say 5 min)
 - At least 5 min after agonist addition that does not cause maximal aggregation after 3 min for most control samples.
 - At least 10 min after agonist addition that does not cause maximal aggregation after 5 min for most control samples.
- A note on Lumi-aggregometry
 - A modified LTA technique that simultaneously measures ADP/ATP release using Luciferin/Luciferase (from Fireflies) Bioluminescence.
 - Because the bioluminescence is in the visible range of light, aggregation measurements are made in the infrared range.
- Interpretation of LTA
 - Transmittance is on the Left Axis; Absorbance is on the Right Axis
 - Aggregation before agonists are added may be an indication of a hypercoagulable state.
 - Initially you may see a slight decrease (step like drop) in absorbance due to dilution of platelets because of agonist addition. This is factored into the reference ranges and there is no need to correct for it.
 - Then there is usually a slight increase (smooth curve increase) in absorbance due to the platelet shape change; only seen with (in our assay):
 - Collagen gives the best responses
 - ADP usually gives mild responses
 - Followed by the primary aggregation wave, which is reversible.
 - A brief plateau may be seen as the release reaction is occurring
 - This is followed by the secondary release reaction (under study conditions it is ADP release that is mainly necessary for secondary aggregation – Apyrase will prevent it), which is irreversible.
 - Computer measured Slope and Maximal Aggregation
 - Slope is measured as the region of the curve that demonstrates the largest decrease in absorbance over 17 seconds, with the result expressed as %/minute. Technologists manually adjust the “start point” after the sudden step-down caused by agonist addition, in order to prevent interference from this artifact.

- Maximal aggregation is calculated as the lowest point reached, not as the end-point. Therefore, maximal aggregation should be valid even if there is disaggregation.
- Agonists {Cattaneo, M. *Semin. Thromb. Hemost.* 35:158-167}:
 - Stimulate primary (reversible) aggregation which induces TxA2 synthesis due to the physical closeness of the platelets, followed by δ -granule Secretion and secondary aggregation (mainly secondary to ADP).
 - Weak Agonists:
 - Include Epi, ADP
 - Stimulate primary aggregation only, at all concentrations (even high). The proof is:
 - If aggregation is blocked (eg. using abciximab or simply by not stirring), weak agonists do NOT stimulate ADP/ATP release as measured by lumi.
 - The effect of low calcium conditions (citrate PRP):
 - Under physiologic calcium conditions (eg. collected into PPACK) weak agonists do NOT cause secondary aggregation in most patients.
 - Ironically, it is only because of the low calcium conditions that weak agonist-induced platelet closeness results in TxA2 synthesis followed by degranulation.
 - Thus secondary aggregation in citrate PRP due to weak agonists is just an artefact, albeit a useful one.
 - Note: at high agonist concentrations (in citrate PRP) you see a unified primary and secondary aggregation wave – but these still are occurring in sequence. Thus by Lumi-aggregometry (in citrate PRP) you see initial aggregation with ADP release following a short time later.
 - Epi is unique because:
 - It is the only agonist (thus excluding Ristocetin) that does not produce a platelet shape change.
 - It does not lose its biphasic appearance at any concentration.
 - Strong Agonists
 - Includes
 - Collagen: Type 1 Fibrillar Collagen from Equine Tendon (aka Horm Collagen)
 - TRAP: Thrombin Receptor Activating Peptide. A 6 amino acid peptide that stimulates Platelet Receptors for Thrombin but does not convert fibrinogen to fibrin.
 - Stimulate primary aggregation, TxA2 synthesis and dense granule release simultaneously. The proof is:
 - If aggregation is blocked (eg. using abciximab or simply by not stirring), weak agonists DO stimulate ADP/ATP release as measured by lumi.
 - At low concentrations they mimic weak agonists, but at high concentrations they are able to overcome blockages in TxA2 or primary aggregation defects, but NOT secondary aggregation defects as occurs in δ -Storage Pool Defects or Storage Pool Release Disorders.
 - Thus, normally at high concentrations, by Lumi-aggregometry you see simultaneous aggregation and ADP release.
 - Arachidonic Acid and U46619
 - Usually classed as Strong agonists, but this is not entirely accurate as they do not induce any primary aggregation.
 - Do not fit in either category, because they bypass primary aggregation altogether and stimulate only secondary aggregation.
 - Thus at all concentrations their effect will be a single wave.
 - Ristocetin
 - Antibiotic drug that activates vWF.
 - NOT an agonist per se, it induces primary agglutination NOT aggregation.

- Does not produce a platelet shape change.
 - Depends only on a functional vWF and GpIb to produce primary agglutination.
 - Thus, even dead (or fixed) platelets will give agglutination.
 - In living platelets, at low concentrations you see a primary agglutination with stimulation of secondary aggregation secondary to physical platelet closeness.
 - Agglutination is absent in only two conditions – Bernard-Soulier Disease and Type III vWD.
 - Responses may be reduced in Type I or II vWD.
 - Agglutination with low dose Ristocetin (0.5 mg/ml) suggests Type 2B vWD or pseudo-vWD (Need Mixing Studies).
 - A 'normal control' should be run with each sample
 - Characteristics that should be evaluated according to ISTH:
 - Lag phase
 - Shape change (from discoid to spiny spheres)
 - Slope of aggregation
 - Maximal amplitude
 - Amplitude at the end of evaluation (late amplitude) 3, 5 or 10 min
 - Evidence of disaggregation
 - Must have secondary response to epinephrine in controls
 - Spontaneous Aggregation {Ref?}
 - Within the first 10 min is associated with High Risk of CVS death in the next 10 years.
 - Within the first 10-20 min is associated with Int Risk of CVS death in the next 10 years.
 - No spontaneous aggregation is low risk.
 - Before interpreting any abnormality ensure you have verified that α -granules are normal via a PBF, as α -Storage Pool Defect can cause a variety of LTA abnormalities.
- Platelet Disorder Classification {Cattaneo, M. *Semin. Thromb. Hemost.* 35:158-167}:
- Abnormalities of platelet receptors for adhesive proteins
 - Bernard Soulier's Syndrome (GpIb/V/IX deficiency or dysfunction)
 - Responds to all assayed (above) substances except Ristocetin. This is also the case in vWD, but this should have been excluded by the time Platelet Aggs are being done.
 - Does not aggregate on exposure to bovine vWF, which doesn't require activation with ristocetin (or shear stress) like human vWF.
 - Also does not respond as well to low dose TRAP as GpIb/V/IX is a receptor for Thrombin (though PAR-1 and 4 are also receptors and so sensitivity is not completely lost).
 - Pseudo-vWD
 - 0.5 mg/ml Ristocetin should NOT induce agglutination of platelets in normal people but patients with Type 2B vWD and pseudo-vWD patients will get agglutination.
 - Mixing studies are then necessary to distinguish 2B/p-vWD:
 - If pt plasma + donor plts + low dose ristocetin causes agglutination then you have Type 2B
 - If pt plts + donor plasma + low dose ristocetin causes agglutination then you have pseudo-vWD
 - Glanzmanns' Thrombasthenia (gPIIb/IIIa deficiency)
 - Complete failure to respond to all above assayed substances; EXCEPT Ristocetin.

- Usually the ristocetin response is decreased because while the initial response is agglutination, the secondary response is aggregation which can't happen with GpIIb/IIIa deficiency.
 - You should be able to see a shape change response with all agonists that normally produce one; this serves as a useful internal control to verify the platelets are functional/living just lack the GpIIb/IIIa.
 - CAUTION: Hypofibrinogenemia/Dysfibrinogenemia could cause the same pattern of defects.
- Collagen and Collagen Receptor Disorders
 - Collagen receptor defects include a variety of syndromes that fail to respond to collagen stimulation.
 - Collagen disorders (eg. Ehler's Danlos Syndrome) fail to respond to stimulation with their own collagen.
- Abnormalities of platelet receptors for soluble agonists
 - TxA₂ Receptor Defects – fail to respond to all concentrations of AA or U46619, other agonists may be variably impaired.
 - α₂-Adrenergic Receptor Defects – complete failure to respond to epi, but otherwise normal responses.
 - P2Y₁₂ Receptor Abnormalities
 - Failure to respond to ADP at all concentrations.
 - More severe than δ-Storage Pool Defects or after Clopidogrel ingestion, in that even at high concentrations of ADP the response is weak and transient or completely absent.
- Abnormalities of platelet granules aka Storage Pool Defects
 - δ-Storage Pool Disease (SPD)
 - Lack of response to both Arachidonic Acid and U46619 (Aspirin only inhibits Arachidonic Acid)
 - Inhibition of secondary responses with both Weak and Strong Agonists at all concentrations
 - However, LTA is not very sensitive to δ-SPD and can be completely normal or only partially abnormal. In a relatively large study of 106 known δ-SPD patients {*Blood*, 1987; 70: 620-623}:
 - 23% had normal platelet aggregation studies
 - 44% had miscellaneous (non-specific) abnormalities
 - 33% showed the aggregation pattern typical of this disorder.
 - Lumi-aggregometry is much more sensitive and specific for the disorder, but this disease can only be distinguished from a Primary Secretion Defect by use of Electron Microscopy.
 - Note that δ-SPD can be acquired (non-heritable) with:
 - Many Hematologic Neoplasms: ALL, AML, MDS, MPN. MDS/MPN, various lymphoproliferative disorders.
 - PNH
 - SLE
 - Endocarditis
 - DIC
 - α-storage pool disease (Grey Platelet Syndrome)

- Key to diagnosis is lack of granulation on PBF +/- non-specific abnormalities on LTA
 - LTA abnormalities are highly variable but can include impaired ADP, Collagen, or TRAP responses.
- $\alpha\delta$ -storage pool disease
- Factor V Quebec (Quebec Platelet Disorder)
 - Autosomal Dominant
 - Caused by a whole gene duplication mutation in the *PLAU* gene which causes a ~100X increase in platelet (but not plasma) levels of uPA (Urokinase Plasminogen Activator) {*Blood* 2010, 115:1264–6; *Blood Rev.* 2011, 25: 169–173}.
 - Typically demonstrate isolated complete lack of epinephrine response:
 - Can have other abnormalities
 - May not demonstrate any abnormalities
- Abnormalities of signal transduction pathways
 - Aspirin (and other COX inhibitors)
 - Note: many herbal remedies, especially those for menstrual cramping, often contain naturally occurring anti-prostaglandin agents.
 - Absent response to Arachidonic Acid, but normal U46619 response.
 - Lack of secondary responses to Weak Agonists; may show normal high dose ADP responses.
 - Normal responses to high dose Strong Agonists (key to distinction from δ -Storage Pool Deficiency/Primary Secretion Defect)
 - Normal ristocetin responses, typically (but may be reduced).
 - Clopidogrel
 - Normal Arachidonic Acid response
 - ADP demonstrates normal primary wave, but no secondary wave and followed by disaggregation.
 - Lipid Mediator Disorders:
 - PLA₂, COX deficiency, Thromboxane Synthetase Deficiency
 - Absent or decreased response to AA and weak agonists.
 - Respond normally to U46619.
 - Ca²⁺ mobilization or signaling disorders: PLC Mutation
- Abnormalities of procoagulant phospholipids
 - Scott Syndrome
 - Activated platelets are unable to translocate phosphatidylserine from the inner to the outer leaflet of the plasma membrane.
 - Results in a functional coagulation defect that is not detectable by PT/PTT (which uses exogenous Phospholipid).
 - Not characterized by any LTA abnormalities

- Miscellaneous abnormalities of platelet function,
 - Primary Secretion Defects (PSD)
 - Similar to δ -SPD but with normal granules as assessed by EM.
 - Diagnosis can only be made by combined lumi-aggregometry and Electron Microscopy.

Platelet Electron Microscopy

- Stock comments that can be used, or modified, to sign-out reports in Copath:
- Normal results after examining platelet whole mounts, only:

MICROSCOPIC DESCRIPTION:

Whole mount electron microscopy was performed on the patient's platelet sample. The dense granule (also known as delta granule) count is derived as an average from 30 intact platelets.

Dense granules = X.X granules/plt (Normal Range=2.7-5.9 granules/plt)

FINAL DIAGNOSIS:

Platelet Whole-Mount Electron Microscopy:

No Evidence of Platelet Dense Granule (Delta Granule) Deficiency.

DIAGNOSIS COMMENT:

- The finding of a mildly increased platelet dense granule count does not have any specific clinical significance.
- The results do not exclude the possibility of a Platelet Secretion Defect (also known as a Primary Secretion Defect), in which the dense granules are present but are not secreted appropriately. Alpha granule content and additional platelet micro-architecture cannot be assessed by the whole mount method. Buffy coat preparations for granulocyte inclusions were not examined.
- There are significant numbers of lysed platelet seen which may indicate a pre-analytic problem that resulted in platelet degranulation and factitiously low dense granule counts. Suggest repeat testing.

- Abnormally decreased platelet dense granules:

MICROSCOPIC DESCRIPTION:

Whole mount electron microscopy was performed on the patient's platelet sample. The dense granule (also known as delta granule) count is derived as an average from 30 intact platelets.

Dense granules = X.X granules/plt (Normal Range=2.7-5.9 granules/plt)

FINAL DIAGNOSIS:

Peripheral Blood, Platelet Whole Mount Electron Microscopy:

- Consistent with Platelet Delta Storage Pool (Dense Granule) Deficiency.

DIAGNOSIS COMMENT:

The findings are consistent with a diagnosis of delta-Storage Pool Deficiency (delta-SPD), which is a platelet function disorder that can be heritable or acquired. [Comment on platelet aggregation results if available; see below for additional comments. May suggest repeat testing if results are borderline]. The heritable forms of this disorder can be isolated, or present as part of the Chediak-Higashi, Wiskott-Aldrich, Hermansky-Pudlak or TAR (Thrombocytopenia with Absent Radii) Syndromes. Though data are limited, studies indicate that a diagnosis of heritable delta-Storage Pool Deficiency is strongly predictive for bleeding problems (Hayward, P.M. *Blood Rev.* 2011, 25:169–173; Hayward, P.M. *et al. J. Thromb. Haemost.* 2009, 7: 676–684). Acquired delta-SPD has been described in association with some hematolymphoid neoplasms, post-cardiopulmonary bypass and in Lupus; it may also be seen in conditions of chronic platelet stimulation.

- ◆ In a relatively large study of 106 known delta-SPD patients (*Blood*, 1987; 70: 620-623), 23% had normal platelet aggregation studies, and only 33% showed the characteristic aggregation pattern.
- ◆ Note that delta-SPD can also be acquired in variety of other disorders, including any hematologic neoplasm (myeloid or lymphoid), PNH, SLE, endocarditis and DIC.

- Booking:

- Clinicians can book at 407-3000.
- Secretary Records on the "Platelet EM Booking and Reporting Form".
- Booking/Reporting form is given to Special Coagulation bench for inclusion in Pathologist sign-out folder.
 - Pathologist on Coagulation Service must be notified immediately if EM is scheduled for the same week in which it is booked.
- Pathologist requests the appropriate tests on the Booking/Reporting form and returns the form to special coagulation.
 - The special coagulation tech faxes the form to the EM lab. This notifies the EM lab of the booking, and serves as their reporting form.
- The faxed and filled out booking/reporting form is returned to the pathologist along with select EM photos for reporting once complete.

- Sample collection and testing process:

- Samples for both Platelet Aggregations and EM are collected on the scheduled date (EM requires very little sample, most of the bulk is for Platelet Aggregations) at ~09:00 in the Outpatient Lab.
- Samples are sent to the Special Coagulation bench who then prepares the necessary samples requested on the Booking/Reporting Form.
- The prepared samples are then sent to the EM lab ASAP.
- The EM Lab carries out all further processing.
- Samples referred in from external labs:
 - Whole mount EM for dense granules cannot be performed as grids must be prepared on freshly collected samples (ideally <2 hours old).
 - Thin sections on buffy coat preparations for platelet and granulocyte morphology can be performed – contact Dr. Szkotak or the EM lab for instructions.

- Reporting:

- The EM lab accessions the report on Copath and records the accession on the Booking/Reporting form; note: the report is assigned to the approving pathologist and will need to be changed by the sign-out pathologist.
- The completed Booking/Reporting form and representative photographs are submitted back to the Special Coagulation bench, and are put into the pathologist sign-out folder.
- The pathologist on for that week of Coagulation signs out the report if whole-mounts were performed only. Consultation will be needed with Dr. Szkotak if thin sections are performed.
- Sign-out will be done in Copath with the reporting pathologist searching using the assigned Anatomic Pathology Accession Number.
- Platelet Aggregation studies performed on the same collection are reported separately and should have a comment appended stating that results of EM are reported under Anatomic Pathology.
- CAUTION: when there are significant numbers of lysed or clumped platelets seen the results may be unreliable. In particular this finding could indicate a pre-analytic problem resulting in platelet degranulation, and therefore factitious granule deficiency.
- Counting dense granules (from NASCOLA):
 - Uniformly dense/dark texture
 - Sometimes there is uniform dark core with a slightly lighter (grey) outer portion. In this case the core should be uniform, and the outer portion should be uniform. The outer portion should still be relatively dark.
 - Round or ovoid with a sharp contour
 - Should not have irregular outline

- Should not have surface blebs/irregularities
- Larger than 100 nm in diameter
 - At UAH we do not follow this criterion strictly because it was not followed strictly during normal reference range validation.
- Variant acceptable forms:
 - Whip like extensions
 - Hairy and fine processes
 - Ring forms
 - Purse/basket forms
- What are not dense granules (from NASCOLA):
 - Alpha granules – large, mostly irregular, fuzzy outline, usually less dense and much more numerous.
 - Clusters or chains
 - Very small (<100 nm diameter) and pale dots.
 - Non-specific dirt or debris.
- Accessing Images:
 - Electronic images can be downloaded from the shared (P:\) drive; access can be obtained from Tom Turner. These are lower quality images than the extremely high resolution images that are captured and stored in the EM lab but are still adequate for presentations.
 - The high resolution images can be obtained from the EM lab on a DVD. Note that the files are in excess of 30 MB each.
- Costing (Marginal Cost: includes Tech time and consumables but not Capital costs) assessed in 2011; calculations based on one sample and one control:
 - Platelet Whole Mounts = \$62.54
 - Thin Sections (either granulocyte or platelet) = \$306.67

Protein C

- Relevant smartphrases that pathologist can append at sign-out:
 - .PCLOW = A single low Protein C level is not diagnostic of deficiency. Repeat assay is recommended. Recollection should be performed while the patient is not on warfarin.
 - .SLVAC = A single low value is not diagnostic of deficiency. Factors such as recent thrombosis, acute systemic illness, surgery, trauma or liver disease can all decrease Protein C, Protein S and Antithrombin levels. Repeat testing after resolution of acute illness is recommended. Protein C and S levels can also be decreased by warfarin. Protein S can be decreased by high estrogen states such as pregnancy.
 - .REPCS = The patient has demonstrated marginally reduced levels on more than one occasion (different blood collection episodes). Such mild decreases are unlikely to indicate true heritable deficiency. However, additional testing and family studies may be indicated if there is a strong personal or family history of venous thromboembolism.
 - Mnemonic appended to all results >1.50 U/mL = CAUTION: Elevated Protein C results (>1.50 U/mL). Partial sample clotting (eg. due to a problematic collection), active Disseminated Intravascular Coagulation, or recent thrombolytic therapy can occasionally produce falsely high results with this assay. Recollection for repeat testing may be of value for confirmation.
- HemosIL (Werfen) chromogenic Protein C Assay:
 - Plasma incubated with Southern Copperhead Viper Venom (from Agkistrodon contortrix contortrix, aka Protac) to activate Protein C to APC.
 - Quantification of activated Protein C with a synthetic chromogenic substrate. The paranitroaniline released is monitored kinetically at 405 nm and is directly proportional to the Protein C level in the test sample.
- Interferences/confounders in HemosIL (Werfen) chromogenic Protein C Assay:

- Direct cleavage of the chromogenic substrate (amidolysis) by FXIIa, Kallikrein, Thrombin, Plasmin, and tPA can INCREASE Protein C levels in the following conditions:
 - Partial activation of coagulation (frankly clotted samples would be excluded from analysis)
 - DIC
 - Therapeutic thrombolysis
 - Not affected by:
 - Heparin (UF or LMW heparin) up to 2 U/mL {product monograph}
 - Free Hemoglobin up to 2 g/L {product monograph}
 - Triglycerides up to 100 mmol/L on ACL TOP {product monograph}
 - Bilirubin up to 359 umol/L on ACL TOP {product monograph}
 - DOACs {*J Thromb Haemost.* 2020;18:271–277}
 - Factor V Leiden {*J Thromb Haemost.* 2020;18:271–277}
 - Lupus Anticoagulant {*J Thromb Haemost.* 2020;18:271–277}
 - Aprotinin (inhibitor of fibrinolysis) is known to inhibit activated Protein C, thus low Protein C activity may be observed in aprotinin treated patients {product monograph}.
 - An elevated PT-INR has a strong probability of being associated with acquired Protein C deficiency due to:
 - Vitamin K deficiency/antagonism
 - liver dysfunction
 - DIC
 - Acute thrombosis.
 - Nephrotic syndrome can increase or decrease Protein C. Effect on anti-coagulant factors:
 - Mainly causes AntiThrombin decrease.
 - Possible milder Protein S deficiency.
 - Protein C may be increased or decreased.
 - Elevated levels may also be seen with:
 - High estrogen states such as OCP, Pregnancy or HRT
 - High lipid levels but no postprandial effect.
 - Type 2b Heritable Protein C Deficiency {*J Thromb Haemost.* 2020;18:271–277}.
 - Type I (~85% of heritable deficiency): Decreased Activity, Decreased Antigen
 - Type II (~15% of heritable deficiency): Decreased Activity, Normal Antigen
 - 2a – failure to cleave FVa, FVIIIa and the HemosIL chromogenic substrate.
 - 2b – failure to interact with Calcium ions, Phospholipid, Protein S, Factor Va, or Factor VIIIa. Not detected by Chromogenic assay but can be detected by clot-based assays. Type 2b is rare.
- Confounding factors seen in clot-based assays:
- DOACs
 - High FVIII levels
 - Factor V Leiden
 - Lupus Anti-Coagulant - Types of Protein C Deficiency:
- Diagnosing Protein C Deficiency {*J Thromb Haemost.* 2020;18:271–277}:
- High Protein C results (>1.5 U/mL) may be secondary to amidolysis (as above):
 - Generally these are addressed with the comment above recommending repeat testing. However, the following investigations may be considered if there is a clinical need.
 - Review other coagulation results, history and medications (especially thrombolytics) as well as adding on a D-Dimer and Fibrinogen. Taken together, these should be able to rule out amidolysis.
 - If amidolysis cannot be ruled out, then a blank can be run, replacing the Viper Venom with water.
 - This would be considered positive if the blank gives a detectable result (>0.10 U/mL) instead of a “fail”.

- In various trials of a suspect plasma or using activated Thrombin to mimic amidolytic interference we were never able to produce such a falsely high (>0.10 U/mL) blank result. They all gave a “fail”.
 - We will wait to trial this on additional patients suspected to have an amidolytic reaction.
 - If amidolytic interference is confirmed, then modify the comment. Also review all special coagulation clot-based assays on the same collection (eg. Lupus Anticoagulant, ATIII, Protein S Activity) to determine if they should be cancelled (or commented upon).
 - Also note that this same mechanism could theoretically cause falsely normal results in a true Protein C deficient patient.
- Low Protein C results should always be confirmed with repeat analysis to exclude pre-analytic issues.
- Allow at least 30 days from Vitamin K Antagonist discontinuation before re-testing.
- May suggest family studies for Protein C deficiency.
- Genetic testing:
 - If available, gene analysis can aid diagnosis of hereditary PC deficiency, but there is no good correlation between thrombotic phenotype and PROC genotype in heterozygous subjects. Approximately 75% of PROC mutations are linked to a single base change, resulting in missense or nonsense mutations, and a smaller number at the exon/intron boundaries resulting in splicing defects. Most of the small deletions or insertions introduce frameshifts, leading to a premature stop codon and truncated protein, and large deletions are identified in around 3% {*J Thromb Haemost.* 2020;18:271–277}.
- Diagnosis of Protein C and S Deficiency while on Warfarin Therapy
 - Though Protein C and Protein S are both affected by warfarin anticoagulation the ratio of the two (Protein C:Protein S) is not consistent even in the stably anticoagulated; probably because of their very different Half-Lives (9 hrs vs 60 hrs, respectively). Thus, a Protein C:S ratio is NOT recommended for distinguishing either Protein C or Protein S deficiency.
 - A variety of other ratios have been examined such as the Protein C:FVII ratio and the Protein S:FII ratio in stably anticoagulated patients, with better results. These likely work much better than the Protein C:Protein S ratio because FVII (6 hrs) and FII (60 hrs) have Half-Lives much more similar to Protein C and S, respectively {O'Brien, *et al. Clin.Lab.Haem.* 20:245–252, 1998}.
 - Patients who are on long term/life-long warfarin anticoagulation can be switched to UFH/LMWH for 4 weeks and be tested.
 - Ideally normal Protein C:FVII and Protein S:FII ratio reference ranges would be established in each lab to account for variations in assay performance (accuracy, precision, etc). While this has not been performed locally, results may still be useful as a rough guide.
 - Based on a review of 5 years of local data using the clot based Stago assay (may not apply equally to HemosIL chromogenic assay):
 - Neither Protein C nor Protein S levels are reliable when INR is even marginally elevated; when INR=1.3 almost 25% of normal individuals will demonstrate low (<0.4-0.5 U/ml) levels. However, a normal Protein C or S level may still be of value for exclusion of deficiency.
 - Neither Protein C nor Protein S levels should be tested when INR≥2.0 as essentially all patients will demonstrate acquired deficiency.

Protein S

- Protein S testing on the ACL TOP workflow:
 - The Free Protein S Antigen assay will be run first.
 - Normal results are Final Verified by the technologist, while decreased results (<0.65 U/mL) are sent for pathologist review.
 - The pathologists is to Final Verify results after applying relevant comments and deciding if functional Protein S testing is needed.
 - The following common scenarios have low Free Protein S that don't need functional testing:
 - INR is increased (>1.2): append .INRHI

- Pregnant or otherwise known to have a high Estrogen state: .PEST
 - Acute Inflammation: .SLVAC
 - Liver Disease: .SLVAC
- The following scenarios don't cause low Free Protein S but may cause factitiously high results in the Protein S Activity assay. In this context, low Protein S Activity is still consistent with deficiency.
 - DOAC therapy
 - Lupus Anticoagulant
- The purpose of this workflow is to reduce the frequent false positive (low) functional Protein S results encountered clinically {Marlar,R.A.; Gausman,J.N. Protein S abnormalities: a diagnostic nightmare. *Am.J.Hematol.*, 2011; 86(5): 418-421}. However, this means that Type II Protein S deficiency will be missed. Fortunately Type II Protein S deficiency is rare, with estimates of prevalence varying from 0.1-5% of all Protein S deficiencies. Sensitivity to Type I and III Protein S deficiency are not affected.
- Coded Comments:
 - INRHI = Patient's PT INR is elevated. Potential causes include Coumadin, vitamin K deficiency, liver dysfunction, infancy and coagulopathy, which are all known to be associated with low Protein C and Protein S levels.
 - PEST = Protein S levels are decreased by high Estrogen states (eg Pregnancy, OCP/HRT, etc) and Acute Phase Reactions (eg. infection, inflammation). In this context a low Protein S level cannot be used as evidence of a heritable deficiency.
 - SLVAC = A single low value is not diagnostic of deficiency. Factors such as recent thrombosis, acute systemic illness, surgery, trauma or liver disease can all decrease Protein C, Protein S and Antithrombin levels. Repeat testing after resolution of acute illness is recommended. Protein C and S levels can also be decreased by warfarin. Protein S can be decreased by high estrogen states such as pregnancy.
 - PSPRG = Compatible with Protein S deficiency as long as estrogen levels are not elevated (Pregnancy, OCP/HRT, etc) and the patient is not demonstrating an Acute Phase Reaction.
 - REPCS = The patient has demonstrated marginally reduced levels on more than one occasion (different blood collection episodes). Such mild decreases are unlikely to indicate true heritable deficiency. However, additional testing and family studies may be indicated if there is a strong personal or family history of venous thromboembolism.
 - DTIXA= "CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban."
- Epidemiology of Protein S Deficiency:
 - Prevalence in healthy caucasian population is 1:700 to 1:3000
 - In selected patients with previous VTE is 2% (1-13%)
 - Appears to be more common in Chinese/Japanese patients
 - Total and free PS levels are lower in women, but total PS levels in women rise to the levels of men by the age of 50 years.
 - ~50% of heterozygotes clot by 45-50 years of age.
- Normal PS:
 - 70 kDa, T1/2=30 hours (sources vary from 30 to 60 hours)
 - Only 40% circulates in free form – potentiates APC activity by 10 Fold
 - 60% is bound non-covalently to C4b-BP and is inactive toward APC; but does appear to function as a TFPI cofactor.
- Genetics
 - Autosomal Dominant inheritance (ie. heterozygous state associated with thrombophilia).
 - Homozygous PS deficiency is rare because it is usually lethal *in utero* or presents at birth as purpura fulminans (usually fatal).
- Types of Protein S Deficiency:
 - Type I: Decreased APC cofactor activity, Decreased total PS, Decreased free PS
 - Type II: Decreased APC cofactor activity, Normal total PS, Normal free PS

- Type III: Decreased APC cofactor activity, Normal total PS, Decreased free PS (some people consider Type I and III to be the same)
- True acquired Protein S (including free Antigen) deficiency:
 - High Estrogen States: Pregnancy, OCP, HRT
 - Acute inflammation causing increased C4b-BP
 - Vitamin K Antagonism/Deficiency
 - Liver Disease
 - Nephrotic Syndrome
 - DIC
- HemosIL (Werfen) Free Protein S Antigen
 - Automated latex ligand immunoassay on citrated plasma. Measures the increase in turbidity produced by agglutination of two latex reagents:
 - Purified C4BP adsorbed onto first latex reagent reacts with free Protein S in patient plasma (in presence of Ca^{2+})
 - A second latex reagent with adsorbed Anti-PS Antibody is added and agglutinates with the first.
 - The Turbidity increases with increasing levels of free Protein S
 - Will not detect Type II Protein S deficiency.
 - Not affected by:
 - UFH or LMWH up to 1.5 U/mL {product monograph}
 - Bilirubin up to 427.5 $\mu\text{mol/L}$ {product monograph}
 - Free Hemoglobin up to 2 g/L {product monograph}
 - Triglycerides up to 16.9 mmol/L {product monograph}
 - Rheumatoid Factor up to 900 IU/mL {product monograph}
 - Factor V Leiden Mutation {product monograph}
 - Product insert does not mention Heterophilic Antibody as an interfering substance or the species of animal it came from. This had been a limitation in the Stago assay, but presumably isn't in the HemosIL assay.
- HemosIL (Werfen) Protein S Activity:
 - PT Clot-based assay:
 - Diluted patient plasma is mixed with a Protein S deficient reagent plasma.
 - Mixed with reagent containing recombinant human Tissue Factor, Phospholipids, Ca^{2+} , and Activated Protein C. Also contains polybrene heparin inhibitor.
 - May show factitiously increased Protein S levels with high levels (low levels are unlikely to be adverse because patient plasma is diluted) of {theoretical}:
 - DOACs (DTI or DXI)
 - Lupus Anticoagulant
 - Not affected by:
 - UFH up to 1.6 U/mL {product monograph}
 - LMWH up to 2.1 U/ml {product monograph}
 - Bilirubin up to 256.5 $\mu\text{mol/L}$ {product monograph}
 - Free Hemoglobin up to 2.5 g/L {product monograph}
 - Triglycerides up to 26.6 mmol/L {product monograph}
 - Rheumatoid Factor at all {theoretical}
 - Factor V Leiden {theoretical} because only the FVIIIa inhibiting function of APC:PS is FV dependent {J Thromb Haemost. 2020; 18:271–277.}
- Diagnosis of Protein C and S Deficiency while on Warfarin Therapy
 - Though Protein C and Protein S are both affected by warfarin anticoagulation the ratio of the two (Protein C:Protein S) is not consistent even in the stably anticoagulated; probably because of their very different Half-Lives (9 hrs vs 60 hrs, respectively). Thus, a Protein C:S ratio is NOT recommended for distinguishing either Protein C or Protein S deficiency.
 - A variety of other ratios have been examined such as the Protein C:FVII ratio and the Protein S:FII ratio in stably anticoagulated patients, with better results. These likely work much better

then the Protein C:Protein S ratio because FVII (6 hrs) and FII (60 hrs) have Half-Lives much more similar to Protein C and S, respectively {O'Brien, *et al. Clin.Lab.Haem.* 20:245–252, 1998}.

- Performing Protein C:FVII or Protein S:FII ratios in select cases is not practical either as normal ranges for these ratios would have to be validated first; not to mention revalidated with each lot# change.
- If a patient requiring testing is on Warfarin, which cannot be safely stopped then LMWH therapy can be substituted for the 4 weeks it takes for factor levels to normalize so that blood samples can be collected.
- Based on a review of 5 years of local data using the old Stago assay:
 - Neither Protein C nor Protein S levels are reliable when INR is even marginally elevated; when INR=1.3 almost 25% of normal individuals will demonstrate low (<0.4-0.5 U/ml) levels. However, a normal Protein C or S level may still be of value for exclusion of deficiency.
 - Neither Protein C nor Protein S levels should be tested when INR≥2.0 as essentially all patients will demonstrate acquired deficiency.

Reptilase Time

- Reptilase® (Batroxobin) is derived from Bothrops *atrox* snake venom

- It only cleaves Fibrinopeptide A (not A and B like Thrombin)
- It does not activate FVIII, FXIII or Platelets (unlike Thrombin)
- Unlike Thrombin, it is insensitive to Heparin or Hirudin.

- Clinical uses:

- Heparin Contamination can be diagnosed by prolonged Thrombin Time with a normal Reptilase Time – now rarely done due to use of Hepzyme/Hepzorb.
- Dysfibrinogenemia {*Arch.Pathol.Lab.Med.* 2002; 126: 1387-1390}:
 - Reptilase Time is not as sensitive as Thrombin Time.
 - Of the hundreds of different heritable dysfibrinogenemias only 3 are known not to prolong the Thrombin Time but may prolong the Reptilase Time.
 - All of these three (Fibrinogen Oslo I, Valhalla and Denver) may be detected as an abnormally shortened Thrombin Time {*Haemophilia* 2006; 12: 393-97}.
 - Thus, Thrombin Time is useful as the screening test for Dysfibrinogenemia and a positive result can be followed up with a Reptilase Time to improve specificity.

- Ordering:

- After discussion with clinical Hematology in October of 2009 we have agreed to send for external testing rather than performing internally. This is because:
 - The test is rarely ordered
 - Proficiency Testing is frequently a problem as this is an enzyme with wide variations in activity depending on lot number, manufacturer, etc.
- The test has been discontinued locally as of Feb. 2010.
- Esoterix lab charges \$140 for the test (as of Oct. 20, 2009)
- If the test is ordered an aliquot will be frozen pending pathologist approval; in addition a Fibrinogen level and Thrombin time will be automatically added on.
- My recommendation for cancelling or approving requests:
 - Reptilase time should not be used as a screen for Dysfibrinogenemia – Thrombin Time (either prolonged or shortened) should be sufficient.
 - Reptilase Time can be of value if Thrombin Time is abnormal to increase the specificity of the finding. In this circumstance a more useful test would be a ratio of functional:antigenic fibrinogen.
 - Reptilase Time may be considered if there is a family history of Dysfibrinogenemia but normal TT.

Thromboelastography (TEG)

- No longer performed in Alberta (previously done at UAH as part of Berlin Heart program)

- Method:

- The timing after putting into a cup depends on presence or absence of citrate:

- No Citrate Collection – must be on analyzer <4 min
 - Citrated Collection – must be on analyzer >6 min but <120 min after collection.
 - At UAH Lab TEG we use a standardized 30 min after collection for citrated samples as R time in particular is affected (shortened) by increasing pre-analytic time. This is most prominent in heparinized patients.
 - Two citrate anticoagulated whole blood samples are placed into one of two cups (both of which contain Kaolin Activator):
 - Plain Cup (CK)
 - Heparinase Bonded Cup (CKH)
 - A torsion wire from the analyzer is placed into each cup.
 - The sample (with-in a cup) twists back and forth.
 - Calcium must be re-added for citrated samples
 - The Thrombus twists the wire with increasing force the stronger the clot is.
 - The plot is of the degree of resistance on the wire in one direction and then the other – these should be equal.
- Measured Values:
- SP (Split Point)
 - The time from the initiation of coagulation to the moment that there is any measurable resistance on the torsion wire.
 - Represents the first 2-3% generation of Thrombin.
 - Average is 4.8 min
 - This is a “research” parameter only, ie. is not supposed to be used for clinical decision making.
 - R (Reaction Time)
 - The time to reach a 2 mm difference between the split arms.
 - Represents the first 5-6% generation of Thrombin.
 - Normal is 5-10 min.
 - This is a measure of coagulation factor function.
 - This will be prolonged by any anticoagulants (heparin, warfarin, DTIs, etc) or depleted factors (anything that will raise INR or PTT)
 - Therapeutic anti-coagulants increase this to ~12-15 min.
 - If R is short it suggests a thrombophilic state.
 - K (Kinetic Time)
 - The time it takes for the clot strength to reach 20 mm
 - Normal is 1-4 min
 - If low then the patient needs FFP or Cryo
 - Angle (α)
 - Normal is 47 to 74 degrees
 - Represents the rate of fibrin generation and cross-link formation
 - If low then the patient needs FFP or Cryo
 - MA (Maximum Amplitude)
 - Reflects the Platelet (normally ~80%) and Fibrin (normally ~20%) amounts/function.
 - Normal is 55 to 73 mm
 - If low then the patient needs platelets.
 - Unfortunately, the MA likely reflects PL content more than functional platelet amounts – hence the need for platelet mapping.
 - G (Shear Elastic Modulus Strength)
 - Normal is 6 to 13.2 Kdynes/sec
 - Note: G increases exponentially as A increases:
 - A=50 mm then G=5 Kdyn/cm²
 - A=67 mm then G=10 Kdyn/cm²
 - LY30 (Lysis over 30 Min once the MA has been defined)
 - Reflects fibrinolysis – percent clot breakdown over 30 min.
 - Measured as the change in the area of the curve.

- Normal is 2-3%, but >8% is considered a significant change.
 - If high then it suggests fibrinolysis:
 - Primary Fibrinolysis (CI<1.0 or negative D-Dimers) treat with anti-fibrinolytics
 - DIC (CI>3.0 or Positive D-Dimers) do NOT treat with anti-fibrinolytics
 - CI (Clotting Index)
 - Proprietary calculation on over-all bleeding/clotting tendency
 - Values of < -3 suggest progressively increasing risk of bleeding
 - Values of > +3 suggest progressively increasing risk of thrombosis
- Comparisons of Cups
- Any difference in the R time between the Heparinase and Plain cups can be attributed to Heparin; useful for:
 - Measuring line heparin contamination.
 - Incomplete heparin reversal by protamine at the bedside
 - ATIII Deficiency can be assessed for by:
 - Add Heparin to the Plain Cup sample
 - If there is no difference between the Plain Cup R time and the Heparinase Cup R time, then the patient is ATIII deficient.
- Troubleshooting
- Any agitation of the analyzer can introduce blips that alter points used for calculation.
- Platelet Mapping for ADP Inhibitors (eg. Clopidogrel) and AA Inhibitors (eg. ASA)
- Was developed in response to the observation that the MA was more dependent on the Phospholipid content than the platelet function.
 - All are based on just the MA measured under different conditions
 - % ADP Inhibition = $1.0 - (MA_{ADP} - MA_{FIB}) / (MA_{Thrombin} - MA_{FIB})$
 - No normal ref due to wide variation
 - Comparisons to baseline recommended
 - % AA Inhibition = $1.0 - (MA_{AA} - MA_{FIB}) / (MA_{Thrombin} - MA_{FIB})$
 - Normal range in adults at UAH is <20%
 - MA_{ADP} and MA_{AA} are the MAs measured when clotting is stimulated by exogenous ADP or AA respectively.
 - MA_{FIB} is a measure of the fibrin contribution to the MA
 - Normally determined by activating with Reptilase and FXIIIa in order to obtain fibrin clot without any platelet activation.
 - In some patients the platelets are hyper-reactive and do not give a clean MA_{FIB} result (ie. polluted by some platelet aggregation). This can be recognized by:
 - Unstable MA_{FIB} ie. one that never plateaus
 - An unusually high MA_{FIB}
 - A biphasic waveform
 - In patients where this occurs you can inhibit the platelets using a GpIIb/IIIa inhibitor:
 - 10 ul of 750 ug/ml stock solution of eptifibatide (Integrilin) {*Am.J.Clin.Pathol.* 2014; 142:331-338}.
 - As of Aug. 2014 this inhibitor will be evaluated in every TEG-Platelet Mapping patient assay (even with a stable MA_{FIB}) even there is sufficient reagent and sample remaining after obtaining clinical results. The MA_{EPF} should give the same results as the MA_{FIB} in most patients unless there is a problem with the latter.
 - $MA_{Thrombin}$ is a routine TEG, where the reaction is started with Kaolin and thus is based on initial thrombin generation.
- ROTEM (Rotational Thrombelastometry) is essentially the same as TEG – just a different manufacturer. There is variation in terminology {*Haemophilia* 2011, 17: 532–537}:
- The same parameters are measured as TEG, these are:
 - Clotting Time (CT) = Reaction Time (R)
 - Clot Formation Time (CFT) = Kinetic Time (K)
 - Alpha = alpha
 - Maximum Clot Formation (MCF) = Maximal Amplitude (MA)

- Lysis Index at 30 min (LI30) = Lysis over 30 min (LY30)
- Area Under the Curve (AUC) = area under the velocity (first derivative) curve ending at the point corresponding to MCF.
- Run under various conditions with the following names (on whole blood):
 - INTEM – activation via the intrinsic pathway
 - HEPTTEM – INTEM assay in presence of heparinase
 - EXTEM – activation using Tissue Factor of the extrinsic pathway
 - FIBTEM – EXTEM assay in presence of potent platelet aggregation to measure fibrinogen.
 - APTTEM – EXTEM assay in presence of aprotinin to inhibit fibrinolysis.

ADAMTS13 Inhibitor (external)

- Prior to July 2014 ADAMTS13 Activity testing was sent out to the Versiti Wisconsin. Done using Fluorescence Resonance Energy Transfer (FRET) assay.
- An ADAMTS13 Inhibitor (at Versiti Wisconsin) can be ordered as a standalone test when an ADAMTS13 Evaluation is ordered in Connect Care.
- ADAMTS13 Inhibitor reports states:
 - Inhibitor activity is determined using mixing studies with normal pooled plasma and residual ADAMTS13 activity is measured using FRET-VWF73 substrate. In patients with acute idiopathic thrombotic thrombocytopenic purpura (TTP), severe ADAMTS13 deficiency is attributed to circulating auto-ADAMTS13 antibody. Publications suggest that inhibitor antibody is observed in 44-93% of such patients. Persistence of inhibitory autoantibody during symptomatic remission of TTP suggests an increased risk for subsequent clinical relapse. Autoantibody is not implicated in the mechanism of congenital ADAMTS13 deficiency (Upshaw-Schulman syndrome). Severe hemolysis (plasma free hemoglobin >2 g/L) and hyperbilirubinemia (total bilirubin >257 umol/L) can cause an artifactually positive ADAMTS13 inhibitor result. Correlation with clinical data and the ADAMTS13 activity result is suggested.
- Note: while the majority of acquired idiopathic TTP is due to an ADAMTS13 Inhibitor, some are due to an antibody that does not have an inhibitor effect but does result in more rapid plasma clearance.

ADAMTS13 (local/UAH)

- The PLASMIC score is a clinical scoring system for predicting ADAMTS13 deficiency:
<https://www.mdcalc.com/plasmic-score-ttp>
- Predicting ADAMTS13 deficiency (<5%) {855-Blood Rev. 2014; 28: 67–74}:
 - Strongest association with:
 - Platelet count $\leq 30 \times 10^9/L$
 - Serum creatinine $\leq 200 \text{ umol/L}$
 - Weaker association of ADAMTS13 deficiency when ANA is positive.
 - When all 3 criteria are met (Plts<30, Creatinine<200, and ANA positive) the specificity for ADAMTS13 deficiency is 98.1%.
- Diapharma TECHNOZYM® ADAMTS-13 ACTIVITY ELISA:
 - 2X 100 ul aliquots at a minimum are needed per patient
 - Functional ELISA:
 - Anti-GST antibody coated ELISA plate
 - Reagent contains vWF73 (73 amino acids fragment) conjugated to GST, which binds to the plate.
 - Plasma is added and any ADAMTS13 cleaves the vWF73 leaving a vWF10 segment.
 - Washes remove any residual plasma.
 - An Antibody conjugated to HRP (HorseRadish Peroxidase) targeting the exposed (ie. cleaved) end of the vWF10 fragment is added.
 - After incubation the excess antibody-HRP conjugate is washed away.
 - An HRP substrate is added and optical density measured at 450 & 620 nm to quantitate the amount of vWF73 that was cleaved.
 - Calibration with known quantities of ADAMTS13 is necessary.

- Citrated samples required. Cannot use EDTA which is a strong inhibitor of ADAMTS13 function.
- Estimated cost per test (in 2014):
 - STAT (individual patient per run) \$348.75 for reagents/patient
 - Routine (multiple patients in a single run) = \$87.19 for reagents/patient
- Interference:
 - Cannot be tested on EDTA samples.
 - Citrated PPP should be frozen within 2 hours of collection.
 - Insensitive up to at least free Hb of 5 g /L
 - Insensitive up to at least Unconjugated Bilirubin of 855 umol/L
 - Insensitive up to at least Conjugated Bilirubin 855 umol/L
 - Insensitive up to at least RF of 50 IU/ml
- Assay validation characteristics at UAH:
 - Accuracy – compared to 3 different Ontario labs (n=42 samples):
 - Correlation $r = 0.9707$
 - Bias = -13%
 - ie. UAH lab gave results 13% lower than these Ontario labs on average.
 - Precision:
 - QC:
 - Normal QC at 0.70 U/ml, SD=0.056, CV=8.4%
 - Low QC at 0.23 U/ml, SD=0.009, CV=4.0%
 - Patient sample near clinical decision limit of 0.10 U/ml
 - Mean = 0.058 U/ml, SD=0.013, CV=22.54%
 - Limit of Blank (LOB) = 0.004 U/ml
 - Limit of Detection (LOD) = 0.03 U/ml (0.025 U/ml to be exact)
- EDTA contamination in ADAMTS13 testing:
 - An ADAMTS13 result <0.10 is quite specific for TTP; however, EDTA contamination is an important pre-analytical consideration which may result in undetectable ADAMTS13
 - This may occur because:
 - Specimen collection into an EDTA tube instead of citrate; the special coagulation lab will not know if only frozen aliquots are received.
 - Incorrect tube draw order (remember that citrate should be collected before EDTA, because of relative strength of calcium chelation).
 - Due to incidents of suspected falsely undetectable ADAMTS13 together with the massive treatment implications of an undetectable result, as of December 2020, additional testing will be performed on any undetectable result:
 - Calcium, Potassium, INR, and PTT will be run any time the result is <0.03 (but these will not be reported).
 - Guidance to pathologists:
 - Compare to patient baseline results.
 - Sodium citrate collections should have $\text{Ca}^{+} > 1.0 \text{ mmol/L}$ and $\text{K}^{+} < 5.0 \text{ mmol/L}$, unless patient is hypocalcemic or hyperkalemic at baseline.
 - INR and PTT should be comparable to historic; be suspicious if the results are markedly different (especially if Vmax)
 - Published data from Linda Stang's studies:

Table 4
Electrolyte results associated with different anticoagulant tubes used
in haemostatic testing

	Sodium level (mmol/L)	Potassium level (mmol/L)	Calcium level (mmol/L)
EDTA plasma	131.6–134.5	18.79–20.89	<0.50
Na Citrate plasma	148.9–156.6	3.11–4.63	1.27–1.42

von Willebrand Disease

- Coded Comments:

- VWFMM = von Willebrand Factor Multimer analysis may be of value, but is not performed locally. If desired please collect two blue top (citrate) tubes and write "ATTN: Clinical Trials, vWF Multimers" on the laboratory requisition.

- vWF:Rco Assay

- Performed like a platelet aggregation study – can be tricky and time consuming
- Final concentration of vWF:RCo is 1 mg/ml (vs. 1.2 mg/ml for Platelet Aggs).
- A standard curve is first made using "normal" pooled plasma at various dilutions (using buffer) – just like one would do for Factor Levels (except that you need not mix with vWF deficient plasma).
- The test mixture is:
 - 200 ul of reconstituted lyophilized platelets
 - 25 ul of diluted patient plasma (25 ul = total after dilution with buffer; typically 1/2, 1/4 or 1/8)
 - 25 ul of Ristocetin ([Working]=10 mg/ml, [Final]=1 mg/ml)
 - 250 ul Total
- Quantitation:
 - Maximum slope is measured at a minimum of two different dilutions.
 - Note: the maximum aggregation is not useful as it remains constant over a large range of vWF levels.
 - Result is read off the standard curve
 - The two different dilutions must check within 20% of the lower value.
 - It may be necessary to redilute the patient sample if the result is too high or the results don't check – which may indicate the presence of an inhibitor.
- vWD Type 2B and pseudo-vWD:
 - Are associated with hyperactive binding – but this does NOT result in higher than normal vWF:Rco.
 - This is because the excessive binding leads to rapid depletion of vWF.
 - Both Type 2 vWD and pseudo-vWD are usually associated with low vWF:Rco levels.

- vWF Multimer analysis (Western Blot)

- Had been done previously at the UAH but required a radio-isotope; though a biotin-avidin method is available now.
- CROT database lists McMaster as our Reference lab, as of Feb. 25, 2008:
 - Two Blue Top Tubes: Sodium Citrate Plasma
 - Two 1.0 ml PPP shipped frozen to Hamilton
 - \$55.00/test
 - Expected TAT=1 month
- Calgary as of May 18, 2010:
 - Does in Molecular Hematology Lab
 - Likely long TAT (only done 1-2X per year)
- Procedure:
 - Run on 1% agarose gel
 - Transferred to membrane
 - Probed with antibody to vWF

- Can be used to distinguish Type 1 vWD (low HMW and LMW) from Type 2 vWD (normal LMW but low HMW)
- Experienced operators can also make distinctions between subtypes of Type 2 based on pattern of multimer distribution.
- Also potentially useful in TTP, which demonstrates increased HMW vWF.
- FVIII binding assay (FVIII:B:vWF) {474-*Zhukov et al. Hemophilia*. 2009, 15:788-796}
 - Estimated frequency of the most common vWD2N allele (R854Q) is ~1% in the USA.
 - Some may respond to DDAVP such as R854Q which has reduced rather than absent FVIII:B activity.
 - Heterozygous vWD2N:
 - Not usually detected by routine vWF:Ag, vWF:RCO and FVIII.
 - Not usually symptomatic.
 - FVIII is usually >0.4 U/ml with FVIII:B:vWF ratio of 0.4-0.7
 - Only the addition of FVIII binding assay is reliable for detecting these individuals.
 - These patients are likely to benefit from genetic counselling.
 - Homozygous vWD2N:
 - These patients can usually be identified by routine vWF:Ag, vWF:RCO and FVIII measurements.
 - Typically FVIII<0.4 U/ml with FVIII:B:vWF ratio of <0.1
 - These patients are usually symptomatic.
 - Compound Heterozygous:
 - Compound vWD1 + vWD2N alleles is likely quite common.
 - Typically low vWF:Ag, with low FVIII:B:vWF and thus very low FVIII.
 - These patients can be symptomatic.
 - Procedure:
 - Antibody against vWF is bound to the ELISA plate.
 - Plasma is adsorbed to the plate such that patient vWF and FVIII are immobilized together.
 - A separation step is carried out to remove the patient's endogenous FVIII leaving only patient vWF.
 - Recombinant rFVIII is adsorbed.
 - rFVIII and patient vWF levels are quantitated separately and the ratio derived.
 - Interpretation:
 - FVIII:B:vWF of ~1.0 is normal ie. NOT vWD2N; but can also be the result in Hemophilia A.
 - FVIII:B:vWF of ~0.5 suggests heterozygosity for vWD2N
 - FVIII:B:vWF of 0.15-0.30 suggests compound heterozygosity for vWD2N and vWD1
 - FVIII:B:vWF of <0.1-0.2 is homozygous for vWD2N

Appendix A – Platelet Active Drugs

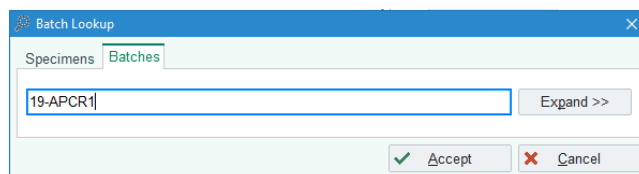
The following list is derived from Kottke-Marchant, K. and Corcoran, G. *Arch.Path.Lab.Med.* 2002, 126: 133-146. Additions are referenced.

- Aspirin and NSAIDs eg. ibuprofen, indomethacin, naproxen, mefenamic acid
- ADP Receptor Antagonists eg. clopidogrel, ticlopidine, prasugrel, cangrelor, ticagrelor
- GpIIb-IIIa Antagonists eg. abciximab, tirofiban, eptifibatide
- Drugs that increase cAMP or cGMP:
 - Prostaglandin I₂ analogues
 - Phosphodiesterase Inhibitors (eg. Caffeine, Theophylline, Dipyridamole)
- Nitric Oxide donors
 - ED Dysfunction drugs – Sildenafil (Viagra), Tadalafil (Cialis), Vardenafil (Levitra)
 - Nitrates – Nitroglycerin, Nitroprusside
- Various Antibiotics especially:
 - Penicillins
 - Cephalosporins
 - Nitrofurantoin
 - Amphotericin
 - Anti-malarials – hydroxychloroquine or chloroquine
- β -Blockers (anything that ends in -olol)
- Diuretics – mainly Furosemide
- Calcium Channel Blockers – Diltiazem, Verapamil, Nifedipine, Amlodipine, etc.
- ACE-I (anything that ends in -pril)
- Anticoagulants (anything that interferes with Thrombin formation)
 - Heparins – UFH, LMWH and Fondaparinux
 - Warfarin
 - Danaparoid
 - Direct Thrombin Inhibitors – Argatroban, Lepirudin, Dabigatran, Rivaroxaban
- Thrombolytics (Streptokinase, tPA)
- SSRIs: Citalopram {*J.Thromb.Haemost.* 10: 1177–1202} and probably others.
- Psychotropics
 - Tricyclic Antidepressants – imipramine, amitriptyline, nortriptyline
 - Phenothiazines – chlorpromazine, promethazine, trifluoperazine
- Local and General Anaesthetics
- Chemotherapeutic Agents – vincristine, asparaginase, plicamycin
 - Ibrutinib – irreversible inhibitor, especially of collagen induced aggregation (Blood 2014, 124: 3991).
- Dextrans and Hydroxyethyl Starch
- Some lipid agents: clofibrate, halofenate
- Guaifenesin (expectorant)
- Radiographic contrast agents
- Vitamins and Food Supplements: Alcohol, Caffeine (methylxanthine), Cold FX, Cumin, Dong quai, Fenugreek, Garlic, Ginseng, Ginger, Ginkgo, Omega-3 supplements including Fish Oil, Onion, Turmeric, Clove, Tamarind, Vitamin C, Vitamin E, Willow Black Tree Fungus (“Chinese Mushroom”).

Appendix B Special Coagulation Pathologist Interpretation and Result Verification

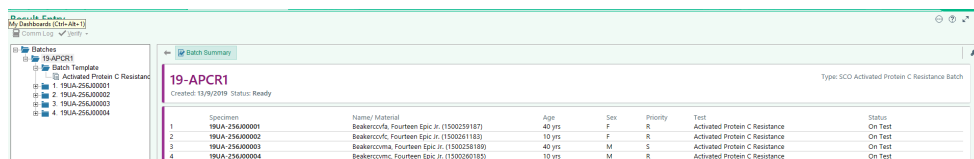
A. Opening Batches for interpretation

1. Select “Result Entry and Verification” (various locations).
2. In the pop up, select the “Batches” tab, enter in the batch number (scan barcode off printout or manually enter), and “Accept”.



The Batch Lookup dialog box has two tabs: "Specimens" and "Batches". The "Batches" tab is selected. A text input field contains "19-APCR1". To the right of the input field is a button labeled "Expand >>". At the bottom right are two buttons: "Accept" (with a green checkmark icon) and "Cancel" (with a red X icon).

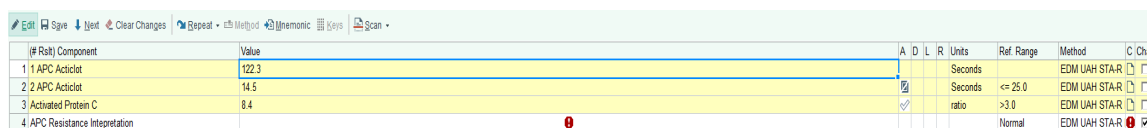
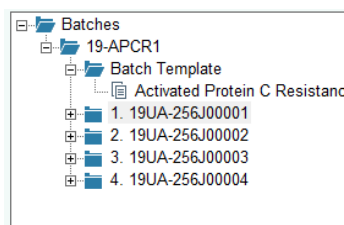
3. Result entry screen window will come up with a list of all samples on the batch.



The Batch Summary screen shows a table of samples for batch 19-APCR1. The table has columns for Specimen, Name/Material, Age, Sex, Priority, Test, and Status.

Specimen	Name/Material	Age	Sex	Priority	Test	Status
1 19UA-256J00001	Beakercoma, Fourteen Epic Jr. (1500259187)	40 yrs	F	R	Activated Protein C Resistance	On Test
2 19UA-256J00002	Beakercoma, Fourteen Epic Jr. (1500261182)	10 yrs	F	R	Activated Protein C Resistance	On Test
3 19UA-256J00003	Beakercoma, Fourteen Epic Jr. (1500258189)	40 yrs	M	S	Activated Protein C Resistance	On Test
4 19UA-256J00004	Beakercoma, Fourteen Epic Jr. (1500260185)	10 yrs	M	R	Activated Protein C Resistance	On Test

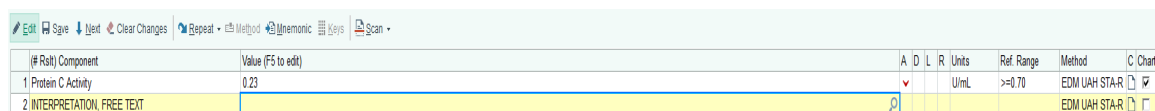
4. To enter interpretations:
 - In the left hand file tree, click on the first sample.
 - Click on “Edit” near the middle of the screen to start entering results. The ID that was highlighted will open.



The result entry screen shows a table with columns: (# Rst) Component, Value, A, D, L, R, Units, Ref. Range, Method, and C Chart. The first three rows are highlighted in yellow.

(# Rst) Component	Value	A	D	L	R	Units	Ref. Range	Method	C Chart
1 APC Actictot	122.3					Seconds		EDM UAH STAR	
2 APC Actictot	14.5					Seconds	<= 25.0	EDM UAH STAR	
3 Activated Protein C	8.4					ratio	>3.0	EDM UAH STAR	
4 APC Resistance Interpretation							Normal	EDM UAH STAR	

- Click on the magnifying glass icon in the required field, enter in desired interpretation/comments, then select “Next” to go to the next sample on the list or “Save” when you are done entering. The type of entry is different for different tests (see step B).
- Interpretations can be entered by residents and left at this point for review by the hempath. Results will not be released until Final Verify is completed (step D).
- Most Special Coagulation tests will have a yellow “Interpretation Free Text” field which allows for additional comments to be added. Once an entry is made in this field, it will change to white and go to the chart.

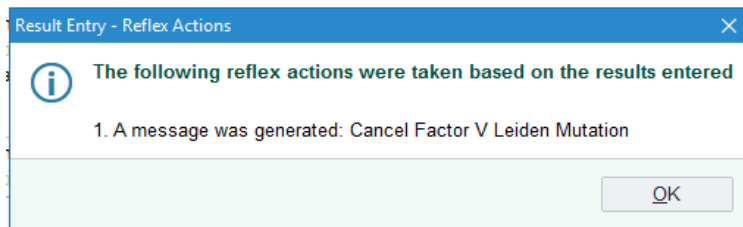


The interpretation free text entry screen shows a table with columns: (# Rst) Component, Value (F5 to edit), A, D, L, R, Units, Ref. Range, Method, and C Chart. The first two rows are highlighted in yellow.

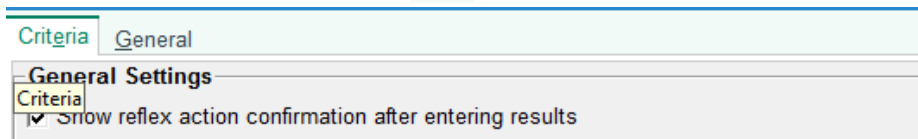
(# Rst) Component	Value (F5 to edit)	A	D	L	R	Units	Ref. Range	Method	C Chart
1 Protein C Activity	0.23					UnitL	>=0.70	EDM UAH STAR	
2 INTERPRETATION, FREE TEXT								EDM UAH STAR	

5. Some tests will have attached files to be reviewed (e.g. HITs) as part of interpretation (see step C).

6. Reflex actions may appear once a result is entered, click OK.



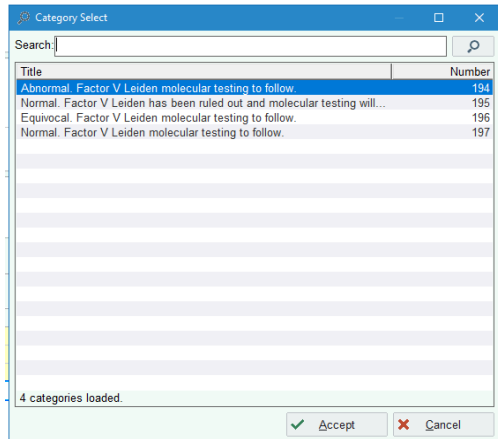
- There is a setting to turn this off when customizing result entry (see "settings" under "views" in menu).



B. Interpretation entry options

1. Categories

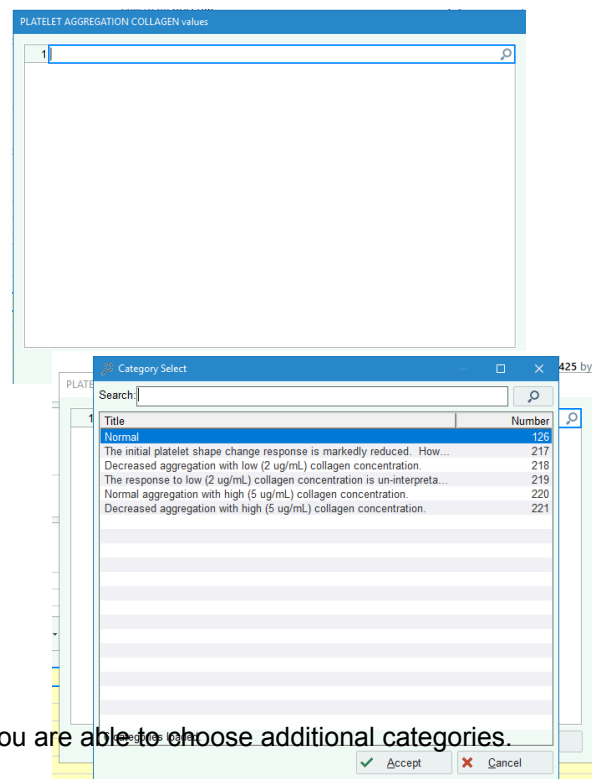
- Select interpretation choice and “Accept”, only one choice allowed.



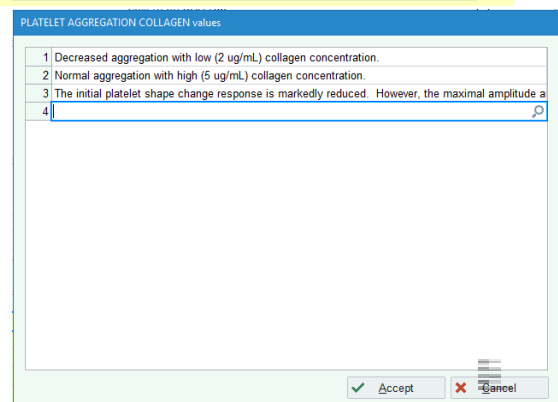
2. Multi-categories

- First screen will come up blank.

- Click on magnifying glass icon to see choices.



- Once the choice is accepted, you are able to choose additional categories.



PLATELET AGGREGATION COLLAGEN values

1 Decreased aggregation with low (2 ug/mL) collagen concentration.

2

Accept Cancel

– Abbreviations of the multiple choices will display in the main entry screen.

#	Roll	Component	Value (F5 to edit)	A	D	L	R	Units	Ref Range	Method	C	Chart
1		PLATELET AGGREGATION COLLAGEN	(Dec 2 Coll)(Norm 5 Coll)(Norm collage)							EDM UAH SPCO(		
2		PLATELET AGGREGATION ADP								EDM UAH SPCO(		
3		PLATELET AGGREGATION RISTOCETIN								EDM UAH SPCO(		
4		PLATELET AGGREGATION ADRENIC ACID								EDM UAH SPCO(		
5		PLATELET AGGREGATION THROMBIN RECEPTOR ACTIV								EDM UAH SPCO(		
6		PLATELET AGGREGATION INTERPRETATION								EDM UAH SPCO(		
7		PLATELET RICH PLATELET COUNT						10 ⁹ /L		EDM UAH SPCO(		

3. Free text / smart phrases

– A free text result can be entered or a smart phrase with a pre-determined comment can be used.

INTERPRETATION, FREE TEXT values

Insert SmartText

Accept Cancel

– To use a smart phrase, type "." before the phrase abbreviation, highlight the desired phrase and hit Enter on the keyboard. Typing in more characters of the phrase narrows the choices to the correct one.

INTERPRETATION, FREE TEXT values

Insert SmartText

.pc

Abbrev	Expansion
☆ PCDEF	Decreased Protein C Activity suggestive of hetero...
☆ PCLL	Moderate lymphocytosis composed of atypical s...
☆ PCLOW	A single low Protein C level is not diagnostic of d...
☆ PCPEPHONE	Displays the department phone number for a PCP
☆ PCPNOTITLE	PCP external name without title
☆ PCT	Displays the patient's care team

Refresh (Ctrl+F11) Close (Esc)

Accept Cancel

INTERPRETATION, FREE TEXT values

Insert SmartText

.pclo

Abbrev	Expansion
☆ PCLOW	A single low Protein C level is not diagnostic of d...

Refresh (Ctrl+F11) Close (Esc)

Accept Cancel

– Once Accept is selected, the entire comment is displayed. Modifications can be made if required.

INTERPRETATION, FREE TEXT values

Insert SmartText

A single low Protein C level is not diagnostic of deficiency. Repeat assay is recommended. Recollection should be performed while the patient is not on warfarin.

Accept Cancel

4. Comments

- Additional comments can be added by selecting the icon under the “C” column. This will allow free text and the use of smart phrases.

C. Reviewing scans (attached files) – 2 options.

(Files will be attached for TEG, Platelet Aggregations, HITs, Inhibitors, and Platelet Mixing Studies.)

1. Before selecting Edit, scroll to the very bottom of the specimen displayed at the bottom of the screen and click on the desired jpg file to view it.

- File name will indicate if it is on the chart or not.

Method: EDM UAH SPCOAG MANUAL METHOD Last received: 27/8/2019 0845

Chart Scans

Scan on 27/08/2019 1505 by Amy Bajer : HIT Lumi example_Page_1.jpg (Not on chart)

Method: EDM UAH SPCOAG MANUAL METHOD Last

Scans

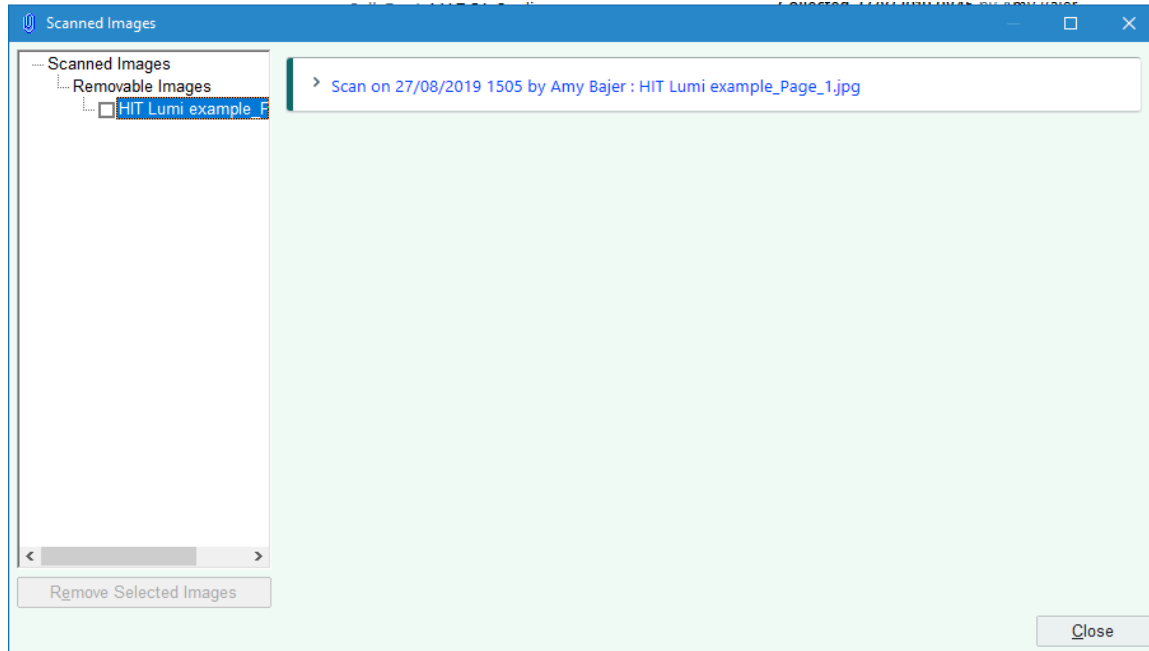
Scan on 13/09/2019 1604 by Amy Bajer : TEG example.jpg (On Chart)

2. While in Edit, click on the Scan icon and choose “View or removed scanned images”. Select the desired jpg file to view it.

Edit Save Next Clear Changes Repeat Method Mnemonic Keys Scan

(# Rslt) Component	Value
1 Heparin-Induced Thrombocytopenia, ELISA	Positive. PLEASE NOTE: Resu
2 Heparin-Induced Thrombocytopenia, Lumi-aggregrometry	(Equivocal)
3 Heparin-Induced Thrombocytopenia Interpretation	

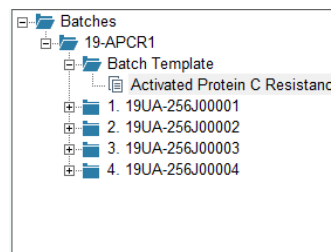
Scan Image View or remove scanned images



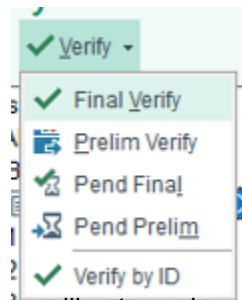
D. Final Verify to release results to the patient chart.

1. Open batch in result entry as per previous instructions.

2. In the left hand file tree, click on the test name under the "Batch Template".



3. Click on the Verify icon to Final Verify (or the arrow to the right of Verify if you want to select a different status).



4. If there are problems with the results or any holds, you will get warnings that you need to deal with or acknowledge before you can continue.

Final Verify

19UA-256J00001: Activated Protein C Resistance Routine X

Instrument ID: 1000083734

Beakerccvfa, Fourteen Epic Jr. (MRN 1500259187)

F, 40 yrs, 23/4/1979
Coll. Dept: UAH Lab

Blood, Blood, Venous
Collected Today 0816 by Amy Bajer
Containers: 1 BLUE 4.5ML
Draw type: Venipuncture

⚠ Not all required fields have been filled in.

Chart	Component	Value	Ref Range & Units
☒	Activated Protein C Resistance Interpretation		

Comments:
CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban.

Resulting Lab: EDM UAH LAB Test method: EDM UAH STA-R Evolution - 32 Status: Resulted

19UA-256J00002: Activated Protein C Resistance Routine X

Instrument ID: 1000083738

Beakerccvfc, Fourteen Epic Jr. (MRN 1500261183)

F, 10 yrs, 23/4/2009
Coll. Dept: UAH Lab

Blood, Blood, Venous
Collected Today 0817 by Amy Bajer
Containers: 1 BLUE 4.5ML
Draw type: Venipuncture

⚠ Not all required fields have been filled in.

Chart	Component	Value	Ref Range & Units
☒	Activated Protein C Resistance Interpretation		

Comments:
Stated reference range/ranges have been derived from normal adults (18 years and above) and may not apply to pediatric patients.
CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban.

Resulting Lab: EDM UAH LAB Test method: EDM UAH STA-R Evolution - 32 Status: Resulted

[Acknowledge Warnings](#)

[Go Back](#) [Confirm Final](#)

- Once warnings are removed, you can scroll through and double check entries if desired. If you do not want to verify a certain result, it can be removed from Final Verify by clicking on the X in the top right corner of that specimen.

Final Verify

19UA-256J00001: Activated Protein C Resistance Routine X

Instrument ID: 1000083734

Beakerccvfa, Fourteen Epic Jr. (MRN 1500259187)

F, 40 yrs, 23/4/1979
Coll. Dept: UAH Lab

Blood, Blood, Venous
Collected Today 0816 by Amy Bajer
Containers: 1 BLUE 4.5ML
Draw type: Venipuncture

Chart	Component	Value	Ref Range & Units
☒	Activated Protein C Resistance Interpretation	Normal. Factor V Leiden has been ruled out and molecular testing will not be performed.	Normal

Comments:
CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban.

Resulting Lab: EDM UAH LAB Test method: EDM UAH STA-R Evolution - 32 Status: Resulted

19UA-256J00002: Activated Protein C Resistance Routine X

Instrument ID: 1000083738

Beakerccvfc, Fourteen Epic Jr. (MRN 1500261183)

F, 10 yrs, 23/4/2009
Coll. Dept: UAH Lab

Blood, Blood, Venous
Collected Today 0817 by Amy Bajer
Containers: 1 BLUE 4.5ML
Draw type: Venipuncture

Chart	Component	Value	Ref Range & Units
☒	Activated Protein C Resistance Interpretation	Abnormal. Factor V Leiden molecular testing to follow. !	Normal

Comments:
Stated reference range/ranges have been derived from normal adults (18 years and above) and may not apply to pediatric patients.
CAUTION: The results of this test may NOT be valid if the patient is receiving any anticoagulant therapy other than warfarin or heparin (UFH and LMWH). The interfering drugs include, but are not limited to, Dabigatran, Argatroban, Lepirudin, Bivalirudin, Fondaparinux, Danaparoid, Rivaroxaban, Edoxaban and Apixaban.

Resulting Lab: EDM UAH LAB Test method: EDM UAH STA-R Evolution - 32 Status: Resulted

19UA-256J00003: Activated Protein C Resistance STAT X

Instrument ID: 1000083742

Beakerccvma, Fourteen Epic Jr. (MRN 1500258189)

M, 40 yrs, 23/4/1979

Blood, Blood, Venous
Collected Today 0818 by Amy Bajer

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- Click on "Confirm Final" to verify all selected specimens. Your name will be logged in the system as performing this task, it is not necessary to add the reviewed by comment.

