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Email: mainc@hhsc.ca
Michelina Bozzo, Editorial Assistant
Email: bozzom@hhsc.ca

🔴 Coagulation Screening Tests – What do we measure? 🔴

The prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and fibrinogen levels are routine coagulation screening assays that are performed in core laboratories (1). The PT (which is often reported as the International Normalized Ratio, or INR) assesses the extrinsic and common pathways of coagulation whereas the APTT assesses the intrinsic and common pathways (1). PT reagents contain a heparin neutralizer so the results for samples containing heparin are often normal unless there is a large amount of heparin (> 1 unit/ml) or other abnormalities (e.g. liver disease, vitamin K deficiency, warfarin) (2). The TT is a qualitative assay that uses thrombin to convert fibrinogen to fibrin and measures fibrin polymerization (1,2). The TT is sensitive to the effects of antithrombins, such as unfractionated heparin, and drugs that directly inhibit thrombin, such as dabigatran (2). The TT is also sensitive to quantitative (hypofibrinogenemia) and qualitative (dysfibrinogenemia) defects in fibrinogen and fibrinogen degradation products that impair fibrinogen polymerization (e.g. post fibrinolytic therapy) (2). It can also be prolonged by valproic acid therapy (3). The Clauss fibrinogen assay provides information on the level of clottable fibrinogen in plasma (2).

Drawing a coagulation sample into the wrong anticoagulant can result in striking laboratory abnormalities. EDTA reduces the ionized calcium level in plasma much more than the sodium citrate anticoagulant used to anticoagulate coagulation samples, and the abnormal results can mimic an inhibitor with a markedly reduced factor V and factor VIII level (4,5,6). Failure to properly mix or anticoagulate coagulation testing samples with sodium citrate can result in clotting ex vivo, which reduces or completely depletes fibrinogen and results in either markedly prolonged or non-coagulable PT, APTT and TT (6).

The PT, APTT, TT and fibrinogen levels are often used to screen for bleeding disorders and other coagulopathies that cause single and/or multiple factor deficiencies, including liver disease, vitamin K deficiency (which can reduce the levels of factors II, VII, IX and X), hemodilution and disseminated intravascular coagulation (DIC) (2,3). An isolated prolonged APTT, with a normal TT, excludes heparin as a cause. If the APTT prolongation fully corrects in immediate 1:1 mixing studies, the findings suggest a deficiency of either factor VIII, IX, XI and/or XII, and less commonly a deficiency of prekallikrein or high molecular weight kinogen. Prolongations of the APTT by

LAB
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Dr. Cheryl Main, Editor, Email: mainc@hhsc.ca; Michelina Bozzo, Editorial Assistant, Email: bozzom@hhsc.ca

contact factor deficiencies (factor XII, prekallikrein and high molecular weight kininogen) can be congenital or acquired but they do not cause bleeding. An isolated APTT, with a normal TT, that does not correct on an immediate 1:1 mixing study suggests the possibility of multiple intrinsic pathway factor deficiencies (e.g. low factor XI and XII from liver disease), a rapidly acting specific factor inhibitor, or a lupus anticoagulant (LA). The APTT test performed in the core laboratory should not be used to rule out a LA as APTT reagents vary considerably in their sensitivity to LA (7). In Hamilton, we currently use an APTT reagent that contains ellagic acid and a high concentration of phospholipid to limit false positive prolongations due to lupus anticoagulants. This can occur transiently in acutely ill patients or reflect phospholipid antibody syndrome. An isolated prolonged PT, with a normal APTT, reflects factor VII deficiency, which can be congenital or acquired, due to vitamin K deficiency, warfarin or liver disease.

Liver disease can result in an isolated prolongation of the PT (due to low factor VII, which has a short half-life), an isolated prolongation of the APTT (often due to lowering of factor XI and factor XII), a prolonged INR and APTT (due to lowering of multiple factors made in the liver), and when severe, fibrinogen levels may also be low. Factor VIII and fibrinogen can be elevated in early liver disease. A prolonged TT, with a normal PT and APTT, is a typical finding with a low or a dysfunctional fibrinogen as the PT and APTT have very poor sensitivity to fibrinogen defects even though clot detection requires fibrin formation (3).

The INR and APTT are often used to monitor anticoagulant therapy with warfarin and unfractionated heparin (UFH) respectively (8,9). Direct thrombin inhibitors can affect multiple coagulation tests, and can markedly prolong the TT (8).

Core laboratory coagulation tests, such as the PT, PTT, TT and fibrinogen level, are not recommended for routine preoperative screening as testing yields far more false positive findings than true positives from congenital or acquired bleeding diatheses (3). Our recent study indicated that when the PT, APTT, TT and fibrinogen assays are used in a panel for bleeding disorder evaluation (of subjects referred to a hematologist for a bleeding disorder assessment), the panel has reasonable specificity (98%) but poor sensitivity (3.7%) to identify bleeding disorders (3). This

is because factor deficiencies and fibrinogen disorders are important, but infrequent causes of bleeding disorders (3). When these tests detect abnormalities, additional investigations (e.g. testing for a factor deficiency) are needed to distinguish true from false positive abnormalities and to determine the nature of the defect. If the findings are normal and a bleeding disorder is suspected, additional tests may be required to investigate delayed bleeding problems (such as mild hemophilia, factor XIII deficiency and a fibrinolytic defect from α_2 antiplasmin deficiency) and immediate bleeding problems, which are often due to von Willebrand disease or a platelet function disorder. The sensitivity of bleeding disorder test panels is improved (to 8.5%) when a von Willebrand disease (VWD) screen is performed in addition to the PT, APTT, TT and fibrinogen level and the addition of light transmittance aggregation studies to these panel tests gives the highest sensitivity (30%) for bleeding disorders (3). Because testing for platelet disorders requires a hematology referral, we recommend that individuals suspected of having a bleeding problem should be referred to a hematologist, to evaluate the bleeding history (for features suggestive of congenital or acquired defects) and coordinate the specialized laboratory testing to properly work up a bleeding problem. Depending on the bleeding history and initial findings, additional tests for platelet disorders may be ordered (e.g. platelet dense granule secretion tests and tests for dense granule deficiency), or if appropriate, additional tests for factor deficiencies and fibrinolytic defects (α_2 plasmin inhibitor deficiency, genetic testing for the tandem duplication of the urokinase plasminogen activator gene in Quebec platelet disorder). About one third of patients that are suspected to have a bleeding disorder, based on their bleeding history, have no cause identified after detailed investigations.

While core laboratory coagulation tests are important for monitoring unfractionated heparin (using the APTT, and less frequently, factor Xa inhibition assays) and warfarin (using the PT/INR), routine laboratory monitoring is not indicated for several new anticoagulants, including Dabigatran (Pradaxa®), a direct thrombin inhibitor, and Rivaroxaban, a drug that inhibits factor Xa activity (8). Dabigatran and Rivaroxaban can sometimes prolong the PT and APTT but they do not have predictable effects on these tests

and normal findings do not exclude the possibility that the patient is anticoagulated (8,10). The TT is a much more sensitive test to determine if the patient's plasma contains Dabigatran (10). The TT is not affected by Rivaroxaban. Clottable fibrinogen levels are not influenced by direct thrombin or factor Xa inhibitors (9,11).

Table 1 summarizes the effect of various congenital and acquired bleeding disorders, DIC, anticoagulant therapy and pre-analytical sample issues on the PT, APTT, TT and fibrinogen. This table also shows the results for the bleeding time, however laboratories have been advised to abandon performing bleeding times due to inability to control the assay and observations that more specific tests for defects in primary hemostasis have much greater sensitivity (12,13). As the routine coagulation tests are influenced by many factors, including pre-analytical issues and anticoagulant therapy, a proper interpretation of the findings needs to consider both the clinical picture and potential diagnoses.

Karen A. Moffat BEd, FCSMLS(D), Supervisor, Special Hematology; Technical Specialist, Coagulation

Catherine P.M. Hayward MD, PhD, Head, Coagulation, HRLMP

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Table 1: DIFFERENTIAL DIAGNOSIS OF COAGULATION DISORDERS

	PT (neutralizes up to approx. 1 U/ml heparin)	APTT	Thrombin Time	Fibrinogen	Bleeding time (not recommended)	PFA-100® Closure time	platelet count
Normal	10-13 sec	Ranges vary	Ranges vary	1.5-4.0 g/L	1-9 minutes	Ranges vary	150-400x10 ⁹ /L
Fibrinogen deficiency or dysfunction	N - ↑	N - ↑	↑	↓	N	N	N
Factor VII deficiency	↑	N	N	N	N	N	N
Factor VIII, IX, or XI deficiency	N	↑	N	N	N	N	N
Factor II-, V-, X-deficiency	↑	↑	N	N	N	N	N
Factor deficiencies not associated with bleeding (Factor XII, High Molecular Weight Kininogen or Prekallikrein deficiency)	N	↑	N	N	N	N	N
Acquired hemophilia & congenital hemophilia with inhibitors	N	↑ †	N	N	N	N	N
Lupus anticoagulant	N - ↑	N - ↑ ‡	N - ↑	N	N ‡	N	N ‡
Heparin - therapy or sample contamination	N - ↑	↑	↑↑*	N	N (↑ with massive doses)	N	N
Direct thrombin inhibitors	N - ↑	N - ↑	↑↑	N	-	-	N
Factor Xa inhibitors	N - ↑	N - ↑	N	N	-	-	N
Liver disease (factors affected 1 st : often VII, XI and XII; low fibrinogen often reflects severe/late effects)	N - ↑	N - ↑	N - ↑	↓ - N - ↑	N - ↑	N - ↑	↓ - N
Vitamin K deficiency	↑	N - ↑	N	N	N	N	N
Fibrinolytic therapy	↑	↑	↑	↓	N (↑ if on ASA)	N (↑ if on ASA)	N
Consumptive coagulopathy	N - ↑	↑	N - ↑	N - ↓	N - ↑	N - ↑	N - ↓
Dilutional coagulopathy	N - ↑	N - ↑	N - ↑	↓ - N	N - ↑	N - ↑	↓
von Willebrand Disease	N	N - ↑	N	N	N - ↑	N - ↑ (↑ usually with both cartridges)	N (↓ in type 2B and platelet type)
Thrombocytopenia	N	N	N	N	N - ↑	N - ↑	↓
Thrombocytopathies	N	N	N	N	N - ↑	N - ↑	↓ - N
Preanalytical error – plasma collected in EDTA [§]	↑	↑	N - ↑	N	-	-	-
Preanalytical error – serum instead of plasma	NC	NC	NC	↓	-	-	-

↑ = elevated levels ↓ = reduced levels

N = normal

NC – no clot

† APTT of an immediate 1: mix of patient and normal plasma can be N to ↑ depending on the features of the specific factor inhibitor. Diagnoses should be made by factor assays and special tests for specific factor inhibitors – mixing studies are just a general guide and will show partial correction with multiple factor deficiencies

‡ APTT reagents have different sensitivities to detect lupus anticoagulants. APTT of 1:1 mix of lupus anticoagulant plasma and normal plasma are usually ↑; the diagnosis should be made by requesting special tests for these inhibitors. Some patients with associated phospholipid antibodies may have thrombocytopenia and prolonged bleeding times

*corrects with heparin neutralization & reptilase time is normal

Note: mild factor deficiencies can be missed by the INR and APTT

§Can mimic combined factor V and VIII deficiency. 1:1 mixing studies typically show partial correction. At higher dilutions, assays for specific factor inhibitors show no inhibitor but low dilutions may suggest a high titre inhibitor is present. Thrombin time is normal if the reagent used contains calcium.

Education News:

The Laboratory Medicine resident training programs are all preparing for their upcoming Internal Reviews.

The dates of the reviews are:

Anatomical Pathology	October 23, 2012
Medical Microbiology	October 29, 2012
General Pathology	Date pending
Medical Biochemistry	Date pending

Your continued support of these programs is greatly appreciated!

For information and the latest news on our residency training programs follow the link: <http://fhs.mcmaster.ca/pathres/news/index.html>

Information on the postdoctoral fellowship:

<http://fhs.mcmaster.ca/pathology/education/postdoctoralfellowshiptraining.html>

Faculty Education:



HAMILTON REGIONAL LABORATORY MEDICINE PROGRAM

**5th Annual HRLMP Rapid Fire Showcase
"Getting Back to Basics"**

This half-day session will be offered

**Saturday November 3, 2012
8:15 am - 12:15 pm**

** Registration @ 7:45 am
Light Breakfast included

Miller Amphitheatre - 2nd floor Juravinski Innovation Tower
St. Joseph's Healthcare Hamilton
50 Charlton Ave. E. Hamilton, Ontario



Get answers to the questions you've been too afraid to ask...or have forgotten....

1. Polymerase Chain Reaction (PCR)
2. Acid/base Chemistry
3. Coagulation Cascade
4. Fluorescence in situ Hybridization (FISH)
5. Microbiology basics.....and so much more!

** Register Early at <http://tinyurl.com/Rapid-Fire-Showcase>
Registration Deadline: October 26, 2012



The Digital Laboratory: Today and Tomorrow

October 27, 2012
Mohawk College, Hamilton, ON

Organized by Hamilton Regional Laboratory Medicine Program (HRLMP), Quality Management Program—Laboratory Services (QMP-LS) and the Institute for Quality Management in Healthcare (IQMH).

Register today:
www.iqmh.org/
TheDigitalLaboratory

Staffing Updates:

Thank you to **Karen Dunnam** who previously held the position of Coordinator of Transportation and Safety. Karen has left the HRLMP and we wish her all the best! **Andrea Tjahja** has assumed this role effective September 19, 2012.

News from Chemistry:

The Canadian Society of Clinical Chemists 2012 Traveling Lectureship took place on Wednesday October 17, 2012 in A4-4 at the Juravinski Hospital

MicroRNAs and other non-coding RNAs as biomarkers
Presented by Dr. George A. Calin, MD, PhD

Mapping epigenomic miRNA and DNA methylation profiles using microarrays Presented by Dr. Bekim Sadikovic

Tracy Carrier has assumed the role of **Acting Supervisor for Point of Care Testing (POCT)**. Congratulations Tracy!

The Annual E.S. Garnett Memorial Lecture will take place on Tuesday October 23, 4:30-5:30, MUMC 1A5.

Dr. Raman Chirakal will be presenting:
Different Avatars* of ¹⁸F-Fluorodopa: A Multi-Purpose, Multi-Target PET Tracer for Diagnostic Imaging.

The specimen requirements for **C - reactive protein** (CRP) have changed. The test now requires:

Adult: 4ml blood in a green heparinized tube

Pediatric: 200µl (0.2ml) blood in a green microtainer

News from Pathology:

ANATOMICAL PATHOLOGY GRAND ROUNDS 2012 / 2013

TIME: 12:30 - 1:30 p.m.

DATE: 2012	SPEAKER:	TOPIC:
September 12 th MDCL – 2232	Dr. R. Kandel Mount Sinai Hospital University of Toronto	Keratin Positive Soft Tissue Tumors: a pathologists dilemma.
October 17 th MDCL – 2232	Dr. A. Pollett Mount Sinai Hospital University of Toronto	Personalized Medicine in GI Oncologic Pathology
November 7 th MDCL – 2232	Dr. V. Chen McMaster University	FNA Salivary Glands for Surgical Pathologists
December	Holiday Season	No Rounds

If you cannot attend Rounds you can join the Anatomical Pathology Grand Rounds live on:

https://maclive.mcmaster.ca/join_meeting.html?meetingId=1265491829299

Dr. Kathryn Urankar joined the forensics team in early September. Dr. Urankar joins us from Australia, where she worked as a forensic pathologist and neuropathologist with the Queensland Health Pathology Services at the John Tonge Centre and the Royal Brisbane Hospital. The forensics group is very pleased with the arrival of Dr. Urankar.

Dr. Harkiran Kaur, has taken on a full time permanent position in anatomical pathology after completing fellowships in breast and gynecological pathology at the University of Toronto. Dr. Kaur replaces Dr. Dean Daya at the JCC site. Welcome Dr. Kaur!

News from Microbiology:

The 2011 Antibigram data for HHS/SJH is now available on the HHS Antimicrobial Stewardship Program web site which can be accessed using the link below:

<http://corpweb.hhsc.ca/body.cfm?id=3056>

News from Special Hematology:

Dr. Ted Warkentin and Dr. Sam Schulman presented the 5th Coagulation Lecture in honour of Dr. Bernadette Garvey, hosted by the Division of Hematology and Oncology at St. Michael's hospital in Toronto. Their presentation on October 10, 2012 was titled "HIT and factor VII inhibitors: two threatening immunological disorders in hemostasis and thrombosis".



Congratulations to **Dr. Mark Crowther** who has been appointed the Associate Chair of Finance and Administration for the Department of Medicine.

Congratulations to **Dr. Jeff Weitz** who has recently been appointed the Associate Chair of Research for the Department of Medicine.

Celebrating Research Success!



Dr. Mark Loeb

Since arriving in Hamilton, Mark Loeb has been fortunate to work with colleagues such as HRLMP members Dr. Marek Smieja and Dr. Jim Mahony on aspects of respiratory viral infections and more recently with Dr. Guillaume Pare on genomics projects. He has also been fortunate to have outstanding research staff, all of which has resulted in continuous funding from CIHR.

A long-standing interest in studying infections in the elderly led to a cluster randomized controlled trial of a clinical pathway to manage nursing home residents on site in the nursing home (1). The trial showed similar clinical outcomes despite site of care and that there is a substantial cost savings (up to \$70 million in Canada and \$831 million in the US) to using the clinical pathway.

More recent research has focused on influenza. This includes a cluster randomized trial of Canadian Hutterite children and adolescents who received either influenza or hepatitis A vaccine, as a control, and community members who did not receive study vaccine in Hutterite colonies in Alberta, Saskatchewan, and Manitoba. Among vaccine non recipients, the protective effectiveness was 61% and 59% among all participants (2). This study offers the most rigorous epidemiological proof for the indirect benefit of vaccinating children against influenza. The study received wide attention,

including an article in the New York Times, and was selected as **2010 paper of the year** by The Lancet.

Another influenza study was a multi-centre randomized trial comparing the effect of N95 respirators to surgical masks in protecting nurses in acute care hospitals from laboratory confirmed influenza (3). This study was influential in the debate of how to protect healthcare workers and was cited by public health authorities in Canada (Public Health Agency of Canada) and CDC in the U.S. It was selected as **one of the 10 most important infectious diseases papers in 2009** by the Infectious Diseases Society of America and one of the most influential infection control papers of the decade by the Society for Healthcare Epidemiology of America.

Another area of interest has been flaviviruses. With funding from the NIH, Dr. Loeb's team led a genome wide association study to assess genetic variants associated with neuroinvasive disease due to West Nile virus. Frequencies of single nucleotide polymorphisms between West Nile neuroinvasive cases and non-neuroinvasive controls showing that a SNP in the gene RFC1 is a potential genetic variant for neuroinvasive disease (4). Current work includes a large NIH study to assess genetic variants associated with severe dengue infection in seven countries in Central America and Southeast Asia as well as another randomized trial in the Hutterite community to compare herd effects of live versus inactivated influenza vaccine.

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Happy Halloween!



from the HRLMP

