LifeLabs®
Medical Laboratory Services

Inside Diagnostics

SCREENING PROGRAM REACHES ONE-YEAR MARK

ColonCancerCheck

The ColonCancerCheck (CCC) program is one-year-old! This is a free colon cancer detection program for Ontarians over 50 years sponsored by the Ministry of Health and Long-Term Care and Cancer Care Ontario that began in April 2008. LifeLabs is supporting CCC by providing fecal occult blood testing. Over the last year, LifeLabs, working with caregivers across the province, has successfully screened thousands of people using mail-in fecal occult blood test (FOBT) kits. For information on how to order free kits for your office visit **www.lifelabs.com** and click on "New Tests" and select *"ColonCancerCheck – Fecal Occult Blood Testing"* or go to: www.lifelabs.com/Lifelabs_ON/News/MDSNews 02250801.asp

About Colon Cancer

Colon Cancer is the second most deadly cancer and Ontario has one of the highest rates in the world. This cancer is highly treatable if detected early through screening. Studies have shown that fecal occult blood testing performed every two years can reduce death from colon cancer by an average of 16% over 10 years of screening. Nearly 80% of susceptible people in Ontario have not been screened for this preventable cancer.

IN THIS ISSUE

CAUTION WHEN ORDERING TESTS WITH THE SAME NAME

Of the two Factor V tests (Factor V Leiden and Factor V Activity) "Factor V Leiden" is the more frequently ordered test in both the hospital and community settings. Factor V Leiden is the most frequently detected inherited abnormality in patients presenting with unexplained venous thrombosis. It is due to a specific point mutation in the Factor V gene. This mutation does not affect the ability of Factor V to clot, but, rather, renders it resistant to breakdown hence promoting thrombosis. Factor V Leiden is tested for by examining DNA harvested from blood cells collected in an EDTA (lavender top) tube.

On the other hand, Factor V Activity is a reflection of the clotting ability of Factor V. This is measured on coagulation analyzers by testing citrated plasma (light blue top tube). "Factor V Activity" can be reduced because of congenital deficiency, DIC, hemodilution, liver disease, or rarely autoantibodies. EDTA plasma cannot be used for clotting assays. Similarly, citrated plasma contains no DNA and cannot be used for detection of mutations.

When ordering one of these two tests, it is imperative that the full name of the test be indicated. Requests marked with "Factor V" only, WILL BE REJECTED.

LABORATORY DETECTION OF NEISSERIA GONORRHOEAE AND CHLAMYDIA TRACHOMATIS

Epidemiology:

In the United States, infections caused by *Chlamydia trachomatis* (CT) or *Neisseria gonorrhoeae* (GC) were the two most common notifiable diseases in 2006 and rates of GC

continued to increase for the second year in a row in 2006 [1]. A similar trend has occurred in Canada [2] where the rate of GC increased 94% from its lowest point in 1997 to a national rate of 28.9 cases per 100,000 in 2004. Males between the ages 20-29 are the most affected. In females 70% of all cases are in the age group 15 to 24. Rates of GC have increased in men who have sex with men (MSM).

Clinical Presentation:
GC and CT cause an acute urethritis in males. In females, GC causes an endocervitis with associated urethral infection in 70-90% and 20% develop an ascending infection such as pelvic inflammatory disease (PID). Infection of the oropharynx and anorectal area can occur related to oral or rectal genital contact. Disseminated infection occurs infrequently and can involve the joints, skin, and rarely endocarditis and meningitis.

CT causes cervicitis and ascending infection is a significant cause of infertility. CT and GC can be transmitted from a pregnant infected mother to the neonate.

C. trachomatis serovars L1, L3, or L3 cause lymphogranuloma venerum (LGV), a disease more commonly seen in Africa, Asia and South America and rarely in North America. However outbreaks have been reported in the US often in MSM and at rectal sites.

Indications for Screening

Primary screening is an essential component of prevention and control of sexually transmitted diseases as GC and CT infection can be asymptomatic in females. A summary of screening recommendations is as follows:

- Screening for CT is recommended in sexually active females < 25 years, males and females with risk factors, and routinely in all pregnant women [3].
- Screening for GC is recommended in sexually active women including those who are pregnant who are at risk for infection: age < 25 years, previous GC or other STD, new or multiple sexual partners, and inconsistent condom use.
- There is insufficient evidence to recommend for or against routine screening for GC in pregnant women [4]. In contrast, routine prenatal screening for both GC and CT is recommended by the Canadian Paediatric Society [5]
- Routine screening for GC and CT is recommended in sexually active men who have sex with men

Secondary screening of patients who have had GC or CT is recommended 3-6 months after treatment for the initial infection due to the risk of re-infection [3, 6].

In patients that present with symptoms suggestive of a STD, testing for both CT and GC is recommended.

Laboratory Methods:

The nucleic acid amplification method (NAA) used at LifeLabs to detect CT and GC from genital and urine specimens is the ProbeTec Strand Displacement Amplification method (BD Diagnostics). Similar to other NAA methods, small amounts of nucleic acid are amplified and detected including non-viable organisms. GC culture from genital specimens is also available.

For detection of CT, a NAA is preferred over culture due to the increased sensitivity [3]. For GC, testing by either NAA or culture is acceptable. Both CT and GC can be ordered on the same ProbeTec swab and urine sample. Advantages of NAA testing include collection of a non-invasive specimen (urine) and stability of the organisms despite transport distances. Viability of the GC in swab transport systems for culture decreases after 12-24 hours. Culture for GC is preferred when antimicrobial susceptibility testing is required and for treatment failure. For both CT and GC, culture is recommended for non-genital sources such as eye, rectal, pharynx which are not sources approved for testing using any NAA method. In cases of sexual assault a culture method with or without a NAA may be required.

For males, urine specimens are preferred for NAA. The sensitivity using the ProbeTec is similar to urethral swabs (>90%) and is more acceptable to the patient. For females, the sensitivity of urine compared to swab is slightly lower (85% versus 95%) [7, 8].

If a test of cure is required and a NAA method is used, testing should be delayed for 3 weeks to avoid false positive results due to non-viable organisms.

Please refer to the Ministry of Health Specimen Collection Guide for information on detection of LGV.

Management

Due to the emergence of fluoroquinolone resistance in GC in both the US and Canada, treatment guidelines have recently been revised. In Canada, the resistance rates were >15% in 2005. Ontario rates are also increasing with quinolone resistance in 28% of isolates sent for culture.(9) Quinolones (Ciprofloxacin and oflaxacin) are not recommended for the empiric treatment of GC. For further information on management of CT and GC refer to Canadian Guidelines on Sexually Transmitted Infections.

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SEROLOGY TESTING FOR VIRAL HEPATITIS

The vast majority of all viral hepatitis is caused by hepatitis A (HAV), B (HBV), and C (HCV). Hepatitis D and E are uncommon in Canada.

	Serological Tests Available	Acute Hepatitis	Chronic Hepatitis	Immunity	Unclear History
Hepatitis A (HAV)	anti-HAV Total			√	√
	anti-HAV IgM	√			√*
Hepatitis B (HBV)	HBs Ag	√	√		√
	anti-HBc Total				√
	anti-HBc IgM	√			
	anti-HBs			√	
	HBe Ag		√**		
	anti-HBe		√**		
Hepatitis C (HCV)	anti-HCV	√	√		√

** (secondary marker, to be ordered only if anti-HAV Total is detected) **(not to be used for diagnosis but to aid in treatment decision)*

When ordering hepatitis serology markers, select the most appropriate option on the OHIP requisition, as follows:

- Acute Hepatitis
- Chronic Hepatitis
- Immunity
  - Hepatitis A
  - Hepatitis B

If **"Acute Hepatitis"** is selected, the initial test performed is ALT. In the absence of elevated ALT (ie: ALT is less than 1.5 times the upper limit of the reference limit), no further testing of acute hepatitis will be performed.

If the ALT value is elevated, anti-HAV IgM, HBs Ag, and anti-HCV will be performed. If the HBs Ag is negative, anti-HBc IgM will be performed to identify those cases of acute Hepatitis B in which anti-HBc IgM is present at an earlier stage than HBs Ag.

Because of the prolonged period of seroconversion, anti-HCV antibodies may not be detectable during the acute stage, when symptoms are present. It may be necessary to specifically test for anti-HCV one to three months later.

If **"Chronic Hepatitis"** is selected, the tests HBs Ag and anti-HCV will be performed. There is no chronic form of Hepatitis A.

Hepatitis B e antigen (HBe) and the antibody to this antigen (anti-HBe) are most useful to help in the decision whether to offer treatment to patients with chronic hepatitis B.

If **"Immunity"** is selected, anti-HAV Total will be tested for Hepatitis A and anti-HBs will be tested for Hepatitis B.

Both of these antibodies can become detected in vaccinated subjects or patients with past infection, however the individual tests do not differentiate between the 2 scenarios. The hepatitis options available on the OHIP requisition are intended to simplify the ordering process, however, specific hepatitis serological markers can be ordered individually, if required. The table below summarizes the situations for which each test is the most helpful.

DOUG TKACHUK MD, FRCPC MEDICAL DIRECTOR, ONTARIO, LIFELABS

Dr. Doug Tkachuk obtained his medical degree at the University of Manitoba in 1982. Doug then spent three years working in family practice in northern and remote communities, including Churchill, Manitoba and the surrounding Arctic regions; Kenora, Ontario; and Shelburne, Nova Scotia. He completed his residency in Anatomical Pathology at the University of Toronto in 1989. Since then, Doug has held a variety of research, teaching and clinical pathology roles in Canada and the United States.

Most recently, Doug was hematopathologist at the University Health Network's Toronto General Hospital (TGH), as well as Associate Professor, Department of Laboratory Medicine and Pathobiology at University of Toronto. While at TGH, he became familiar with LifeLabs, as a reference laboratory conducting flow cytometry. In January 2009, Doug joined LifeLabs as Medical Director, Ontario.

He continues to be actively involved in textbook writing. The second edition of his "Wintrobe's Atlas of Clinical Hematology" is due to be published by Lippincott in 2010 and translated into three languages. Doug is also the president and founder of Objective Pathology, an organization which helps to educate the industry about how information technology affects laboratory testing through developing and distributing educational and diagnostic digital pathology products.

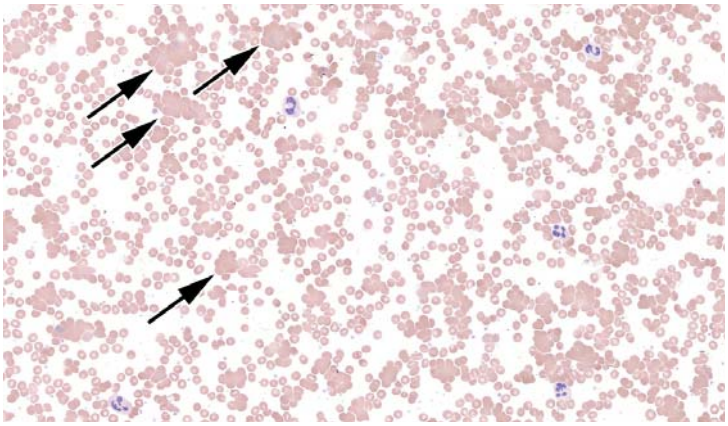
In his spare time, Doug enjoys cooking, photography, and participating in sports such as snowboarding, cross-country skiing, and windsurfing. Doug is married with two children aged 7 and 10.

PROTHROMBIN TIME/INR ALERT: FALSE ELEVATIONS CAUSED BY A NEW ANTIBIOTIC, DAPTOMYCIN (CUBICIN)

Daptomycin (trade name Cubicin) is a lipopeptide belonging to a new class of antibiotics. It is a bactericidal antibiotic exerting its effect through disruption of the cell membrane and is administered by intravenous injection only. While it is used primarily in the hospital setting, it is possible that patients may continue to receive it, through indwelling central lines following discharge from hospital.

It is now known that clinically relevant plasma levels of daptomycin can cause a significant concentration-dependent false prolongation of the prothrombin time (INR) when measured with certain reagents. This is true both for normal plasma, as well as plasma from patients on oral anticoagulants. The mechanism for this interference has not been elucidated yet but is clearly an in-vitro phenomenon only. Such interference and false elevation of the INR has been reported for the reagent used at LifeLabs.

Although, as stated above, this does not cause an in-vivo effect, it does create a problem for the monitoring of oral anticoagulation with warfarin. In this circumstance, the INR should be followed in a laboratory whose reagent is not affected by daptomycin, or the patient should be switched to treatment with low molecular weight heparin with re-institution of oral anticoagulation after the antibiotic has been stopped.



Photomicrograph of a blood film at low magnification showing agglutination of red cells (arrows) from cold agglutinins. The agglutinated red cells in this case disappeared upon warming to 37C.

COLD AGGLUTININS AND THE CBC

Cold agglutinins are cold reacting IgM anti red blood cell (RBC) antibodies that cause RBC agglutination at low temperatures (room temperature or lower). They occur frequently in the healthy population, most commonly in response to viral or mycoplasma infections, in which case they tend to be transient and usually not cause immune hemolysis.

They can also occur in autoimmune diseases, malignant conditions, or be idiopathic. To cause in vivo hemolysis, these antibodies have to be of a sufficiently high titer (> 1:64), have high thermal amplitude (i.e.: agglutinate RBC at temperatures close to 37 C), and then fix complement.

Laboratories encounter cold agglutinins frequently in the course of performing a CBC. It is often suspected when the MCHC is elevated with or without an elevation in the MCV/MCH, and/or when there is discordance between the RBC count and the hemoglobin value. Examination of a blood smear will confirm the presence of agglutination, which looks very different than rouleaux formation (see photomicrograph).

Before releasing any results, the CBC and blood smear are repeated after a period of incubation of the blood in a 37 C water bath. One of three different scenarios may then occur depending on the characteristics of the antibody.

In most cases encountered in the laboratory, the CBC and smear abnormalities disappear completely, and the results are released with a message indicating that analysis was done after a period of warm incubation with dispersion of the agglutinates.

Less frequently, some agglutination persists on the blood smear, but this usually does not affect the CBC results which are reported, however, with a cautionary message.

Lastly, there are situations where the agglutination is quite strong and only a partial CBC report can be provided. In this latter circumstance, results for the WBC, hemoglobin, and platelets will be provided with a cautionary message, however, no MCV, MCH, MCHC values can be given.

FAREWELL TO DR. WAHBI HAMMOUDA

Please be advised that Dr. Wahbi Hammouda has left LifeLabs to pursue other opportunities. Dr. Hammouda is now at St. Michael's Hospital in Toronto as Head, Division of Hematopathology, Department of Laboratory Medicine. In addition to responsibility for the laboratory there, Wahbi will have teaching and research responsibilities. LifeLabs extends best wishes to Wahbi in his new role. Should you have questions regarding Hematology, please contact Dr. Doug Tkachuk, Medical Director at 416-675-4530 ext. 2990 or doug.tkachuk@lifelabs.com.

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