Inside Diagnostics

TO FAST OR NOT TO FAST... LIFELABS' REVISED PATIENT PREPARATION FOR SAMPLE COLLECTION

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The need for fasting presents a significant inconvenience to patients, is a challenge for some (particularly seniors, children and diabetic patients), and impacts compliance. LifeLabs' recent review of collection methods, the scientific literature, and clinical guidelines has led to changes to patient fasting requirements for a number of assays.

Table 1 lists tests that must still be performed using a fasting specimen and those for which a fasting specimen is preferred but not required. All others may be collected using a non-fasting specimen.

Table 1: LifeLabs' Fasting Collection Conditions for Select Tests*

Fasting Required	Fasting Preferred
Adiponectin	Apolipoprotein E
Bile Acids	C1Q Binding Activity
Free Fatty Acids	Calcitonin
Gastrin	Calcium
Gestational DM Confirmation	C-Peptide
Glucose Fasting	Cryofibrinogen
Glucose Tolerance Test	Cryoglobulins
Growth Hormone	Homocysteine
Insulin Fasting	Phosphate
Insulin Glucose Challenge Test	Protein Electrophoresis
Lactose Tolerance Test	
Lipoprotein (a)	
Urea Breath Test	

**Please refer to Healthcare Clients Ontario – Test Information Directory on the LifeLabs Ontario Website (http://tests.lifelabs.com) for detailed information on pre-test preparation instructions, including duration of fasting required for each test listed above.*

Changes to Patient Preparation Recommendations for Lipid Testing

Note the absence of lipids in the table. There are a number of arguments in the literature both for and against the need for fasting samples to determine lipid levels. Many research groups have studied and validated the usefulness of non-fasting lipid measurements - some suggest non-fasting specimens may actually be better at predicting cardiac risk and in making therapeutic decisions.[1,7] Other authors have recorded the reasons why fasting levels are still needed.[8-10] A summary follows when considering the option of using non-fasting specimens:

What is the Rationale for Use of Fasting Specimens for Lipid Assessment?

The majority of clinical guidelines and clinical intervention trials for lipids, completed with diabetic and non-diabetic populations, are based on fasting samples. Trials focused on monitoring of statin therapies have used fasting lipids for the most part although two major trials have published study outcomes using non-fasting specimens.[11, 12]

The 2012 Canadian Cardiovascular Society (CCS) Guidelines for the Diagnosis and Treatment of Patients with Dyslipidemia classify patients and treatment goals based on risk category and lipid measurements.[3] Treatment approach and targets are defined for each cardiovascular disease (CVD) risk category based on LDL-Cholesterol (LDL-C), non-HDL-Cholesterol (non HDL-C) and apolipoprotein B (Apo B) concentrations. LDL-C concentration is calculated by Friedewald equation, which includes measured triglycerides, total cholesterol (TC) and HDL-C levels. Food intake influences measured triglyceride levels and therefore calculated LDL-C.

What is the Rationale for Use of Non-Fasting Specimens for Lipid Assessment?

The CCS 2012 guidelines endorse non-HDL-C as a new lipid target. Non-HDL-C is calculated by subtracting HDL-C from total cholesterol and reflects the total cholesterol concentration transported within atherogenic lipoproteins. Non-HDL-C is unaffected by fasting status, lending support to use of non-fasting specimens.

In 2012, Sidhu and Naugler reported outcomes from a large community based population study (n=209,180 with 53% females and 47% males) and found differences between fasting and non-fasting measurements for triglycerides to be less than 20% and the LDL-C to be less than 10%.[4] Triglycerides increase at hours 2-6 post meal and then decline to baseline levels but differences were not statistically significant. Calculated LDL-C levels were decreased up to 3 hours post meal, but were also not statistically significant from fasting values. The authors illustrated that the change in measured total cholesterol and HDL–C for a fasting vs. non-fasting specimen is approximately 2 %. After 1 hour fast, no statistical differences between fasting TC, HDL-C, LDL-C were observed. These data reflect an earlier study by Langsted *et al.* [6]

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What is the Clinical Impact of Using Non-Fasting Lipid Measurements?

Epidemiological data illustrate that non-fasting data may be a more significant predictor of CVD, independent of post prandial time.[1] Since patients spend most of their day in a postprandial state, use of non-fasting lipids has been determined by most to be acceptable for initial assessment.

Other important considerations when making the decision to use and interpret non-fasting lipid results include:

- **CVD Risk Calculation:** Since the Framingham CVD risk calculations are based on total and HDL-C, the small change in fasting vs. non-fasting values will have minimal impact to the risk score.
- **CVD Prognosis:** After adjustment for risk-factors in a study of 2,809 male subjects, Eberly reported that non-fasting and fasting triglycerides produced very similar hazard ratios for 25 year mortality, and for 8 year fatal or nonfatal Congestive Heart Disease CHD, making non-fasting samples equally valuable.[5]
- **Triglyceride and LDL-C Concentrations:** Non-fasting triglyceride levels may be up to 0.20 mmol/L higher than fasting levels on matched patients with normal food intake, leading to lower calculated LDL-C.[2] The triglyceride bias appears to be acceptable in the general population, but increasing concentrations of triglyceride leads to more significant negative bias in LDL-C. The correlation between fasting and random LDL-C is found to be worse if triglycerides are greater than 2.08 mmol/L.[8]
- **Diabetic Patients:** In 2011 Lund reported on the outcomes from a study of 66 type 2 diabetics. This work suggested that up to 38% of diabetic patients, and 63% of those on statins (n=8), would be misclassified as at decreased risk for CVD, when using non-fasting sample analyses.[8]
  Another study group evaluated 58,434 individuals including 2,270 diabetics and reported no excessive lipemia following normal food intake in those with diabetes.[2] A decrease in LDL-C (-0.3 mmol/L in non-diabetics and -0.5 mmol/L in diabetics) was found to be associated with food combined with hemodilution due to fluid intake.[4] These results were supported by Van Dieren's study.[7] This 8 year review of 1,337 individuals with diabetes concluded that cardiovascular risk assessment was not affected by post prandial collections and, with the exception of triglycerides, changes to total cholesterol, HDL-C and LDL-C were not significant after a normal meal.

When are Fasting Samples for Lipid Analyses Still Recommended?

Good clinical judgment is encouraged to appropriately instruct patients on fasting vs non-fasting requirements for lipid testing in consideration of their individuals' clinical presentation and care.
Continued use of fasting specimens should be considered when:

- Assessing those at high risk for CVD.
- Initiating or altering drug therapy. A fasting specimen is recommended as clinical interventions are presently based on fasting lipid measurements.
- Non-fasting triglycerides are greater than 2.00 mmol/L. This warrants review and fasting analyses to rule out metabolic syndrome, familial hyperlipidemia or diabetes.

Will There Be Changes in Result Reporting?

Modifications to patient lipid reporting will be implemented including:

- Number of hours fasting will be included on the patient's lipid report to facilitate interpretation
- Non-HDL-Cholesterol calculation will be added to all reports
- Interpretative messages, primary and alternate lipid treatment targets will be updated to reflect CCS 2012 guideline (Table 2)
- Ratio of Apo A1/ Apo B will be removed from the report.

Note: TC/HDL-C ratio is not included in the 2012 CCS guideline as a treatment target although it is still recognized as a significant risk indicator if > 6.0.

Table 2: Summary of the 2012 Canadian Cardiovascular Society Treatment Initiation Levels and Lipid Treatment Target Values.[3]

10 Year Risk of CVD	Consider Initiation of Therapy	Primary Lipid Treatment Targets	Alternate Lipid Treatment Targets
High Risk High risk features* FRS ≥ 20%	Consider in all	LDL-C < 2.00 mmol/L *or* ≥ 50% decrease in LDL-C	Non-HDL-C ≤ 2.6 mmol/L Apo B ≤ 0.80 g/L
Intermediate Risk No high risk features FRS = 10% -19%	A. LDL-C ≥ 3.50 mmol/L *OR* B. LDL-C < 3.50 mmol/L *Consider if* Non-HDL-C ≥ 4.3 mmol/L *or* Apo B ≥ 1.2 g/L	LDL-C < 2.00 mmol/L *or* ≥ 50% decrease in LDL-C	Non-HDL-C ≤ 2.6 mmol/L Apo B ≤ 0.80 g/L
Low Risk No high risk features FRS < 10%	LDL-C ≥ 5.00 mmol/L	≥ 50% decrease in LDL-C	

**Risk features: clinical vascular disease, abdominal aortic aneurysm, diabetes, Chronic kidney disease (CKD), high risk hypertension (HT).*
CKD is defined as: *eGFR < 45 or Albumin Creatinine Ratio (ACR) ≥ 30 or eGFR < 60 with an ACR ≥ 3.*
High risk HT is defined as: Hypertension plus 3 risk factors.
FRS: *Framingham Risk Score*

POINTS TO REMEMBER

- **Patient reports will be revised to include the CCS 2012 target values for treatment assessment.**
- **Non-HDL-C, total cholesterol, and HDL-C are unaffected by fasting status, making non-fasting sample collection an option for select patients. Physicians are encouraged to instruct their patients about fasting vs. non-fasting requirements for lipid testing in consideration of patients' clinical presentation and care.**
- **Fasting is still recommended for those at high risk for CVD, or at initiation or change in statin therapy.**

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LIFELABS OFFERS PANORAMA™ NON-INVASIVE PRENATAL TESTING

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Effective September 9, 2013 LifeLabs is pleased to offer Panorama™, the non-invasive prenatal test, to its clients.

What is a Non-Invasive Prenatal Test (NIPT)?

Non-invasive prenatal testing is a new, highly accurate screen for specific chromosome abnormalities like trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome), and monosomy X (Turner syndrome).

How Does It Work?

During pregnancy a small amount of cell-free fetal DNA (cffDNA) crosses into the mother's bloodstream. Panorama™ uses sophisticated DNA sequencing technology and bioinformatics to analyze the fetal DNA in the mother's blood and detect aneuploidies (abnormal chromosome numbers).

How Early In Pregnancy Can Panorama™ Be Done?

The test can be done on a maternal blood sample as early as 9 weeks gestation.

Who Should Be Tested?

The leading American and Canadian prenatal associations (ACOG[1], SOGC[2]) have recommended that NIPT be offered as a screening test to women at increased risk of fetal aneuploidy.

Indications for NIPT include:

- advanced maternal age (≥35years of age or ≥40 years of age, depending on the province)
- a positive prenatal serum screen
- personal or family history of aneuploidy
- abnormal ultrasound finding suggestive of an increased risk of aneuploidy

The accuracy of NIPT has not been validated in low-risk women. Studies are ongoing.

What is the Follow-up for a Positive NIPT Result?

Although it is highly accurate, it is recommended that positive NIPT results be confirmed by diagnostic testing (chorionic villus sampling or amniocentesis). Genetic counselling is also recommended.

How Does Panorama™ Compare to Other NIPT and Amniocentesis?

Table 1 summarizes how Panorama™ performs in comparison to other NIPT as well as amniocentesis.

Is NIPT Covered Under Provincial Health Plans?

Unlike amniocentesis, NIPT is not currently covered under any provincial health plan. The pregnant mother is responsible for the cost of the test. However, Panorama™ is one of the most affordable NIPT in Canada.

Table 1: Performance Features of Panorama™ NIPT Offered by LifeLabs

Features	Panorama™ [3,4]
Earliest Testing Possible	9 weeks
Sensitivity for Down Syndrome (T21) False Positive rate	>99% 0%
Sensitivity for Edwards Syndrome (T18) False Positive Rate	>99% 0%
Sensitivity for Patau Syndrome (T13) False Positive Rate	>99% 0%
Sensitivity for Monosomy X (45,X) False Positive Rate	91.7% 0%
Detection of Triploidy	Yes
Risk of Miscarriage	0
Results Turnaround Time	Up to 10 days

POINTS TO REMEMBER

PANORAMA™ NIPT test is:

- **Simple and safe –performed on a maternal blood sample with no risk of miscarriage**
- **Highly accurate for the detection of Trisomies 21, 18, and 13 – significantly reduces the number of unnecessary invasive procedures like amniocentesis after a positive serum screen**
- **The only NIPT that can detect triploidy**
- **Can be performed as early as 9 weeks gestation**
- **One of the most affordable NIPT in Canada**

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LIFELABS REPATRIATES TESTING FOR PROTEIN C AND PROTEIN S DEFICIENCY

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Protein C and protein S are both vitamin K dependent plasma proteins that act as natural anticoagulants that downgrade the pro-coagulant cascade by inhibiting activated factor V and factor VIII, thereby reducing the rate of thrombin production. Testing for protein C and protein S deficiency are typically ordered as part of a general thrombophilia investigation. Inherited partial (heterozygous) deficiencies of both protein C and protein S result in a mild to moderate risk for venous thromboembolism (VTE) in affected patients.

Protein C Deficiency

Inherited protein C deficiency is classified as quantitative (type 1) or qualitative (type 2). Type 1 deficiency is associated with decreased protein production while type 2 involves production of a dysfunctional protein with reduced activity. Inherited protein C deficiency is a relatively rare disorder and is present in approximately 2-5% of all patients presenting with VTE.

Acquired protein C deficiency is much more common than inherited forms and has several causative factors (Table 1). Acquired causes of protein C deficiency must be excluded before inherited deficiency is diagnosed.

Testing for Protein C

Testing for protein C includes functional (clot based) and antigen assays. It is recommended that functional assays are used as an initial screening tool while antigen assays are generally used to differentiate type 1 deficiency from type 2. However, distinguishing between type 1 and type 2 deficiencies is generally not indicated, as this information is rarely needed for clinical treatment.

Chromogenic functional assays are the most common method used for screening and are favoured because they are less affected by interferences typically seen with clotting methods.

If initial testing is normal, no further testing is warranted. If the initial screen is low, an INR should be performed, and if elevated the reason should be investigated - protein C deficiency does not cause an elevated INR so an acquired cause should be strongly suspected and excluded. Confirmation of protein C deficiency should be established by repeat analysis on a new plasma sample. Screening first degree relatives is also useful to support the diagnosis.

Protein S Deficiency

There are three known types of inherited protein S deficiency that result in either qualitative or quantitative defects. Type 1 deficiency accounts for up to 70% of all inherited protein S cases and is associated with a decreased production of protein. Type 2 is associated with dysfunctional protein production, and type 3 is associated with increased binding affinity of protein S for C4bBP protein limiting the amount of bioavailable protein S. Inherited protein S deficiency is a rare disorder accounting for only 3-6% of people with recurrent thrombosis.

Just like protein C, acquired protein S deficiency is more common than inherited and has several causes (Table 1). Acquired causes should be excluded before a diagnosis of inherited protein S deficiency can be established.

Testing for Protein S

Testing for protein S include clot based functional or antigen assays. Free protein S antigen is the preferred method to screen for protein S deficiency according to the North American Specialized Coagulation Laboratory Association (NASCOLA).

If initial testing is normal, no further testing is required. Similar to protein C testing, if the initial screen is low an INR should be performed and if elevated, acquired causes excluded. In addition, confirmation of a low result should be repeated using a new plasma sample or by screening first degree relatives.

When should this testing not be performed?

Testing for any inherited thrombophilia disorder, including protein C and S deficiency, is compromised by certain clinical situations. Testing should not be performed during an acute thromboembolic event or other medical, surgical, or trauma related acute illness. Protein C and S may be decreased in these situations. Additionally, patients currently on Coumadin therapy or those who have stopped Coumadin therapy less than 30 days prior to testing should not be tested. Protein C and S levels can remain low up to 30 days after cessation of Coumadin treatment.

Testing for Protein C and S at LifeLabs

Starting September 9th, LifeLabs will be performing these tests as part of its routine in-house test menu - protein C deficiency screening with a chromogenic functional assay, and protein S deficiency screening with a free antigen assay.

Please note that LifeLabs' reference ranges will be adjusted to reflect this change - refer to LifeLabs' report for these.

Table 1: Acquired Causes of Protein C and S Deficiency

Decreased synthesis
• Coumadin therapy • Vitamin K deficiency • Liver disease • L-asparaginase therapy
Increased clearance
• DIC • Acute thrombosis • Trauma or acute illness
Hemodilution
• Post-hemorrhagic resuscitation
Other (protein S only)
• Protein S decreases with oral contraceptive use, hormone replacement therapy, and in 2nd and 3rd trimesters of pregnancy

POINTS TO REMEMBER

- **Protein C and S deficiency result in mild to moderate increased risk of venous thromboembolism.**
- **Functional or antigen assay methods are used to quantitate protein C and S.**
- **LifeLabs is pleased to announce the repatriation of these tests to our own testing facility; please refer to your laboratory report for reference range changes.**

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