LIFELABS INTRODUCES A NEW METHOD OF ESR DETERMINATION

Miranda Wozniak, MD, FRCPC
Hematologist, LifeLabs ON

The erythrocyte sedimentation rate (ESR) is widely used as an indicator of inflammation. The traditional Westergren method measures the rate at which red cells sediment in plasma over one hour, and the results are influenced by the overall charge (or zeta potential) of the red cells. The net negative charge on red cells causes them to resist each other, while the net positive charge of serum proteins encourages adhesion causing a faster rate of sedimentation and therefore a higher ESR (i.e., during inflammation and often due to fibrinogen).

Although easy to perform and inexpensive, the Westergren method takes at least 30 minutes to perform in the laboratory. The method is imprecise and its specificity can be reduced by several factors unrelated to the inflammatory process, often making the results unreliable and imprecise.

LifeLabs recently introduced ESR testing by a new methodology that is faster and easier to perform. The new method analyzes the aggregation capacity (or zeta potential) of the red cells using optical density and has reduced analysis time to 20 seconds (more details available on request). Moreover, this new technology eliminates the interferences often encountered by Westergren method and has been proven in studies to better correlate with inflammation when compared to that method.

In April 2013, LifeLabs introduced this new methodology for ESR analysis. Reference interval changes were implemented at that time and interpretative message attached to all reports. This message will remain in the patient reports for 6 months.

POUNTS TO REMEMBER

• ESR determined by the Westergren method is slow and results are non-specific.
• On April 8th 2013, LifeLabs introduced a new method for ESR determination that is more specific and faster to perform.
• Due to the change in methodology, please refer to your laboratory report for the change in reference intervals.

REFERENCES

LIFELABS' NEW COMBINED CYTOLOGY AND HPV REQUISITION

Quality and Regulatory Affairs, LifeLabs ON

To better serve clients, LifeLabs has combined its current Cytology and HPV requisitions into a single-page requisition.

Effective immediately, please start using the new requisition and discard all previous Cytology and HPV requisitions. Future orders will be in order pads. Orders may be placed by using LifeLabs’ Physician’s Order Forms, located on LifeLabs website: www.lifelabs.com/Lifelabs_ON/Health_Care/Specimen-Collection-Supplies.asp

The MOHLTC issued OHIP INFOBulletin #4585 on January 15th, 2013, announcing the Schedule of Benefits for Physician Services was being amended to reflect the CCO’s new guidelines for cervical cancer screening.

LifeLabs’ new Cytology/HPV Requisition will allow clinicians to indicate when a Pap Test is an uninsured service.

Check the “Patient Pay” box and ensure your patient is informed that they will receive an invoice for $35.00 from LifeLabs and be responsible for payment.

There is no change in the ordering of HPV Tests. This test is still uninsured and the charge remains the same. The new Cytology and HPV Requisition, no longer requires the patient’s credit card information prior to submitting the specimen for testing. The patient will receive an invoice.

Check the “HPV Testing” box and ensure the patient is informed that they will receive an invoice for payment.

LifeLabs thanks you for assisting in communicating these charges to your patients.
POINTs TO REMEMBER
• LifeLabs has combined its current Cytology/HPV requisitions into a single-page requisition.
• LifeLabs’ new Cytology/HPV Requisition will allow clinicians to indicate when a Pap Test is an uninsured service.
• There is no change to the ordering process for HPV tests.

CHANGES TO TSH AND FOLATE METHODS USED BY LIFELABS

Peter Catomeris, PhD, FCACB
Clinical Biochemist, LifeLabs ON

In the near future, LifeLabs will implement changes to methods used to quantify TSH and folate. The changes in testing technology will result in modifications to reference intervals. An interpretive comment explaining the change will be included in patient reports.

TSH
The current TSH method will be replaced by a third-generation assay, with improved analytical sensitivity, but will be performed on the same testing platform. The current assay standardization is traceable to the World Health Organization (WHO) 2nd International Standard for human TSH (IRP 80/558), while the new assay standardization is traceable to the WHO 3rd International Standard for human TSH (IRP 81/565).

With the change to the method, TSH results are expected to decrease by about 20% and reference intervals will be adjusted accordingly.

Serum and RBC Folate
Serum and RBC folate methods will be transferred to a new testing platform. As no international standard exists for folate, the methods are traceable to internal standards for each manufacturer.

With the changes to the methods, results for serum folate are expected to increase by about 30%-40% and results for RBC folate are expected to double. Reference intervals for both tests will be adjusted accordingly.

Please contact one of the Clinical Biochemists on LifeLabs’ Medical/Scientific Team for more information.

POINTS TO REMEMBER
• In the near future, LifeLabs will implement changes to methods used to quantify TSH and folate.
• Reference intervals for both methods will be adjusted to reflect the change in methodology.
• An interpretive comment explaining the change will be included in patient reports.

URINE DRUG TESTING: OPIOIDS

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Common questions received at LifeLabs are related to interpretation of drugs of abuse reports, the majority of which are related to opioids. This article will discuss test methods for detection of opioids in urine, their utility and limitations.

The opioids are a class of drugs that have analgesic, antitussive, and antidiarrheal properties. They are most commonly used in acute and chronic pain management, but they also have high potential for abuse or misuse. Opioids may be divided into three main classes: a) natural (from opium), i.e., morphine, codeine; b) semi-synthetic, i.e., hydromorphone, hydrocodone, oxycodone, oxymorphone, heroin, and buprenorphine; and, c) synthetic, i.e., meperidine, fentanyl, methadone, tramadol, propoxyphene, tapentadol.

The metabolism of common opioids is reflected in Table 1. Understanding basic opioid metabolism will enhance urine drug test result interpretation.

Table 1: Metabolism of Common Opioids

<table>
<thead>
<tr>
<th>Parent drug</th>
<th>Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>Hydromorphone</td>
</tr>
<tr>
<td>Heroin</td>
<td>6-monooacetylmorphine</td>
</tr>
<tr>
<td></td>
<td>(short-lived metabolite)</td>
</tr>
<tr>
<td></td>
<td>Morphine</td>
</tr>
<tr>
<td>Codeine</td>
<td>Morphine (major metabolite)</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>Oxymorphone</td>
</tr>
</tbody>
</table>

Please note that information discussed in this article can be found in the latest Community Laboratories Guidelines for Ordering Urine Testing for Drugs-of-Abuse: Targeted and Screening Tests (CLP013) published by the Ontario Association of Medical Laboratories in March 2013.1

Methods for Detection of Opioids in Urine
a) Opioid immunoassay (IA) screens

Opioid IA screens are designed to detect opioids that are structurally similar to morphine, including codeine, hydrocodone, and hydromorphone (Table 2). Results are qualitative and drug concentrations are not provided. Results equal to or above the screen cutoff of 300 ug/L are reported as “Detected”, results below the screen cutoff are reported as “Not Detected”.

In the emergency care setting qualitative opiate IA screens are used to evaluate potential drug abuse or overdose. In the community setting opioid IA screens are most commonly used to identify use of undisclosed opioids, to determine compliance with prescribed opioid, and to uncover diversion of prescribed opioid.

LifeLabs welcomes your feedback and continuous improvement. Keep the suggestions coming!

SEMINAL FLUID ANALYSIS REPORTING IMPROVEMENTS

Shubhra Mohan
Quality and Regularity Affairs, LifeLabs ON

Clients have recently reported that they have some difficulty reading and understanding LifeLabs’ post-vasectomy patient reports. Based on their feedback, LifeLabs revised not only post-vasectomy reports, but also other seminal analysis reports for content, layout and accuracy.
Table 2: Examples of Opioids Commonly Detected by Urine Immunoassay Screen and Broad Spectrum Toxicology Screen

<table>
<thead>
<tr>
<th>Opioid Immunoassay (IA) Screen* (Order on its own or with DOA screen)</th>
<th>Broad Spectrum Toxicology Screen* (Chromatography-Mass Spectrometry technology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>Morphine</td>
</tr>
<tr>
<td>Morphine metabolite (Morphine-3-glucuronide)</td>
<td>Morphine metabolite (Morphine-3-glucuronide)</td>
</tr>
<tr>
<td>Codeine</td>
<td>Codeine</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>Hydrocodone</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>Hydromorphone</td>
</tr>
<tr>
<td>6-acetyl morphine (heroin metabolite)</td>
<td>6-acetyl morphine (Heroin metabolite)</td>
</tr>
<tr>
<td>Dihydromorphone</td>
<td>Dihydromorphone</td>
</tr>
<tr>
<td>Levorphanol</td>
<td>Levorphanol</td>
</tr>
<tr>
<td></td>
<td>Morphine metabolite (Morphine-3-glucuronide)</td>
</tr>
</tbody>
</table>

*Opioid IA Screen shows poor cross-reactivity with oxycodone, methadone, and methadone metabolite (EDDP). LifeLabs offers specific immunoassay tests that detect these opioids in urine.

# Broad Spectrum Toxicology Screen method can detect additional opioids not listed in Table 2.

How to order: Opioid IA screen may be requested either on its own or included in a ‘Drugs-of-Abuse (DOA) Screen’.

b) Confirmation testing by chromatography-mass spectrometry

Table 2 summarizes opioids commonly detected by IA screen and Broad Spectrum Toxicology Screen, respectively.

Confirmatory opioid testing is used to determine the specific opioid abused by patients, identify the specific opioid that cannot be reliably detected with the IA screen due to poor cross-reactivity (i.e., synthetic opioids like meperidine and fentanyl), or to identify the presence of metabolites if there is a suspicion that a patient added the medication directly to their urine to simulate compliance (i.e., fentanyl present but not norfentanyl, methadone present but not EDDP).

In these situations, it is recommended that the initial IA screen is followed by a more specific technique such as chromatography coupled to mass spectrometry to identify, or confirm the presence, or absence of a specific opioid and its metabolites.

How to order: Confirmatory test can be ordered by requesting a ‘Broad Spectrum Toxicology Screen’. If a specific drug(s) is of interest, the drug name should be noted in the ‘Other Tests’ section of the OHIP lab requisition.

To request confirmation testing on a urine specimen that tested positive by opioid IA screen, please call LifeLabs at 416-675-4530 ext. 3339.

Limitations of Urine Opioid Screens

a) A false-negative opioid IA screen: An opioid is present in the patient’s urine but the IA screen test is negative

The most common causes of false-negative results are:
• Drug concentration is present below the cutoff (< 300 ug/L)
• The specific drug is not recognized by the IA screening assay
• The patient took the drug too remotely from the time of urine collection
• Specimen adulteration

Some semi-synthetic and most synthetic opioids will have variable cross-reactivity in opioid immunoassays routinely available in the laboratory. In these circumstances, either specific IA for drugs such as oxycodone and methadone/methadone metabolite can be ordered, or broad spectrum toxicology confirmatory screen for drugs like hydromorphone, meperidine, and fentanyl may be requested.

It is important to note the window for detection of opioid in urine is approximately 2 to 4 days after last use. This depends on many factors including method and frequency of opioid ingestion, opioid half-life, fluid intake, and the patient’s metabolism and physical condition. The duration of positive results for the drug should be considered in interpreting both negative and positive screen results. A detailed discussion of this topic is presented in LifeLabs Inside Diagnostics article published in July 2011. (Please see our website: www.lifelabs.com)

b) A false-positive opioid IA screen: An opioid is absent from the patient’s urine, an IA screen is positive, and a confirmatory test is negative.

The most common causes of false-positive results are:
• Poppy seed ingestion (poppy seed cookies or bagels can cause a positive opioid IA screen due to presence of morphine and codeine in the seed.) Codeine and morphine containing analgesic medications can produce positive opioid screen results. These are both examples of true-positive but misleading results.
• Limitations in specificity of the antibodies used in the analytical method. The antibodies used in opioid IA screens bind to drugs that are structurally related to the opioids but many also cross react with compounds that are not structurally-related to the opioids that the assay is designed to detect. For example, therapeutic doses of quinolone antibiotics (levofloxacin, ofloxacin) that are not structurally related to the opioid can produce false positive results on opioid screens. Other agents that can cause false-positive results include dextromethorphan, diphenhydramine and verapamil (methadone IA only), rifampin, chlorpromazine, and quinine.

For assistance in the interpretation of urine opioid test results and for more information on the available testing methods, please contact one of the Clinical Biochemists on LifeLabs’ Medical/Scientific Team.
WHAT IS DIENTAMOEBA FRAGILIS?

Abdel Belhaj, MD, FRCP
Consultant Microbiologist, LifeLabs ON

Dientamoeba fragilis is an anaerobic intestinal protozoan parasite. Historically, this organism was amongst a group of enteric protozoan parasites beginning with Giardia lamblia that were initially believed to be commensals and not capable of causing symptomatic illness. As more information became available and antimicrobial agents were developed with activity against these parasites, it became clear that D. fragilis is an active infection, although not always symptomatic.

Microbiology

Although initially identified as an amoeba, ultrastructural, immunologic and genetic analyses place this organism in the family of protozoan flagellates, which includes Trichomonas. Unlike other intestinal protozoan organisms that have both trophozoite and hardy cyst stages, Dientamoeba fragilis apparently exist only as trophozoites. Trophozoites measure 7 to 12 micrometers in diameter, contain one or two nuclei, lack flagella, and are minimally motile.

Epidemiology

Infections with Dientamoeba fragilis undoubtedly are acquired by the fecal-oral route, but how fragile trophozoites survive outside of the body and do not succumb to stomach acid following ingestion is unknown. Some earlier investigators suggested that trophozoites might survive within and be ingested with the eggs of the pinworm, Enterobius vermicularis.

It is distributed globally; the prevalence varies with geography. D. fragilis represents about 50 percent of all pathogenic parasites identified in the parasitology laboratory at LifeLabs. About 2-3% of all stool samples that are sent to our laboratory for parasitology testing is shown to have D. fragilis. (based on data from Jan-March 2013).

Clinical Manifestations

The incubation period prior to the onset of symptoms following infection is unknown, as is the proportion of infected people who remain asymptomatic. The parasite localizes in the colon and can cause colitis. Most common symptoms are: abdominal pain, diarrhea, anorexia, fatigue, nausea, weight loss and vomiting. Patients with D. fragilis may present with peripheral eosinophilia and/or eosinophilic colitis.

REFERENCES


Diagnosis

The diagnosis is made via detection of D. fragilis trophozoites in microscopic examinations of appropriately fixed and stained stool samples (Figure 1). Examination of formed, as well as watery, stools increases the diagnostic yield.

Figure 1: D. fragilis in a Stool Sample Stained with Kinyoun/Hematoxylin

Treatment and Prevention

Infections with Dientamoeba fragilis should be treated when the organism is found as a sole pathogen in stool samples from patients with abdominal pain or diarrhea lasting for more than one week.

There are several regimens that may be used to treat D. fragilis infections including: Paromomycin, Metronidazole and Iodoquinol.

The literature is suggestive of Paromomycin as a more efficacious agent than Metronidazole.

Since knowledge is currently lacking about how Dientamoeba fragilis infections are transmitted, there are no specific guidelines on measures to prevent acquisition of infection with this parasite other than good hand hygiene.

POI NTS T O R E ME M B E R

• Dientamoeba fragilis is an anaerobic intestinal protozoan parasite. It is a flagellate that produces trophozoites; cysts have not been identified.
• Infection may be symptomatic or asymptomatic.
• Infection is transmitted by the fecal-oral route.
• Common symptoms include abdominal pain and recurrent diarrhea.
• Patients with D. fragilis may present with peripheral eosinophilia and/or eosinophilic colitis.

REFERENCES