



# CMPT Enteric Parasitology Program

Innovation, Education, Quality Assessment, Continual Improvement

## Challenge 0904-3

April 2009

*No ova or parasites seen*

### CMPT QA

This sample was verified by two reference laboratories. Both laboratories reported no ova or parasites seen.

### SURVEY RESULTS

All but one reporting laboratories reported no ova or parasite seen. One laboratory reported *B. hominis*.

Proficiency Testing samples are to be processed as routine samples; failure to report results even when there is a staff shortage or high workload will result in Unacceptable grade.

### METHODS

#### Microscopic examination

Currently, microscopic examination of stool samples is the primary method used by the laboratory to diagnose parasite in-

fections. The limitations of each procedure must be taken into consideration and a combination of concentration and permanent stained smear methods is required for accurate results <sup>1</sup>.

The microscope should allow examination at magnification X100, X400, as well as X500 and X1000 (oil immersion). An eyepiece micrometer must also be used to accurately measure any suspect parasite found.

#### Concentration Wet Mount

This method is necessary to recover protozoan cysts, coccidian oocysts, and helminth eggs and larvae. Preserved stool samples are acceptable for testing. If iodine is used helminth eggs may be confused with debris.

The slide should be systematically examined with the low-power objective (10X); any suspicious objects may then be examined with the high dry objective (40X).

#### Permanent Stained Smear

### Grading

All the laboratories received an Acceptable grade.

One of the laboratories reported *Blastocystis hominis*. This parasite was not observed by any other lab.

It is always unacceptable not to process a PT sample.

**Table 904-3-1:** Combined results received – No ova or parasites seen challenge

Reports	No. Reported	%	Grade
No ova or parasites	<b>24</b>	92	Acceptable
and WBC (n=5)			Acceptable
and CLC (n=1)			Acceptable
<i>B. hominis</i>	1	4	Acceptable
No report due to high workload	1	4	Unacceptable
<b>Total</b>	26		

WBC= white blood cells CLC= Charcot-Leyden Crystals

**Table 904-3-2:** Historic results – No ova or parasites seen challenge

Challenge	904-2	807-2	701-1	604-1	310-1	210-2
Results (%)	92%	100%	96%	100%	100%	96%

This method is used to recover and identify the intestinal protozoan trophozoites and cysts, excluding coccidian oocysts and microsporidia. At least 300 OIF should be examined with the 100X objective (total magnification, x1000).

### Combination Method

Modified Iron-Hematoxylin Stain incorporating the Carbol Fuchsin step allows the microscopist to screen for acid-fast organisms (*Cryptosporidium*, *Cyclospora* and *Isospora*) in addition to other parasites

### Modified Acid-Fast Staining

This method is used to recognize and identify coccidian oocysts, e.g., *Cryptosporidium parvum*, *Cyclospora cayetanensis*, and *Isospora belli*.

### Fecal leukocytes

CLSI M28-A2 recommends fecal leukocytes be quantitated and reported as this information can suggest some etiologies<sup>3</sup>. Studies from Italy and the U.S. agree that the unit of reporting should be leukocytes per high power field (WBC/HPF). Regardless of examination for bacterial or parasitic pathogens, or cytotoxin, fecal leukocyte reporting has its “best” sensitivity for identification of fecal pathogens (57%) with a threshold of >1 WBC/HPF; the specificity is also poor (87%)<sup>4,5</sup>.

*Caution:* A pseudo outbreak of intestinal amebiasis was attributed to fecal leukocytes being mistaken for amoebae<sup>6</sup>.

### Charcot Leyden Crystals (CLC)

CLCs are commonly reported in samples examined for parasites. They are a host product derived from proteins from eosinophils and basophils<sup>7</sup>. CLCs are a pathogenic feature of respiratory and sinus and also colon-based allergic reactions<sup>8,9</sup>. Eosinophils play a protective role in the host response to helminthic infections and *Entamoeba histolytica*<sup>10</sup>. Neither peripheral eosinophilia (greater than 6%) nor the presence of CLCs is a sensitive or specific marker for parasitic infection<sup>7</sup>.

There is no common convention for the reporting units of CLCs. As there is no infor-

mation to suggest a quantitative relationship between CLCs and parasites, for those laboratories that report their presence, it is reasonable to report them on a

## CLINICAL RELEVANCE

The technologist must be able to identify pathogenic parasites, differentiate pathogenic and nonpathogenic species, and discriminate various artifacts that may be present. Reporting a false positive could lead to unnecessary and costly treatment of the patient. Unwarranted prescription of an antimicrobial exposes patients to potentially adverse effects of the drug<sup>1,2</sup>.

It is necessary to be aware of the parasites found, not only in Canada or parasites in a particular geographic location, but to dose worldwide due to increased world travel and immigration.

In addition, be aware that certain patient population, e.g., immunocompromised groups may be more likely to become infected with parasites than immunocompetent individuals.

Each laboratory must have set protocols to ensure that appropriate O&P collection kits with instructions are available for their patients. It's suggested that instructions for the patients be in the languages for the community the laboratory is serving or presented as simple easy-to-understand graphics.

Until recently, 3 sequential stool samples have usually been ordered for diagnosis of parasitic infections. The multiple sample recommendation arose from epidemiological studies aimed to the diagnosis of asymptomatic *E. histolytica* excretion. This guidelines are not applicable to developed countries where the incidence of *E. histolytica* is low. (**See recommendations depending on province in colored sidebars**).

Collect stool samples before radiologic procedures that use barium as it will interfere with trophozoite detection and may do so for several weeks. It is recommended that samples for parasite testing are collected one to two weeks after radiologic procedures with barium.

### Infectious diarrhea BC guideline for ordering stool specimens Mar 2009<sup>12</sup>

Ova and Parasite examination is recommended for patients at-risk for parasitic infections.

These include:

- travel to or immigration from an endemic area
- prolonged diarrhea (> 2 weeks)
- consumption of unsafe food or untreated water
- children attending day-care
- swimming in unsafe water
- men who have sex with men

In low-risk populations, it is reasonable to submit a single specimen, with a follow-up specimen submitted if initial results are negative and symptoms persist.

For patients at high-risk for parasitic infection, two stool specimens collected at least one day apart are recommended. If submitted using the same requisition, high risk status must be indicated.

If two stool specimens are ordered on the same requisition, high risk status must be indicated.

More than two stool specimens per requisition requires consultation with the laboratory physician.

## Alberta Clinical Practice Guidelines Program<sup>13</sup>

### Evidence for the Single Sample Rule

Work from the Alberta Children's Hospital has also demonstrated the high efficiency for diagnosis of enteric infections of a single initial stool culture and stool parasite examination in both hospitalized and ambulatory children.

Most pediatric cases of enterocolitis (190 of 194, 98%) are confirmed from a single stool culture, and a second sample is seldom required.

Most clinically relevant protozoan infections (102 of 112, 91%) were also detected in the first stool specimen examined.

Infections which would have been missed on a single stool O & P specimen included:

- 4 children with Giardiasis
- 4 children with *Dientamoeba fragilis*
- 2 children with *Blastocystis hominis* and
- 2 children with Cryptosporidiosis.

However, due to the retrospective design of the study, the clinical significance of any of these cases in terms of patient symptoms or institution of treatment could not be ascertained.

## Ontario Association of Medical Laboratories<sup>14</sup>

### Recommendations

Testing for Ova and Parasites should only be ordered when clinically indicated.

The patient must be instructed on the proper collection of the specimen.

One specimen only, properly collected and preserved, should be submitted initially.

The clinical status of the patient should be reviewed by the physician with the results of the initial test before further tests are ordered.

Subsequent testing should be requested if clinically indicated and the results of the first test are negative or if positive results do not match the clinical situation.

The laboratory diagnostic procedures should meet the standards of excellence expected for good clinical care.

Laboratory reports should clearly indicate the pathogenic status of all protozoan parasites identified based on current scientific knowledge.

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