



CMPT Enteric Parasitology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge 0907-3

July 2009

No ova or parasites seen

CMPT QA

This sample was verified by two reference laboratories to contain no ova or parasites.

SURVEY RESULTS

As shown in table 1, all, but one, laboratories reported no ova or parasite seen. One laboratory reported *Blastocystis hominis*.

Historically laboratories have performed very well when a negative sample has been sent as a PT challenge (Table 2).

The Committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.

METHODS

Microscopic examination

Currently, microscopic examination of stool samples is the primary method used by the laboratory to diagnose parasitic infections. 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.

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.

It is recommended the technologist spends at least 10 minutes examining a slide (wet mount and permanent smear) before reporting the

sample as negative.

Concentration Wet Mount

The concentration wet mount method is used 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

The permanent stained 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 ¹.

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

The CLSI document, M28-A2, recommends that fecal leukocytes be quantitated and reported as this information can suggest some etiologies⁸. Studies from Italy and the U.S. agree that the

Grading

The sample was reported negative for parasites and ova by the reference laboratories. Those participants reporting negative results were given an acceptable grade. The laboratory reporting *B. hominis* was also graded as acceptable.

No ova or parasites seen. Historic results

Table 2

Challenge	Results
907-3	100%
904-2	92%
807-2	100%
701-1	96%
604-1	100%
310-1	100%
210-2	96%

Table 1: Combined results received – “no ova or parasites seen” challenge

Reported	No of labs	%	Grade
no ova or parasites	22	85	Acceptable
no ova or parasites, leukocytes	3	12	Acceptable
<i>B.hominis</i>	1	4	Acceptable
Total	26	100	

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%)^{2,3}.

Charcot Leyden Crystals (CLC)

CLCs are commonly reported in samples examined for parasites. They are a host product derived from proteins of eosinophils and basophils⁴. CLCs are a pathogenic feature of respiratory and sinus and also colon-based allergic reactions^{5,6}. Eosinophils play a protective role in the host response to helminthic infections and *Entamoeba histolytica*⁷. Neither peripheral eosinophilia (greater than 6%) nor the presence of CLCs is a sensitive or specific marker for parasitic infection⁴.

There is no common convention for the reporting units of CLCs. As there is no information to suggest a quantitative relationship between CLCs and parasites, it is reasonable to report them on a presence/absence basis only for those laboratories that report their presence.

CLINICAL RELEVANCE

The technologist must be able to identify pathogenic parasites, differentiate pathogenic from nonpathogenic species, and discriminate various artifacts that may be present. Reporting a false positive could lead to unnecessary treatment of the patient. Unwarranted prescription of an antimicrobial exposes patients to potentially adverse effects of the drug¹.

It is necessary to be aware of the parasites found, not only in Canada or parasites in a particular geographic location, but of those of worldwide distribution 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 ova and parasites 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. These guidelines are not applicable to developed countries where the incidence of *E. histolytica* is low.

It is important to remember that in developed countries most of the samples analyzed in a clinical microbiology laboratory are negative. In British Columbia, laboratories have reported that around 80% of the examined samples are negative for parasites, while only 5-8% of samples are positive for pathogenic parasites.

REFERENCES

1. Garcia LS. Collection, preservation and shipment of fecal specimens. In: *Diagnostic Medical Parasitology*. 5th ed. Washington, DC: ASM; 2007:761.
2. Giacometti A, Cirioni O, Balducci M, et al. Epidemiologic features of intestinal parasitic infections in Italian mental institutions. *Eur J Epidemiol*. 1997;13:825-830.
3. Savola KL, Baron EJ, Tompkins LS, Passaro DJ. Fecal leukocyte stain has diagnostic value for outpatients but not inpatients. *J Clin Microbiol*. 2001;39:266-269.
4. Ackerman SJ, Weil GJ, Gleich GJ. Formation of Charcot-Leyden crystals by human basophils. *J Exp Med*. 1982;155:1597-1609.
5. Lertanekawattana S, Wichatrong T, Chaisari K, Uchikawa R, Arizono N. Immunological characteristics of patients infected with common intestinal helminths: Results of a study based on reverse-transcriptase PCR. *Ann Trop Med Parasitol*. 2005;99:71-80.
6. Pantanowitz L, Balogh K. Charcot-Leyden crystals: Pathology and diagnostic utility. *Ear Nose Throat J*. 2004;83:489-490.
7. Lopez-Osuna M, Arellano J, Kretschmer RR. The destruction of virulent *Entamoeba histolytica* by activated human eosinophils. *Parasite Immunol*. 1992;14:579-586.
8. M28-A2: Procedures for the recovery and identification of parasites from the intestinal tract; Approved Guideline. 2005 2nd ed. Vol. 25 No. 16, CLSI. Wayne, Pa.
9. Plorde JJ. *Clinical Parasitology*. In: *Clinical Infectious Diseases: A Practical Approach*. Kr Root, F. Waldvogel, L. Corey, we Stamm, editors. Oxford University Press, New York, 1999

False negatives

False-negatives may occur due to low numbers of parasites in the specimen, intermittent shedding of the parasite, inappropriate transport and/or storage, poor concentration, or insufficiently thorough microscopic examination.

Certain drugs and compounds, including purgatives, antidiarrheal agents, antacids, antibiotics and contrast agents, interfere with the detection of stool parasites. Barium, magnesium, kaolin, and bismuth compounds produce crystalline or particulate debris that can obscure parasite and alter the appearance of trophozoites. Oil globules from castor or mineral oil interfere with microscopic examination. Antibiotics and cathartics temporarily decrease the protozoan population to a level where organisms may not be detected.

Fecal specimens should be collected in a manner that precludes contamination with urine, water, dirt, or soil. Urine and water will destroy trophozoites that may be present, and dirt interferes with the examination. Further, soil may contain free-living larvae or other organisms that can be confused with human pathogens⁹.