



PARTICIPANT STATISTICS

PARASITOLOGY

SECOND QUADRIMESTER 2011

Specimen 1

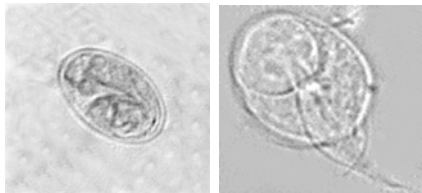
Organisms	Referees	Frequency	Ext 1	Ext 2
18 <i>Giardia lamblia</i>	18	Few to Many	15	56
98 Parasites found, refer for ID	1		11	
99 No parasites found			2	2
33 <i>Entamoeba coli</i>				1
29 <i>Blastocystis hominis</i>			1	1
31 <i>Endolimax nana</i>			1	
TOTAL POPULATION	18		30	60

Extent 1 flagging appears for failure to report 18 or 98.

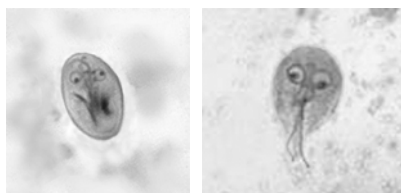
Extent 2 flagging appears for failure to report 18.

Flagging also appears in both extents for reporting other than 18 or 98.

FORMALIN - This specimen contains *Giardia lamblia*. The *G. lamblia* cyst morphology was very typical with a round to oval shape and the presence of multiple nuclei, curved median bodies, and linear axonemes. Representative images can be seen below. The internal structures often appear very refractile in the wet preparation examination. Although some of the cysts are shrunk within the cyst wall, there are plenty of organisms that can be easily identified.



Very High Magnification
G. lamblia cyst *G. lamblia* trophozoite
 Wet Mounts



G. lamblia cyst *G. lamblia* trophozoite
 Permanent Stained Smear

Specimen 2

Organisms	Referees	Frequency	Ext 1	Ext 2
60 <i>Diphyllobothrium latum</i>	18	Few to Moderate	15	56
98 Parasites found, refer for ID	1		11	
48 <i>Cryptosporidium</i> sp.			1	
99 No parasites found			1	1
70 <i>Ascaris lumbricoides</i>				1
31 <i>Endolimax nana</i>			1	1
TOTAL POPULATION	18		29	59

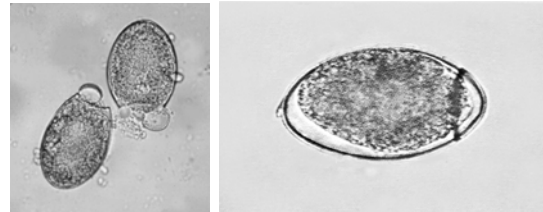
Extent 1 flagging appears for failure to report 60 or 98.

Extent 2 flagging appears for failure to report 60.

Flagging also appears in both extents for reporting other than 60 or 98.

FORMALIN - The specimen was a fecal suspension in 10% formalin for direct wet mount examination; concentration was not necessary. The specimen was to be examined for all parasites unstained, with iodine or other acceptable wet mount stain.

This specimen contains *Diphyllobothrium latum* eggs. They are very typical with an operculum and no opercular shoulders. The eggs measure 58-75 by 40-50 microns.



In the eggs on the left, you can see the open opercula; the egg on the right has an operculum line and "knob" at the posterior end of the egg. Note: these eggs do not have opercular shoulders (like *Paragonimus* or *Clonorchis*).

Specimen 3

Organisms	Referees	Frequency	Ext 1	Ext 2
70 <i>Ascaris lumbricoides</i>	16	Rare to Moderate	15	45
99 No parasites found	2		2	12
98 Parasites found, refer for ID	1		11	
84 <i>Trichuris trichiura</i>				2
18 <i>Giardia lamblia</i>			1	
48 <i>Cryptosporidium</i> sp.			1	
31 <i>Endolimax nana</i>				1
TOTAL POPULATION	18		30	60

No flagging appears due to the lack of participant and referee consensus required for grading by CLIA '88 (80%).

FORMALIN - This specimen contains *Ascaris lumbricoides* eggs. Remember, if you shake the vial, allow the fecal material to settle out prior to taking the specimen (several minutes). Also, when you place the pipette down into the vial of fluid, do not make bubbles, but carefully remove some of the material from the bottom of the vial and place it on a slide.

Specimen 4

Organisms	Referees	Ext 1	Ext 2
34 <i>Entamoeba hartmanni</i>	16	12	39
98 Parasite found, refer for ID	1	6	
36 <i>Entamoeba histolytica/E. dispar</i>		4	3
35 <i>Entamoeba histolytica</i>			
99 No parasites found		1	
TOTAL POPULATION	16		

Extent 1 flagging appears for failure to report 34 or 98.

Extent 2 flagging appears for failure to report 34.

Flagging also appears in both extents for reporting other than 34 or 98.

DIGITAL IMAGE - This specimen contains *Entamoeba hartmanni* trophozoites and cysts (very few cysts, but they are present - no cysts were selected in the boxes). *E. hartmanni* is a nonpathogen, and the organisms were very typical, with the trophozoites having the characteristic nucleus (evenly arranged nuclear chromatin with central compact karyosome - resembles a "bull's eye"); with rare exceptions (see examples 5, 7, and 9 below) the trophozoites measure <12 microns. The cysts have four nuclei, but frequently cysts containing only two nuclei are seen. Also, these cysts often contain numerous chromatoidal bars (smooth, rounded ends); the cysts measure <10 microns. Although these organisms resemble *Entamoeba histolytica/E. dispar*, both the trophozoites and cysts of *E. hartmanni* are smaller. Remember when measuring the cysts on permanent stained smears, one should measure the total distance, including the clear "halo" that represents shrinkage during staining.

Some of the trophozoites (Examples 5, 7 and 9) measured >12 microns; thus the correct response would be *Entamoeba histolytica/E. dispar*.

When examining the permanent stained smears, it is important to read at least 300 fields using the oil immersion objective (100X objective) for a total magnification of X1000. This examination is in contrast to the concentration sediment wet preparation, for which at least 1/3 to 1/2 of the coverslip should be examined using the high dry objective (40X) and the entire 22x22 mm coverslip should be examined using the low power objective (10X).

Typical *E. hartmanni* parasites can be seen in the following:



Note: The nucleus is very typical with evenly arranged chromatin and the central, compact karyosome (resembling *Entamoeba histolytica*/*E. dispar*, but smaller); the trophozoites measure <12 microns, while the cysts measure <10 microns. Often, cysts are seen that may contain only two nuclei, rather than the four that are found in the mature cyst. Also, it is common to see multiple, small chromatoidal bars within the cysts.

It is incorrect to identify any of these organisms as *Entamoeba histolytica*; none of the trophozoites contain ingested red blood cells and the overall size is too small (with the exception of Examples 5, 7, and 9)!

Example 1 contains one small trophozoite, in which the nuclear chromatin is evenly arranged - the karyosome is a bit eccentric. The morphology is very typical and characteristic of *E. hartmanni*.

Example 2 also contains one trophozoite, with typical morphology; however, the "target" nucleus is not as clear as some of the other examples. This troph measures approximately 8.3 microns.

In **Example 3** the trophozoite measures approximately 8.9 microns; the target or bull's eye nucleus is very typical.

In **Example 4** the trophozoite displays very typical morphology (evenly arranged nuclear chromatin, central karyosome). The organism measures approximately 7.2 microns).

In **Example 5** the trophozoite is somewhat elongate and measures approximately 12.5 microns. However, the overall morphology is that of *E. hartmanni*. **The fact that occasionally one may see a trophozoite measure a bit beyond the 12 micron limit is usually linked to the position of the organism (somewhat elongate, rather than the more typical round or oval shape). Although the measurement is a bit over the limit, the overall morphology of the organism is definitely *E. hartmanni*.** When reviewing the slides, one needs to remember that you are looking at a "population" of organisms, and not every organism will exactly match the size guidelines, particularly if the organism is "stretched out" - overall morphology is still consistent with *E. hartmanni*. However, due to the measurement, *Entamoeba histolytica*/*E. dispar* would be acceptable. **Although the red structure resembles a chromatoidal bar, it is probably just debris (see similar structures in the background of the slide).**

In **Example 6**, the trophozoite measures approximately 9.1 microns and demonstrates a good "target" nucleus.

In **Example 7** the trophozoite measures approximately about 12 microns and has an excellent "bull's eye" nucleus with the central karyosome. This is another example like Example 5: it is probably *E. hartmanni*, but due to the size could also be identified as *Entamoeba histolytica*/*E. dispar*.

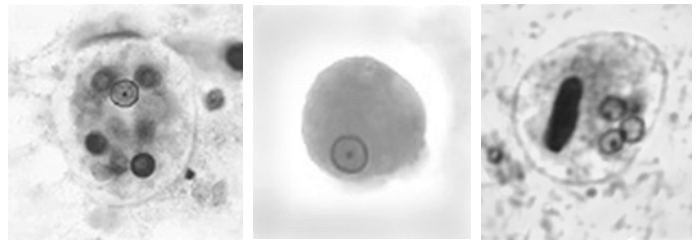
In **Example 8**, the trophozoite measures approximately 6.2 microns; the nuclear chromatin is evenly arranged, but the karyosome is a bit eccentric. However, the overall morphology is very typical for *E. hartmanni*.

Example 9 demonstrates a trophozoite that is a bit like Example 5; the overall measurement is a bit over the 12 micron limit, but the organism is a bit stretched out. However, the overall morphology is definitely that of *E. hartmanni*. **A couple of organisms were specifically selected to show you the same morphology, although the size was a bit over the normal limit - this is typical of what would be seen in any patient specimen similar to this slide.** However, due to the measurement, *Entamoeba histolytica*/*E. dispar* would be acceptable.

In **Example 10**, the trophozoite measures approximately 9.5 microns and has a good target nucleus configuration, typical for *E. hartmanni*.

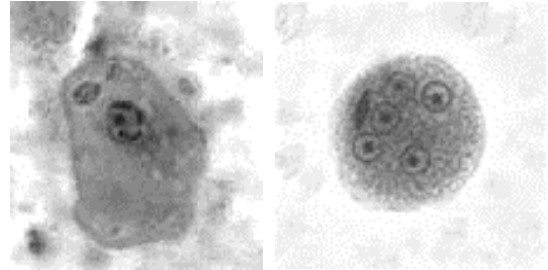
Example 11 shows a typical trophozoite of *E. hartmanni*, with an excellent target nuclear structure.

In **Example 12**, the trophozoite measures 10.4 microns and has the typical nucleus (evenly arranged nuclear chromatin and central dot-like karyosome).



Entamoeba histolytica
(Ingested RBCs)

Entamoeba histolytica/*E. dispar*
(NO ingested RBCs)



Entamoeba coli
(Uneven nuclear chromatin, more diffuse karyosome;
cyst with 5 or more nuclei)

Specimen 5

Organisms	Referees	Ext 1	Ext 2
18 <i>Giardia lamblia</i>	16	19	46
98 Parasite found, refer for ID			3
99 No parasites found			
TOTAL POPULATION			

Extent 1 flagging appears for failure to report 18 or 98.

Extent 2 flagging appears for failure to report 18.

Flagging also appears in both extents for reporting other than 18 or 98.

DIGITAL IMAGE - This stained stool slide demonstrates excellent examples of *Giardia lamblia* trophozoites and cysts. The overall morphology is excellent with nuclei, median bodies, and axonemes being visible. Remember that these organisms are very three-dimensional; it is mandatory that when reviewing patient material, you focus up and down. You may want to increase the magnification and contrast when examining these organisms.

Example 1 contains an excellent *Giardia* trophozoite. Both nuclei are visible as are the pink/purple median bodies and the linear axonemes.

Example 2 contains several trophozoites (typical teardrop shape) and one cyst; the overall morphology is very typical.

Example 3 contains a *Giardia* cyst; note the cyst wall is visible.

Example 4 contains two excellent *Giardia* trophozoites. Also note the large number of organisms outside of the marked box.

Example 5 contains two trophozoites; note the "face/eyes" looking back at you.

Example 6 contains a very typical *Giardia* trophozoite; two of the four nuclei are visible, as are the median bodies and linear axonemes.

Example 7 contains a very typical trophozoite; note the nuclei, overall shape, and reddish median bodies.

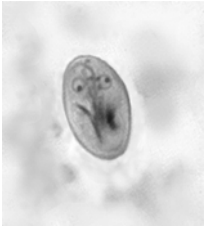
Example 8 contains five *Giardia* trophozoites. Note the teardrop shape from the front and the curved "spoon" shape in two of the upper organisms (seen from the side).

Example 9 contains a cyst on the left and a trophozoite on the right, both of which are typical.

Example 10 contains a very typical *Giardia* trophozoite; note the other organisms in the background (outside of the marked box).



Very high magnification
G. lamblia cyst *G. lamblia* trophozoite
Wet Mounts



G. lamblia cyst

G. lamblia trophozoite

Permanent Stained Smear

GENERAL COMMENTS:

If you are currently using one of the stool fixatives that contains a mercuric chloride substitute (zinc sulfate, etc.), remember that the proficiency testing specimens you receive for permanent staining have been preserved in PVA using the mercuric chloride fixative base. If you use the Trichrome or iron hematoxylin staining method for your mercuric chloride substitute fixatives, you may have eliminated the 70% alcohol/iodine step and the following 70% alcohol rinse step from your method. However, when you stain the proficiency testing fecal smears, you will need to incorpo-

rate the iodine step plus the next 70% alcohol rinse back into your staining protocol prior to placing your slides into the Trichrome stain or iron hematoxylin stain. These two steps are designed to remove the mercury from the smear and then to remove the iodine; therefore, when your slide is placed into the Trichrome or iron hematoxylin stain, both the mercury and iodine are no longer present in the fecal smear. If you fail to incorporate these two steps into your staining protocol, the quality of your proficiency testing stained smears will be poor.

With very rare exceptions, the organisms in any of the proficiency testing (PT) specimens that you are asked to identify will be few to many in number. The presence of a very rare organism probably reflects something that was not seen in the screening process. The purpose of the PT specimen is to provide sufficient numbers (few to many) so that ALL of the participants see the same organisms. It is neither realistic nor practical to expect participants to find and identify organisms that are rare or very rare in number; this is not the purpose of the program. We appreciate the fact that in a patient specimen you would indicate all organisms seen, regardless of the numbers. However, in the PT specimens, you are being tested on those organisms that are present in "few" numbers or greater.

You may be asked to quantitate the organisms as a "quality control check" on the "aliquotting" process used to prepare participant vials prior to shipment. The information provides data for review related to the consistency of organism numbers throughout the aliquotting process. **In a clinical setting, quantitation of most of these organisms is not relevant and this information would not be added to the patient report.**