



## PARTICIPANT STATISTICS

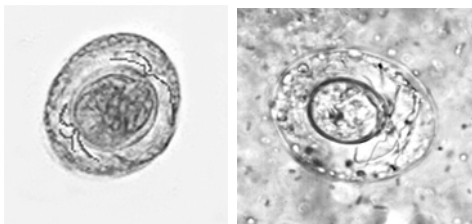
Specimen 1	Referees	Frequency	Ext 1	Ext 2
Organisms				
64 <i>Hymenolepis nana</i>	22	Few to Many	15	53
98 Parasites found, refer for ID			14	
63 <i>Hymenolepis diminuta</i>				1
99 No parasites found			1	
31 <i>Endolimax nana</i>				1
75 Hookworm			1	
TOTAL POPULATION	22		31	55

Extent 1 flagging appears for failure to report 64 or 98.

Extent 2 flagging appears for failure to report 64.

Flagging also appears in both extents for reporting other than 64 or 98.

**FORMALIN** - This specimen contains *Hymenolepis nana* eggs. The typical six-hooked oncosphere with the polar filaments and thin eggshell are clearly seen. Participants should have no problems identifying the eggs; however, in a few of the eggs, the polar filaments are a bit harder to see. The specimen also contains artifact material and a few root hairs. As mentioned before, helminth egg morphology is much easier to see in a direct wet mount or a concentration wet mount.



**Note:** thin eggshell, six-hooked oncosphere (embryo), and polar filaments (stringy filaments that lie between the embryo and the eggshell).

Specimen 2	Referees	Frequency	Ext 1	Ext 2
Organisms				
31 <i>Endolimax nana</i>			1	
99 No parasites found	18		27	25
TOTAL POPULATION	18		27	25

Flagging appears in all extents for reporting other than 99.

**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.

There are no parasites in this specimen. Artifact material and/or yeast cells can be somewhat confusing when reviewing the wet preparation using the low power and even high dry power objectives. However, there is nothing present that can be specifically identified at 100X and 400X magnifications as a parasite, either helminth or protozoan. When having trouble seeing possible internal structures and/or morphologic details, tap the coverslip and get things to move around a bit. Also, reduce the light intensity if you're not using iodine and drop the condenser to increase contrast. Iodine can be used to provide a bit more contrast; some laboratories routinely use iodine, while others do not. Too much light for wet preparations may prevent you from seeing parasites, particularly protozoa, which might be present in the specimen. **Although occasionally a formalin preparation may contain very rare organisms, specimens selected for proficiency testing tend to have moderate to many organisms that are present for identification.**

Specimen 3	Referees	Frequency	Ext 1	Ext 2
Organisms				
97 <i>Schistosoma haematobium</i>	22	Few to Many	17	54
98 Parasites found, refer for ID			12	
96 <i>Schistosoma mansoni</i>				1
TOTAL POPULATION			29	55

Extent 1 flagging appears for failure to report 97 or 98.

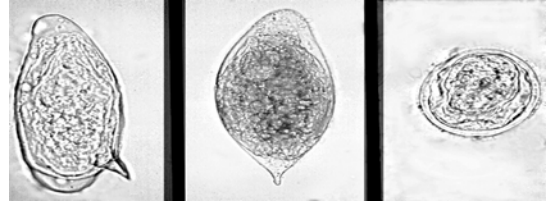
Extent 2 flagging appears for failure to report 97.

Flagging also appears in both extents for reporting other than 97 or 98.

## PARASITOLOGY

## FIRST QUADRIMESTER 2011

**FORMALIN** - This specimen contains *Schistosoma haematobium* eggs. This egg has a distinct terminal spine that is usually very easy to see.



The image on the left is *Schistosoma mansoni* (large lateral spine); the center image is *S. haematobium* (large terminal spine) and the image on the right is *S. japonicum* (small lateral spine or knob - often difficult to see). The small lateral spine on *S. japonicum* can be seen about 11:00 at the surface of the egg shell. Remember, when attempting to diagnose schistosomiasis, both urine and stool should be examined and should be collected using no preservatives (need to determine egg viability if eggs found).

Specimen 4	Referees	Ext 1	Ext 2
Organisms			
33 <i>Entamoeba coli</i>	11	6	29
98 Parasites found, refer for ID		9	
35 <i>Entamoeba histolytica</i>	2	5	5
36 <i>Entamoeba histolytica/E dispar</i>	3	3	9
40 <i>Iodamoeba butschlii</i>			3
12 <i>Chilomastix mesnili</i>			2
52 <i>Plasmodium</i> sp. refer for ID			1
TOTAL POPULATION	16	23	49

Extent 1 flagging appears for failure to report 33, 36 or 98.

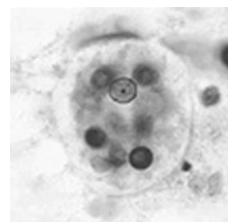
Extent 2 flagging appears for failure to report 33 or 36.

Flagging also appears in both extents for reporting other than 33, 36 or 98.

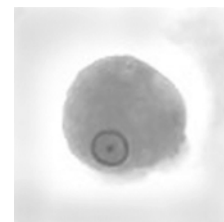
**DIGITAL IMAGE** - This specimen contains *Entamoeba coli* trophozoites and cysts (very rare cysts not selected for ID). Also, some of the trophozoites tend to resemble *Entamoeba histolytica/E. dispar*, so this response was also accepted. A *E. coli* is a nonpathogen, and most of the organisms were very typical, with the trophozoites having the characteristic nucleus (unevenly arranged nuclear chromatin with eccentric diffuse karyosome). There are rare cysts, none of which were boxed for ID. Also, when cysts are visible, they tend to have five or more nuclei; chromatoidal bars are less common than in *Entamoeba histolytica/E. dispar*. When chromatoidal bars are present they have sharp ends. Although some of these organisms resemble *Entamoeba histolytica/E. dispar*, overall the appearance is that of *E. coli*. Remember when measuring the cysts on permanent stained smears, one should measure the total distance, including the clear "halo" that represents shrinkage during staining.

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).

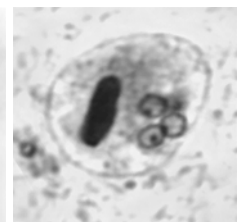
Typical *E. coli* parasites can be seen below. Also note the morphology for the other organisms as shown.

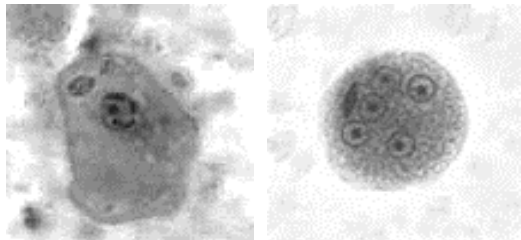


*Entamoeba histolytica*  
(Ingested RBCs)



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





*Entamoeba coli*

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

It is incorrect to identify any of the organisms in SAMPLE 4 as *Entamoeba histolytica*; none of the trophozoites contain ingested red blood cells!

NOTE: These trophozoites contain a lot of debris in the cytoplasm and there tend to be a lot of vacuoles; this appearance is typical of *E. coli*. Also, pseudopods are sometimes seen and they appear to be quite granular - typical for *E. coli*. The karyosomes tend to be blotlike and diffuse (rather than dot-like and compact) emphasize the correct identification as *E. coli*. Although the nuclear chromatin is usually uneven and sometimes clumpy, occasionally it may mimic the *Entamoeba histolytica/E. dispar* group and be more even. Also, the position of the karyosome (central or eccentric) is less important that whether it is dot-like or diffuse and blotlike: when the karyosome is quite diffuse, it is difficult to tell whether it is central or eccentric. The overall morphology can be enhanced by increasing the contrast and brightness. Because some of the organisms had characteristics that somewhat resemble *Entamoeba histolytica/E. dispar*, this response was also accepted along with *E. coli*.

**Example 1** contains one trophozoite, in which the nuclear chromatin is unevenly arranged - the karyosome is a bit eccentric and is somewhat blotlike (approximately 14.5 microns). The morphology is typical and characteristic of *E. coli*, with the cytoplasm containing a lot of debris. **Example 2** also contains one trophozoite, with typical morphology; however, the peripheral nuclear chromatin is not that uneven. The nucleus is eccentric and somewhat diffuse. This trophozoite measures approximately 15.9 microns, including the pseudopod that is visible at the bottom of the trophozoite. Like examples 6 and 9, this organism could be confused with *Entamoeba histolytica/E. dispar* however, when the total population of organisms is examined, they overall exhibit characteristics of *E. coli*.

In **Example 3** the trophozoite measures approximately 18.4 microns; note that the nuclear chromatin is very uneven and the karyosome is eccentric and very diffuse.

In **Example 4** the trophozoite displays very typical morphology (clumpy nuclear chromatin and diffuse karyosome). The organism measures approximately 13.2 microns. In this trophozoite, the karyosome appears to be eccentric. This trophozoite is a bit smaller than others on the slide.

In **Example 5** the trophozoite is somewhat large and measures approximately 19.5 microns. However, the overall morphology is that of *E. coli*. The nuclear chromatin tends to be clumpy and the karyosome is diffuse, not dot-like as seen in *E. histolytica/E. dispar*. As with previous examples, the cytoplasm contains a lot of debris and some vacuoles.

In **Example 6**, the trophozoite measures approximately 16.5 microns. This example tends to have a more dot-like karyosome - thus, this could be confused more with *Entamoeba histolytica/E. dispar*, although overall, the total population of organisms is more like *E. coli*.

In **Example 7** the trophozoite measures approximately 15.5 microns (including the pseudopod that is visible at the left bottom of the organism. Although the nuclear chromatin is a bit more even, the karyosome is very diffuse - typical of *E. coli*).

In **Example 8**, the trophozoite that measures approximately 15.8 microns; the nuclear chromatin is somewhat uneven and the karyosome is very diffuse and large. The overall morphology is very typical for *E. coli*.

**Example 9** demonstrates a trophozoite measures approximately 18.9 microns. The nuclear chromatin is somewhat even, but the karyosome is more diffuse and not dot-like. Like examples 2 and 6, in spite of the large amount of debris in the cytoplasm and very large size, the nuclear characteristics could be interpreted as being more representative of the *Entamoeba histolytica/E. dispar* group. However, in reviewing all the examples - all organisms exhibit characteristics of *E. coli* more than the other *Entamoeba* species.

In **Example 10**, the trophozoite measures approximately 18.7 microns and contains a typical nucleus - the peripheral chromatin is somewhat irregular and the karyosome is very large and diffuse.

**Specimen 5**

Organisms	Referees	Ext 1	Ext 2
54 <i>Plasmodium falciparum</i>	4	5	16
52 <i>Plasmodium</i> sp. refer for ID	8	9	9
53 <i>Plasmodium</i> sp. undetermined	3	6	6

98 Parasites fund, refer for ID		5	
44 <i>Babesia</i> sp.		5	
35 <i>Entamoeba histolytica</i>			4
99 No parasites found			1
40 <i>Iodamoeba butschlii</i>			1
56 <i>Plasmodium ovale</i>		1	
TOTAL POPULATION	15	31	37

Extent 1 flagging appears for failure to report 52, 53, 54 or 98.

Extent 2 flagging appears for failure to report 52, 53 or 54.

Flagging also appears in both extents for reporting other than 52, 53, 54 or 98.

**DIGITAL IMAGE** - This stained thin blood film demonstrates excellent examples of *Plasmodium falciparum* ring forms. The nucleus appears as a dark dot, while the cytoplasm appears as a paler "ring" structure. Since *P. falciparum* tends to infect all ages of RBCs, the infected RBC sizes vary within the smear. There are no larger rings, developing schizonts, or gametocytes on this blood film. Typically with *P. falciparum*, only the ring forms and gametocytes are seen in the peripheral blood; however, this specimen was drawn prior to the patient having gametocytes in the blood - this is typically seen with a traveler who becomes symptomatic early in the infection before the gametocytes are formed. Although many participants did not identify the organism to the species level, they would refer the specimen for identification or the organisms were identified as *Plasmodium* sp, undetermined.

NOTE: When *Plasmodium* sp. Is diagnosed, a report comment should always accompany the report: *Plasmodium* species seen - UNABLE TO RULE OUT *PLASMODIUM FALCIPARUM*.

**Example 1** contains two ring forms, one within the RBC and one that is in the appliqué or accolé form (the ring form looks like it is lying on the side of the RBC membrane).

**Example 2** contains two ring forms, both of which are within the RBCs. Note, there are a number of other ring forms visible in the background.

**Example 3** contains two rings within a single RBC. If you look closely, the nucleus and cytoplasm of each ring is visible.

**Example 4** contains several infected RBCs. One of the rings in the upper left is in the appliqué or accolé form and one RBC appears to contain two ring forms (about right/middle of the box).

**Example 5** contains one trophozoite in the appliqué or accolé form; the ring form lies along the side of the RBC.

**Example 6** contains two infected RBCs. In the left RBC, the ring form appears to be protruding from the RBC. This appearance is typical of *P. falciparum*.

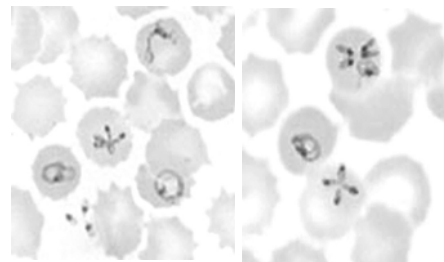
**Example 7** contains a ring form that is configured like "headphones" with two chromatin dots, one at each end of an open ring. This "headphone" appearance with two chromatin dots is also typical for *P. falciparum*.

**Example 8** contains four infected RBCs, each one containing a malarial ring form (same as early trophozoite). The chromatin dot and cytoplasm can be seen in all four rings. Occasionally one may see the RBC appearing to be crenated (or resemble a fimbriated edge); however, this appearance is not consistent and is much less pronounced that the fimbriated edges seen in RBCs infected with *Plasmodium ovale* (also have enlarged RBCs)

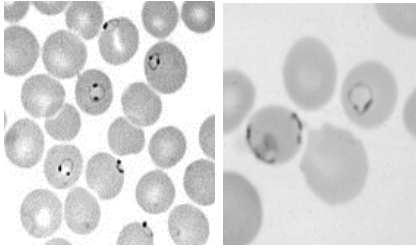
**Example 9** contains two infected RBCs, one of which contains two ring forms. This appearance is typical of *P. falciparum*.

**Example 10** contains one infected RBC containing two ring forms, both of which appear to be protruding from the RBC. Again, this is typical of *P. falciparum*.

For the very few participants who identified the specimen as *Babesia* sp, review the images below:



*Babesia* sp. Rings



*Plasmodium falciparum* rings

**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 incorporate 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.**