PROFICIENCY TESTING SERVICE
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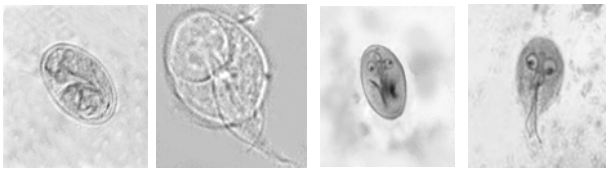
PARTICIPANT STATISTICS — PARASITOLOGY — SECOND QUADRIMESTER 2016

Specimen 1

Organisms	Referees	Frequency	Ext 1	Ext 2
524 Parasite(s) found referred for ID			14	
525 No parasites found			3	2
534 *Giardia lamblia*	11	Many	9	29
541 *Blastocystis hominis*				1
544 *Endolimax nana*	1	Few		1
TOTAL POPULATION	12		26	33

Extent 1 flagging appears for failure to report 534 or 524.
Extent 2 flagging appears for failure to report 534.
Flagging also appears in both extents for reporting other than 534 and 524.

PERMANENT SMEAR FOR STAINING - 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. There were some rare trophozoites present. Note - in some publications, you may see *Giardia lamblia* designated as either *G. intestinalis* or *G. duodenalis*. For the present, we will continue to the species name as *G. lamblia*.



Very high magnification
G. lamblia cyst — *G. lamblia* trophozoite — Wet Mounts
G. lamblia cyst — *G. lamblia* trophozoite — Permanent Stained Smear

Specimen 2

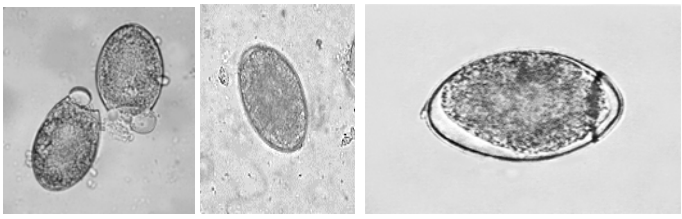
Organisms	Referees	Frequency	Ext 1	Ext 2
524 Parasite(s) found referred for ID			14	
544 *Endolimax nana*	2	Few to Many		2
545 *Entamoeba coli*				1
546 *Entamoeba hartmanni*				1
563 *Diphyllobothrium latum*	10	Few to Many	9	30
568 *Taenia saginata* proglottid				1
571 *Ascaris lumbricoides*			2	
TOTAL POPULATION	12		25	35

Extent 1 flagging appears for failure to report 563 or 524.
Extent 2 flagging appears for failure to report 563.
Flagging also appears in both extents for reporting other than 563, 544 and 524.

FORMALIN - The referees correctly identified this specimen as positive for few to many *Diphyllobothrium latum* eggs. was graded on the basis of the direct wet mount examination; identification of *Diphyllobothrium latum* or Parasite(s) found, referred for. They are very typical with an operculum and no opercular shoulders. The eggs measure 58-75 by 40-50 microns. Two of the referee laboratories reported *Endolimax nana*; however, these organisms are normally identified on the permanent stained smear, not the wet mount.

METHOD REMINDER: Allow the vial contents to settle out for at least 5 min. Then, WITHOUT CREATING A BUBBLE WITH THE PIPETTE (WILL STIR UP SEDIMENT AGAIN), carefully remove material at the bottom of the vial. This approach will help ensure that organisms present will be visible on the wet coverslip preparation. If you create a bubble, allow the vial contents to resettle again before taking the specimen for examination.

Note: the typical *Diphyllobothrium latum* eggs below (operculated with no opercular shoulders). In the image on the left, the opercula are open. When reviewing a wet mount, if you tap on the coverslip, you may get the operculum of this egg to pop open.

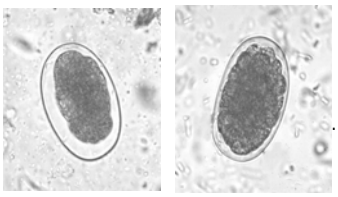


Specimen 3

Organisms	Referees	Frequency	Ext 1	Ext 2
524 Parasite(s) found referred for ID			18	
545 *Entamoeba coli*	9	Many	3	10
574 Hookworm	11	Many	12	30
589 *Schistosoma mansoni*			1	
TOTAL POPULATION	20		34	40

Extent 1 flagging appears for failure to report 574, 545 or 524.
Extent 2 flagging appears for failure to report 574 or 545.
Flagging also appears in both extents for reporting other than 574, 545 or 524.

FORMALIN - This specimen contains Hookworm eggs. The typical broadly oval egg is seen with a thin shell, a developing embryo at about the 8-16 ball stage of development, and a clear space between the shell and developing embryo (particularly visible when using iodine to enhance the color). As mentioned before, helminth egg morphology is much easier to see in a direct wet mount or a concentration wet mount. Often, helminth eggs appear darkly stained and shrunk on a permanent stained fecal smear



Note: thin eggshell, developing embryo, and clear space between the developing embryo and shell.

Specimen 4

Organisms	Referees	Ext 1	Ext 2
524 Parasite(s) found referred for ID		6	
555 *Plasmodium* sp. refer for id to species level	1	2	3
556 *Plasmodium* sp. undetermined	1	1	1
557 *Plasmodium falciparum*	9	8	23
TOTAL POPULATION	11	17	27

Extent 1 flagging appears for failure to report 557, 555, 556 or 524.
Extent 2 flagging appears for failure to report 557, 555 or 556.
Flagging also appears in both extents for reporting other than 557, 555, 556 and 524.

DIGITAL IMAGE - This stained blood film demonstrates excellent examples of *Plasmodium falciparum* gametocytes. The overall morphology is excellent with the shape and chromatin configuration very typical of female macrogametocytes (compact chromatin) and male microgametocytes (dispersed chromatin). This is a good digital image to scan for practice - see if you can find other *Plasmodium falciparum* organisms that are not boxed.

- Example 1 contains an excellent *Plasmodium falciparum* gametocyte. Although the shape is not yet crescent-shaped, the RBC outline can be seen at the top of the organism.
- Example 2 contains a *Plasmodium falciparum* gametocyte. Although the shape is not yet crescent-shaped, the organism is not quite mature and has more of a simple oval shape.
- Example 3 contains a *Plasmodium falciparum* gametocyte that is somewhat more elongated.
- Example 4 contains an excellent *Plasmodium falciparum* gametocyte. Note the dispersed chromatin (male microgametocyte) and the presence of the RBC membrane at the bottom of the organism.
- Example 5 contains an excellent *Plasmodium falciparum* female microgametocyte (compact chromatin). Part of the very pale RBC outline can be seen at the upper right of the organism.

●Example 6 contains an excellent *Plasmodium falciparum* male microgametocyte. The very pale RBC outline can be seen at the bottom of the organism. Although the shape is not quite crescent-shaped, this demonstrates the range of morphology that can be seen.
●Example 7 contains an excellent *Plasmodium falciparum* gametocyte; note the RBC outline at the left side of the organism.
●Example 8 contains an excellent *Plasmodium falciparum* female microgametocyte (compact chromatin). Part of the very pale RBC outline can be seen at the top of the organism.
●Example 9 contains a *Plasmodium falciparum* gametocyte that is somewhat more curved with the RBC outline seen at the right.
●Example 10 contains an excellent *Plasmodium falciparum* male microgametocyte (dispersed chromatin). The very pale RBC outline can be seen at the bottom of the organism.
●Example 11 contains a *Plasmodium falciparu*m gametocyte that is typical; note the RBC outline at the top of the organism.
●Example 12 contains an excellent *Plasmodium falciparum* female microgametocyte (compact chromatin). Part of the very pale RBC outline can be seen at the bottom of the organism.
●Example 13 contains an excellent *Plasmodium falciparum* female microgametocyte (compact chromatin). Part of the very pale RBC outline can be seen at the left side of the organism.

NOTE: There are many other gametocytes throughout the slide - by scanning the image, you can see the range of morphology that can be seen in a patient preparation.

Specimen 5

Organisms		Referees	Ext 1	Ext 2
524	Parasite(s) found referred for ID		9	
545	*Entamoeba coli*	2	4	9
546	*Entamoeba hartmanni*			1
547	*Entamoeba histolytica*	*4*	5	8
548	*E. histolytica/Entamoeba dispar*	5		14
	TOTAL POPULATION		18	32

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

DIGITAL IMAGE - Although the correct response is *Entamoeba histolytica/Entamoeba dispar*, this specimen was ungradable due to the lack of consensus among the referees or participants. UNLESS INGESTED RBCS ARE SEEN IN THE CYTOPLASM OF THE TROPHOZOITES, THE CORRECT RESPONSE IS ENTAMOEBA HISTOLYTICA/ ENTAMOEBA DISPAR.

THERE ARE NO ENTAMOEBA HISTOLYTICA ORGANISMS (true pathogens containing ingested RBCs within the cytoplasm) IN THIS SPECIMEN (SEE IMAGES BELOW).

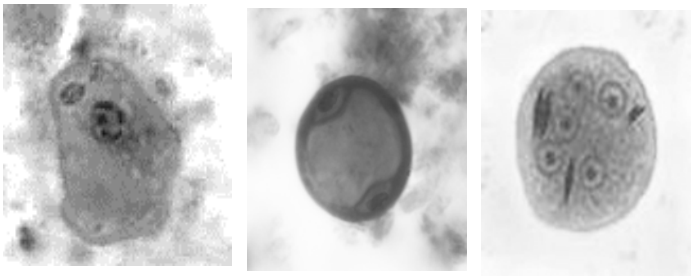
NOTE: IT CAN BE DIFFICULT TO IDENTIFY AN ORGANISM TO GENUS/SPECIES BASED ON A SINGLE ORGANISM; MULTIPLE EXAMPLES NEED TO BE EXAMINED AND THE COMBINED CHARACTERISTICS USED TO IDENTIFY THE PARASITE CORRECTLY. All organisms measured above 12 microns. Also note there are no RBCs anywhere in the background of the stool smear.

●Example 1 contains a trophozoite (over 12 microns); the nucleus contains a centrally located karyosome and evenly arranged nuclear chromatin. The pink structure at the left of the organism is debris - not an RBC.
●Example 2 contains a trophozoite, measuring over 12 microns. Although the nuclear karyosome is eccentric; it is fairly compact with evenly arranged nuclear chromatin. The shape of the karyosome is more important than the position (see images below).
●Example 3 contains a trophozoite, measuring over 12 microns. The nuclear karyosome is fairly compact with evenly arranged nuclear chromatin surrounding the karyosome. Although the nuclear chromatin is pale, it is very typical. Note the debris and small vacuoles within the cytoplasm, also typical of some trophozoites.
●Example 4 contains a trophozoite, measuring over 12 microns. The karyosome is compact, centrally located, with evenly arranged nuclear chromatin.
●Example 5 contains a trophozoite, measuring over 12 microns. As with the other trophozoites, the karyosome is compact, central, and is surrounded by evenly spaced nuclear chromatin.
●Example 6 contains a trophozoite, measuring over 12 microns. The karyosome is compact, central, and is surrounded by evenly spaced nuclear chromatin. Although the nuclear peripheral chromatin is not the same density all around, it does surround the karyosome, rather than being very clumpy and irregular as seen in Entamoeba coli.
●Example 7 contains a precyst, measuring over 12 microns. The precyst contains a single nucleus, often enlarged prior to division into multiple nuclei for the cyst form. Although the karyosome is somewhat eccentric, the location is less important than the fact that it is in a single nucleus and is compact.
●Example 8 contains a trophozoite, measuring over 12 microns. The karyosome is compact with evenly arranged nuclear chromatin.
●Example 9 contains a precyst, measuring over 12 microns. Note the nucleus is somewhat enlarged, the karyosome is compact and the nuclear chromatin is evenly arranged. Note in the precysts the cytoplasm area appears to be almost clear (see also example 7).
●Example 10 contains a precyst, measuring over 12 microns. Note the single nucleus is quite enlarged (prior to nuclear division into the more mature cyst), the karyosome is compact and the nuclear chromatin is evenly arranged. Note in the precysts the cytoplasm area appears to be almost clear (see also examples 7 and 9).
●Example 11 contains a trophozoite, measuring over 12 microns. Although the karyosome is a bit eccentric, it is compact and the nuclear chromatin is evenly arranged on the membrane. The reddish object at the top of the field is not an RBC, but debris (see also example 1).
●Example 12 contains a trophozoite, measuring over 12 microns. As with the other trophozoites, the karyosome is compact, somewhat central, and is surrounded by evenly spaced nuclear chromatin.
●Example 13 contains a trophozoite, measuring over 12 microns. As with the other trophozoites, the karyosome is compact, central, and is surrounded by evenly spaced nuclear chromatin.

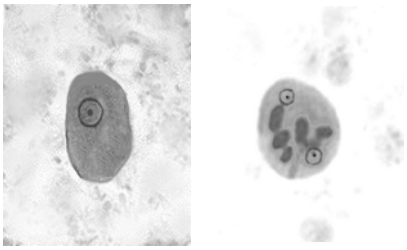
OVERALL CONSIDERATIONS FOR CORRECT IDENTIFICATION:

- All trophozoites measure over 12 microns (eliminating *Entamoeba hartmanni*)
- Nuclear karyosomes tend to be compact and are mostly centrally located (eliminating *Entamoeba coli*, which tend to have a blot-like diffuse karyosome).
- Nuclear chromatin is evenly arranged on the nuclear membrane.
- There are no RBCs present within the trophozoites or in the background of the fecal smear (eliminating *Entamoeba histolytica*/the true pathogen).
- The cyst contains a chromatoidal bar with smooth, rounded ends; the overall cyst measures approximately 10 microns. If the cyst measured approximately 8-9 microns or smaller, *Entamoeba hartmanni* would be have to be considered. However, it is unlikely we would send a mixed challenge unless the two organisms were distinctly different and there were plenty of examples of both organisms marked for examination.
- ALL of these characteristics validate the identification of *Entamoeba histolytica/Entamoeba dispar.*

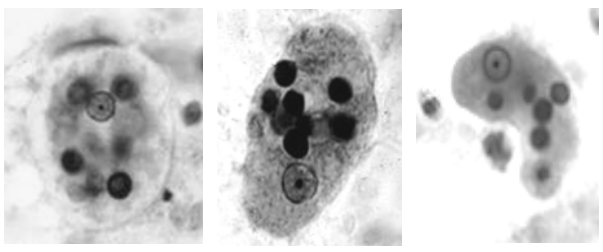
Examples of other related amebae are seen below:



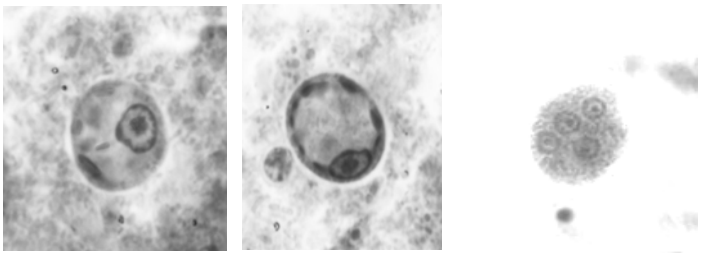
Entamoeba coli trophozoite, precyst (2 nuclei), and cyst



Entamoeba hartmanni trophozoite and cyst



Entamoeba histolytica trophozoites containing ingested RBCs



Entamoeba histolytica/Entamoeba dispar precysts and cyst (right image)
NOTE: Precysts (single nucleus) and cysts cannot be identified morphologically other than Entamoeba histolytica/E. dispar.

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 parasite 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.

We encourage participants to report *Blastocystis* spp; however, these organisms are much easier to identify correctly from a permanent stained smear. Blastocystis is an extremely common parasite with a worldwide distribution. It is not uncommon for it to be the most frequently isolated parasite in epidemiological surveys. Prevalence varies widely from country to country and within various communities of the same country. In general, developing countries have higher percentages of the parasite than developed countries, and this has been linked to poor hygiene, exposure to animals, and consumption of contaminated food or water. Based on PCR-based genotype classification data, there may be approximately 10 or more different subtypes within the genus. Some subtypes are pathogenic and some are non-pathogenic. If no other pathogens are found, *B. hominis* may be the cause of patient symptoms. Confirmation of these subtypes and their pathogenic status may also explain why some patients are asymptomatic and some have clinical symptoms. In the future, it will be recommended that these organisms be reported as *Blastocystis* spp.

Two report comments that should be used when this organism is reported are as follows:

1. The name *Blastocystis hominis* contains approximately 10 different organism subtypes, none of which can be differentiated on the basis of organism morphology; some subtypes are pathogenic and some are non-pathogenic. If no other pathogens are found, *B. hominis* may be the cause of patient symptoms. The proper designation is *Blastocystis* spp.
2. Other organisms capable of causing diarrhea should also be ruled out.