

Q1 2018 Parasitology



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Q1 2018 Parasitology Specimen 1

Organisms	Referees	Frequency	Extent 1	2	Total
525 - No parasites found	11		19	26	45
544 - Endolimax nana			0	1	1
TOTAL POPULATION			19	27	46

Flagging for all extents appear for reporting other than 525.

Specimen 1

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. Flagging appears in all extents for reporting other than "No Parasites Found" – participants performed very well in the examination of Sample 1 with an overall 97.8% correct response.

Specimen 1

PERMANENT STAIN:

The permanent stained smear contains no parasites. Artifact material is rarely consistent in either size or shape. This specimen contains the normal stool debris, most of which has no standard shape or size. In contrast to examination of the wet preparation, remember to use plenty of light when examining the permanent stained smears and to use the oil immersion objective (100X). You need to cover at least 300 oil immersion fields before you call the stained smear negative. Flagging appears for reporting other than No parasites found (**Referees 11/11, 100% and participants 97.8%**).

Specimen 2

Organisms	Referees	Frequency	Extent 1	2	Total
578 - Strongyloides stercoralis / sp.	11	Few to Many	4	28	32
524 -parasite(s) found referred for ID			12	0	12
544 - Endolimax nana			0	1	1
525 - No parasites found			1	0	1
574 - Hookworm			1	0	1
573 - Enterobius vermicularis			1	0	1
TOTAL POPULATION			19	29	48

Extent 1 flagging appears for failure to report 578 or 524.

Extent 2 flagging appears for failure to report 578.

Flagging also appears in both extents for reporting other than 578 or 524.

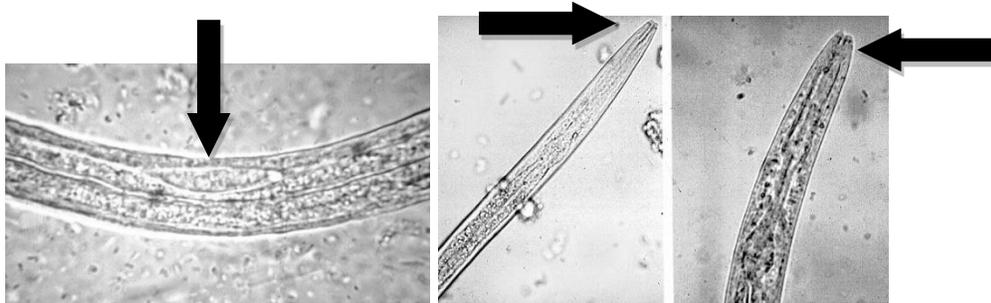
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SPECIMEN 2:

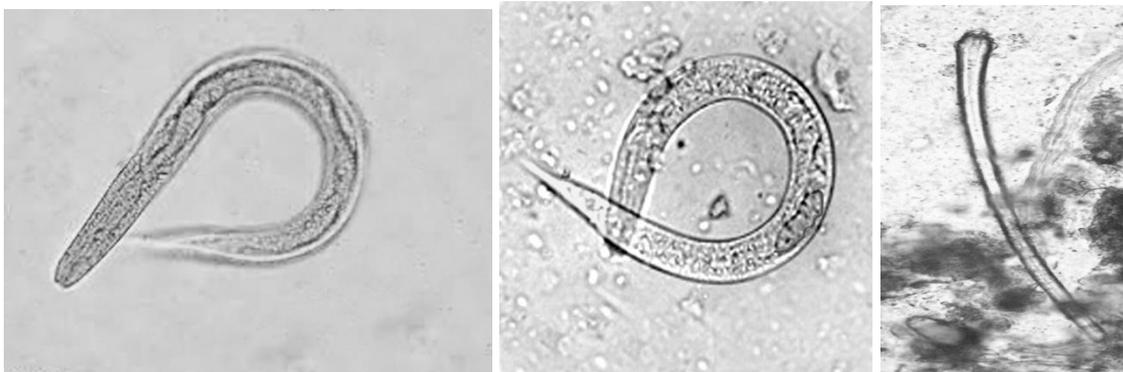
FORMALIN:

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 *Strongyloides stercoralis* rhabditiform (non-infective) larvae. The *Strongyloides* rhabditiform larvae were very typical with the short mouth (buccal) opening and the large packet of genital primordial cells. Although some of the larvae were a bit distorted, the overall morphology was very clear, and the organism identification was relatively easy. Flagging in extent 1 for failure to report *Strongyloides stercoralis* or parasite(s) found, referred for ID. Flagging occurs in extent 2 for failure to report *Strongyloides stercoralis*. Flagging appears in both extents for reporting other than *Strongyloides stercoralis* or "Parasites found, referred for ID" – participants performed very well in the examination of Sample 2 with an overall 95.7% correct response. The referees reported 100% (11/11) for *Strongyloides stercoralis*. One each laboratory reported incorrectly hookworm or *Enterobius vermicularis*.



(Left image) Note the large packet of genital primordial cells; (Right paired image) hookworm long mouth opening (left), *Strongyloides* short mouth opening (right) (see arrows). Two additional rhabditiform (non-infectious) larvae in wet mounts are seen below. Note the artifact (root hair) below on the right (no internal structure).



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Specimen 3

Organisms	Referees	Frequency	Extent 1	2	Total
574 - Hookworm	10	Few to Many	6	29	35
524 - parasite(s) found referred for ID			14	0	14
545 - <i>Entamoeba coli</i>	5	Few to Many	1	11	12
TOTAL POPULATION			21	40	61

Extent 1 flagging appears for failure to report 574 or 524.

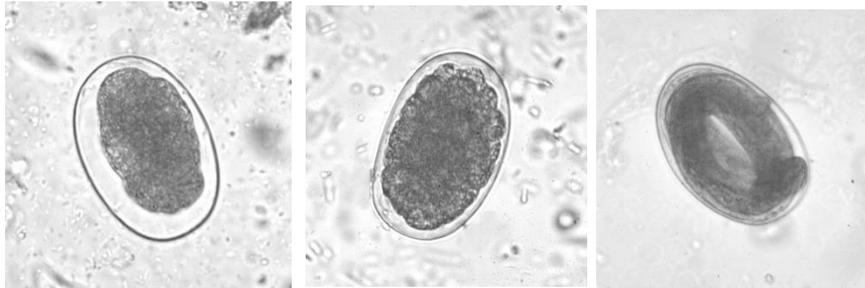
Extent 2 flagging appears for failure to report 574.

Flagging also appears in both extents for reporting other than 574, 545, or 524.

SPECIMEN 3 FORMALIN

This specimen contains Hookworm eggs and *Entamoeba coli*. 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). The referee labs reported 10/11 (90.9%) hookworm eggs. Extent 1 flagging appears for failure to report Hookworm, or Parasites found, referred for ID. Extent 2 flagging appears for failure to report Hookworm. Flagging also appears in both extents for reporting other than Hookworm, Parasites found, referred for ID, or *Entamoeba coli*. Overall, participants performed well with this challenge. As mentioned before, helminth egg morphology is much easier to see in a direct wet mount or a concentration wet mount. Helminth eggs appear darkly stained and shrunk on a permanent stained fecal smear.

Referee laboratories (10/11, 90.9%) reported few to many hookworm eggs. Five referee labs reported *Entamoeba coli*. Note the hookworm egg images below; the one on the left is less mature and is about the 8-ball stage of development. The image on the right show an egg that contains a larval form; this can happen if eggs are held too long prior to preservation. If they hatch, these larvae can be confused with those of *Strongyloides stercoralis* (see specimen #2 above). Note the clear space between the thin egg shell and developing embryo.



Per CMS regulations, participants are required to report 5 samples unless the sample type or organism is not reported. If you are having trouble viewing Samples 4 and 5, please contact AAB Technical Support. Would refer is not an appropriate response unless it is an organism or represents a sample type your laboratory would refer.

Specimen 4

Organisms	Referees	Extent 1	2	Total
547 - <i>Entamoeba histolytica</i>	8	7	16	23
548 - <i>Entamoeba histolytica</i> / <i>Entamoeba dispar</i>	3	3	4	7
524 - parasite(s) found referred for ID		6	0	6
541 - <i>Blastocystis hominis</i>		0	1	1
534 - <i>Giardia lamblia</i>		0	1	1
553 - <i>Cryptosporidium</i> sp.		0	1	1
544 - <i>Endolimax nana</i>		0	1	1
TOTAL POPULATION		16	24	40

Extent 1 flagging appears for failure to report 547, 548 or 524.

Extent 2 flagging appears for failure to report 547 or 548.

Flagging also appears in both extents for reporting other than 547, 548 or 524.

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SPECIMEN 4 (Digital Image):

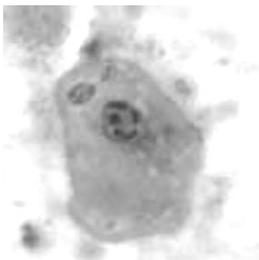
This permanent stained fecal smear demonstrates excellent examples of the true pathogen *Entamoeba histolytica*. Since a couple of the organisms selected do not contain ingested RBCs within the cytoplasm, *Entamoeba histolytica*/*E. dispar* group is also considered a correct response. Extent 1 flagging occurs for failure to report *Entamoeba histolytica*, *Entamoeba histolytica*/*E. dispar*, or parasite(s) found, referred for ID. Flagging occurs in Extent 2 for failure to report *Entamoeba histolytica* or *Entamoeba histolytica*/*E. dispar*. Flagging also appears in both extents for reporting other than *Entamoeba histolytica*, *Entamoeba histolytica*/*E. dispar* or parasite(s) found, not identified referred for ID. **THE MAJORITY OF THE ORGANISMS ARE TYPICALLY *ENTAMOEBIA HISTOLYTICA*.**

If a positive fecal immunoassay specific for the true pathogen, *Entamoeba histolytica*, is positive or the trophozoites on the permanent stained slide contain ingested RBCs within the cytoplasm, it is acceptable to identify any of these organisms as *Entamoeba histolytica*; in this specimen most of the trophozoites contain ingested red blood cells. However, without the confirmation of a positive appropriate immunoassay, even with ingested RBCs, some experts indicate the correct identification could also be: *Entamoeba histolytica*/*E. dispar* (or *Entamoeba histolytica*/*E. dispar* group). However, if you report with both organism names rather than *Entamoeba histolytica*, a comment should be added indicating ingested RBCs were seen within the organism cytoplasm. If the RBCs are present, then the true pathogen, *Entamoeba histolytica*, can be reported.

- **Example 1** contains a trophozoite containing ingested RBCs, has a single nucleus with even nuclear chromatin, and a central karyosome that appears neat and compact. Note the pleomorphic RBCs in the background.
- **Example 2** contains a single trophozoite with the nucleus having evenly arranged peripheral chromatin and a central, compact karyosome. No ingested RBCs were seen within the cytoplasm, however, note the many RBCs throughout the background.
- **Example 3** contains ingested RBCs in the cytoplasm, and has a single nucleus with even chromatin and a compact central karyosome (below center). Again, note the numerous RBCs in the background.
- **Example 4** contains a very pale staining trophozoite. Note the typical nucleus and ingested RBCs
- **Example 5** is a single trophozoite containing ingested RBCs and a typical nucleus with even chromatin and a compact central karyosome.
- **Example 6** contains ingested RBCs and a single typical nucleus.
- **Example 7** contains two ingested RBCs and a typical nucleus with even chromatin and a central, compact karyosome.
- **Example 8** contains numerous ingested RBCs and a typical nucleus. Note the PMN directly to the right of the organism (lobed nucleus).
- **Example 9** does not appear to contain ingested RBCs, but note the typical nucleus and RBCs in the background.
- **Example 10** contains numerous ingested RBCs in the cytoplasm and a typical nucleus (note the karyosome is a bit out of focus).

RESULTS: Congratulations to the referee laboratories (100%) and participants (90.8%) that identified *Entamoeba histolytica* and *Entamoeba histolytica*/*E. dispar* as being present. **See additional tips below:**

1. Note that the ingested RBCs are being digested within cytoplasmic vacuoles, so they will appear smaller than the RBCs found in the background of the fecal smear.
2. Although the nuclear peripheral chromatin may not always go all the way around (*Entamoeba histolytica* or *Entamoeba histolytica*/*E. dispar*, it is not clumpy and uneven like that seen in *Entamoeba coli*).
3. While the karyosome seen in *Entamoeba coli* tends to be somewhat large and blot-like, karyosomes seen in *Entamoeba histolytica* tend to be compact and neat. **NOTE THAT THE POSITION OF THE KARYOSOME (CENTRAL or ECCENTRIC) IS NOT AS IMPORTANT AS THE APPEARANCE OF THE CHROMATIN AND KARYOSOME.**



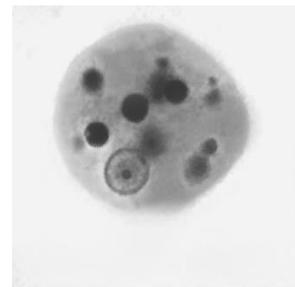
Entamoeba coli



E. coli



Entamoeba histolytica/*E. dispar*



Entamoeba histolytica
Ingested (RBCs)

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Specimen 5

Organisms	Referees	Extent 1	2	Total
533 - <i>Dientamoeba fragilis</i>	11	8	20	28
524 - parasite(s) found referred for ID		7	0	7
545 - <i>Entamoeba coli</i>		0	2	2
547 - <i>Entamoeba histolytica</i>		0	1	1
546 - <i>Entamoeba hartmanni</i>		1	0	1
552 - <i>Cyclospora cayetanensis</i>		0	1	1
TOTAL POPULATION		16	24	40

Extent 1 flagging appears for failure to report 533 or 524.

Extent 2 flagging appears for failure to report 533.

Flagging also appears in both extents for reporting other than 533 or 524.

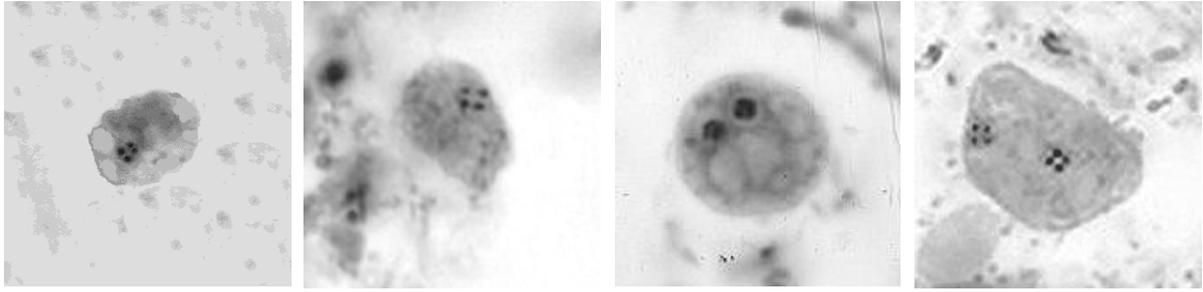
SPECIMEN 5 (Digital Image):

This specimen (digital image of routine trichrome stain) contains *Dientamoeba fragilis* trophozoites. The referees (11/11) reported correctly, identifying *Dientamoeba fragilis*. NOTE: *Dientamoeba fragilis* can often mimic *E. hartmanni* or *E. nana*, particularly when the *D. fragilis* trophozoite contains only a single nucleus. Extent 1 flagging appears for failure to report *Dientamoeba fragilis* or parasite(s) found referred for ID. Extent 2 flagging appears for failure to report *Dientamoeba fragilis*. Flagging also appears in both extents for reporting other than *Dientamoeba fragilis* or parasite(s) found referred for ID. Overall, participants performed very well with this challenge. Although the cyst of this organism has now been confirmed, the number of cysts seen in patient specimens is very low. Identifications will continue to be based on the trophozoite stage.

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).

- **Example 1** contains a single trophozoite, which contains a single nucleus (fragmented karyosome). Note there are also granules/debris within the cytoplasm. This is typical for *D. fragilis*.
- **Example 2** contains two trophozoites, both of which contain two nuclei. The nuclear chromatin appears to be somewhat fragmented, very typical for *D. fragilis*.
- **Example 3** contains two trophozoites with two nuclei, both of which appear to be somewhat fragmented. In the lower trophozoite, the two nuclei are very close together and more difficult to differentiate.
- **Example 4** contains one trophozoite that is somewhat larger. The nuclear chromatin is fragmented. Note that the cytoplasm varies from light to dark, both areas containing granules.
- **Example 5** contains a single trophozoite, with two nuclei (one top, one bottom); note the cytoplasm contains debris and the nuclear chromatin is not yet fragmented.
- **Example 6** contains two trophozoites, with two nuclei (organism on the right is more in focus. The cytoplasm appears to be vacuolated, also typical in *D. fragilis*.
- **Example 7** contains a single trophozoite (somewhat larger). Note the nucleus is fragmented into two parts. Also, note the debris within the cytoplasm.
- **Example 8** contains a single trophozoite with one fragmented nucleus (upper right). There are also two clumps of debris that can mimic nuclei.
- **Example 9** contains one trophozoite with one nucleus. The karyosome is beginning to fragment. Note the pleomorphic shape of the organism (typical). And the granular debris in the cytoplasm.
- **Example 10** contains a single trophozoite with two nuclei. Note the cytoplasm also contains granular material and debris.

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Dientamoeba fragilis trophozoites