Laboratory diagnosis of amebiasis

Entamoeba histolytica and *Entamoeba dispar*

DPDx
Laboratory Identification of Parasites of Public Health Concern

Basic guidelines

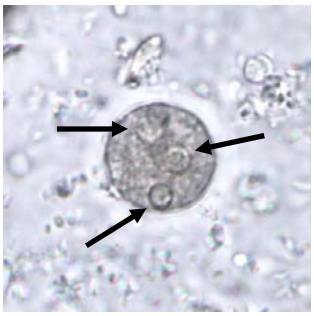
A. Multiple stool samples (at least 3) should be tested before a negative result is reported.

B. To maximize recovery of cysts, stool samples in formalin, or other fixatives, should be concentrated prior to microscopic examination (e.g.,10 min at 500 × *g* when using the formalin-ethyl-acetate concentration procedure). ***Exception:*** *Specimens to be used for EIA or rapid cartridge assays should NOT be concentrated because antigens are lost during the procedure!*

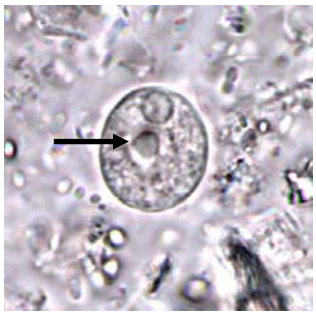
C. Choice of diagnostic techniques depends on available equipment and reagents, experience, and considerations of time and cost.

1. Wet mount

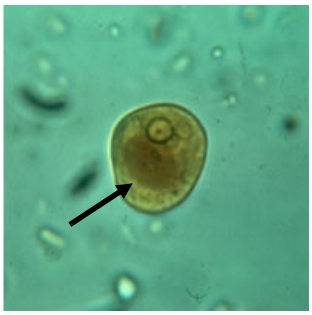
Entamoeba histolytica and *Entamoeba dispar* are morphologically identical species. In bright-field microscopy, *E. histolytica*/*E. dispar* cysts are spherical and usually measure 12 to 15 µm (range may be 10 to 20 µm). A mature cyst has 4 nuclei while an immature cyst may contain only 1 to 3 nuclei. Peripheral chromatin is fine, uniform, and evenly distributed. Elongated, chromatoid bodies with bluntly rounded ends may sometimes be found. Glycogen can be diffuse or absent in mature cysts while clumped in immature cysts. Wet mount preparations and trichrome stained smears of stool specimens are the recommended procedures for identification of *E. histolytica/E. dispar*.



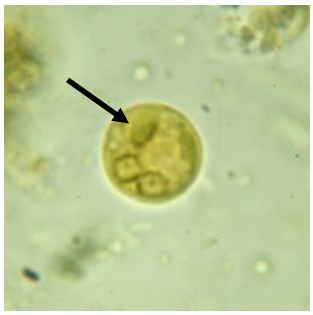
E. histolytica/E. dispar cyst with three visible nuclei (arrows).



E. histolytica/E. dispar cyst with one visible nucleus and a glycogen vacuole (arrow).



E. histolytica/E. dispar cyst in iodine with one visible nucleus and a glycogen vacuole (arrow).



E. histolytica/E. dispar cyst in iodine with two visible nuclei and a chromatoid body (arrow).

2. Trichrome stain

Trophozoites in trichrome stained smears usually measure 15 to 20 µm (range may be 10 to 60 µm). Presence of one nucleus with evenly arranged chromatin on the nuclear membrane and a small, centrally located karyosome are morphological features of trophozoites. The cytoplasm is finely granular and few ingested bacteria or debris may be present. Presence of red blood cells within the cytoplasm of trophozoites is a diagnostic feature for the identification of *E. histolytica*. Ingested RBCs are not frequently seen; in the absence of this diagnostic characteristic *E. histolytica/E. dispar* should be reported. Cysts usually measure 12 to 15 µm (range 10 to 20 µm) and have 1 to 4 nuclei. Chromatoid bodies with bluntly rounded ends may also be present.

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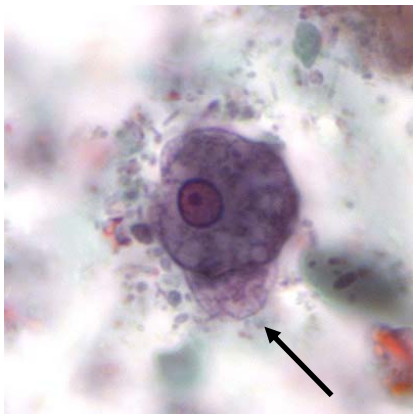
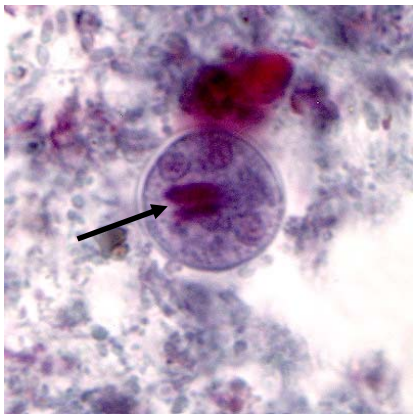
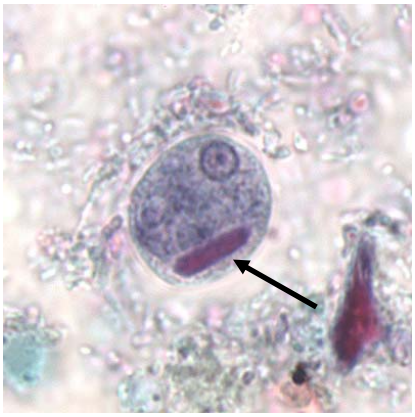


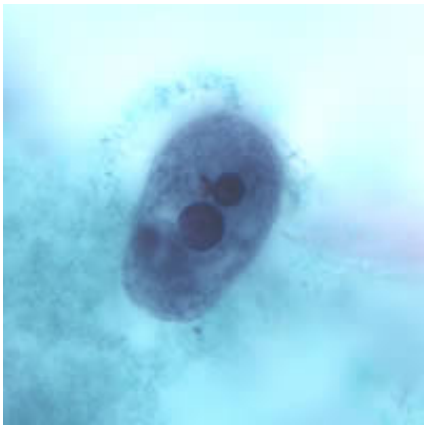
E. histolytica/E. dispar trophozoite with a progressive pseudopod (arrow).



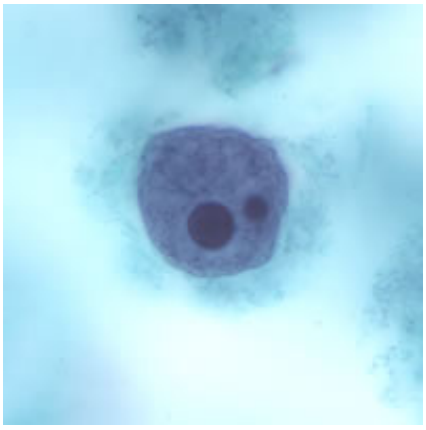
E. histolytica/E. dispar cyst showing chromatoid bodies with bluntly rounded ends (arrow).



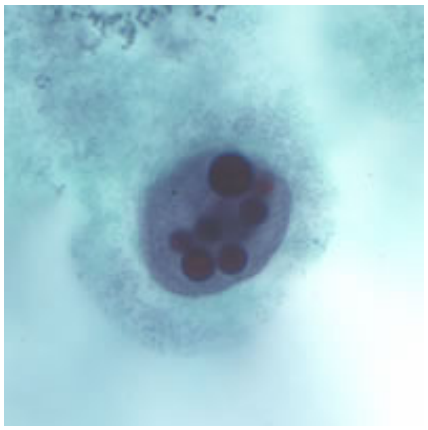
E. histolytica/E. dispar cyst showing a chromatoid body with bluntly rounded ends (arrow).



E. histolytica trophozoite with an ingested RBC (arrow).



E. histolytica trophozoite with an ingested RBC (arrow).



E. histolytica trophozoite with six ingested RBCs in the focal plane (arrows).

3. Enzyme immunoassays (EIA)

Immunoassay kits are commercially available that detect *E. histolytica.* Currently, these tests require the use of fresh or frozen stool specimens and **cannot** be used with preserved specimens.

4. Rapid immunochromatographic cartridge assay

A rapid cartridge is available that detects antigens of *E. histolytica*/*E. dispar*, however this assay does not distinguish between *E. histolytica* and *E. dispar*. This assay also detects antigens of *Giardia* and *Cryptosporidium.* Stool samples must be fresh or frozen and should not be concentrated prior to testing. Borderline positives and questionable negatives obtained with this technique should be further confirmed by additional testing. This assay is quick and easy to perform and no special equipment is needed.

The quality of reagents in commercially available kits may be variable or deteriorate under storage conditions; for that reason external controls are necessary to determine whether the kit is properly performing.