Laboratory diagnosis of parasitic diseases

(Amoebiasis)

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Laboratory Diagnosis of Amoebiasis:

- *E. histolytica* must be carefully differentiated from other non-pathogenic, intestinal amoeba.
- Microscopic identification of cyst and trophozoites in the stool is the common method for diagnosing *E.histolytics.*
- The non-pathogenic *Entamoeba dispar* is morphologically identical to *E. histolytica*.
- Trophozoites with ingested red blood cells in fresh stool or other specimens and trophozoites in tissue biopsies are both strongly correlated with the presence of *E.histolytica* and invasive disease.
- The differentiation between *E. histolytica E. dispar* must be based on isoenzymatic or immunologic analysis.
- Cysts in stool samples may be identified from direct saline wet smears or smears stained with iodine or trichrome with or without concentration of stool samples.
- Trophozoites will have to be demonstrated in fresh stool without concentration.

Microscopy of Amebae

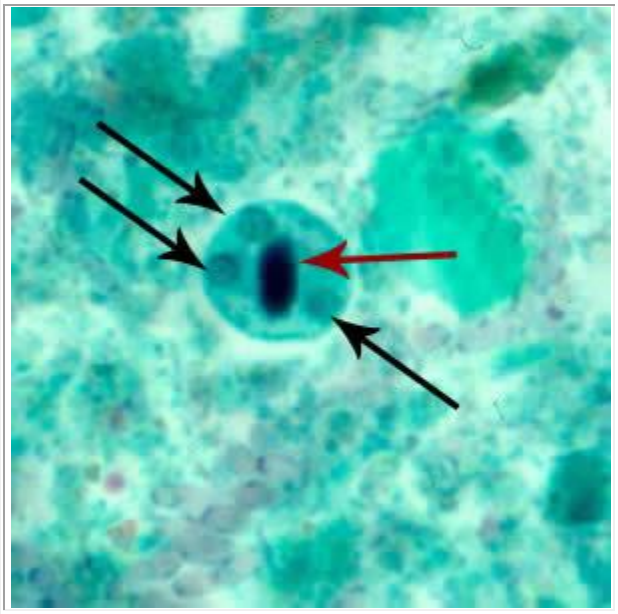
Table1.1. Comparison of Ameabae (cyst)

			NUCLEUS			CYTOPLASM	
Species	**Size (Diameter or length)**	**Shape**	**Number**	**Peripheral Chromatin**	**Karyosomal Chromatin**	**Chromatoid Bodies**	**Glycogen**
Entamoeba histolytica	10-20 □m	Round	4 in mature cyst.	Peripheral chromatin present. Fine, uniform granules, evenly distributed.	Small, discrete, usually centrally located.	Present. Elongated bars with bluntly rounded ends.	Usually diffuse. Concentrated mass often present in young cysts. Stains reddish brown with iodine.
Entamoeba coli	10-35 □m	Round	8 in mature cyst.	Peripheral chromatin present. Coarse granules irregular in size and distribution,.	Large, discrete, usually eccentric	Present, but less frequently seen than in *E. histolytica*. Usually splinter-like with pointed ends.	Usually diffuse, but, occasionally well defined mass in immature cysts. Stain reddish brown with iodine.
Endolimax nana	5-10 □m.	Spherical or ovoidal.	4 in mature cysts. Immature cysts are rarely seen.	None	Large (blot-like), usually central.	Not present	Usually diffuse. Concentrated mass seen occasionally in young cysts. Stains reddish brown with iodine.
Iodamoeba buetschlii	5-20 □m. m	Ovoidal, ellipsoidal, triangular, or other shapes.	1 in mature cyst.	None	Large, usually eccentric. Refractile, achromatic granules on one side of karyosome. Indistinct in iodine preparations.	Not present	Compact, well-defined mass. Stains dark brown with iodine.

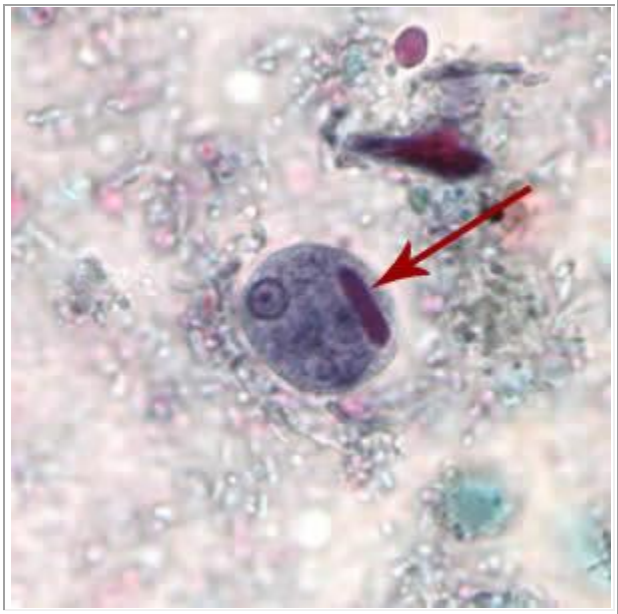
A

B

A: Cyst of *E. histolytica*/*E. dispar* in an unstained concentrated wet mount of stool. Notice the chromatoid body with blunt, rounded ends (arrow).
B: Cyst of *E. histolytica*/*E. dispar* in a concentrated wet mount stained with iodine.



C



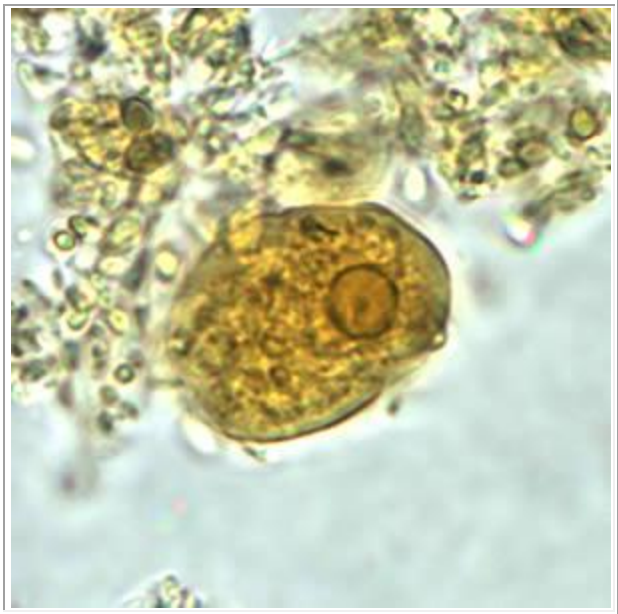
D

C: Cyst of *E. histolytica*/*E. dispar* stained with trichrome. Three nuclei are visible in the focal plane (black arrows), and the cyst contains a chromatoid body with typically blunted ends (red arrow).
D: Cyst of *E. histolytica*/*E. dispar* stained with trichrome. Notice the chromatoid body with blunt, rounded ends (arrow).

Table.2 Comparison of Amebae (trophozoite)

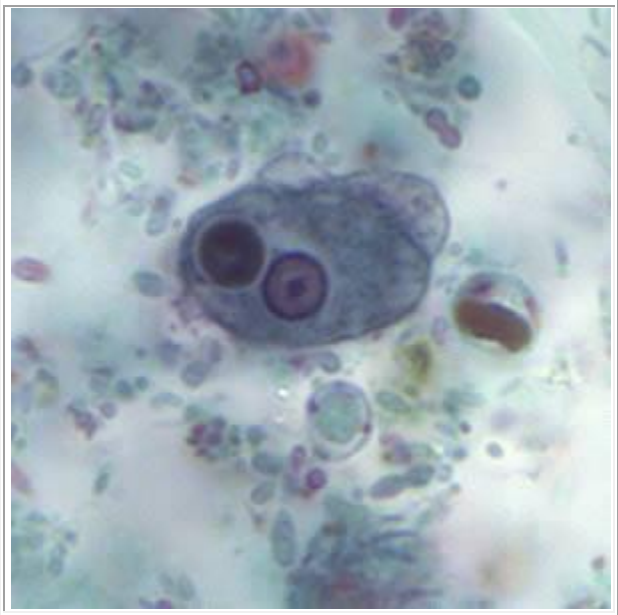
Species	**Size (µm)**	**Motility**	**Cytoplasm**	**Nuclear structure**
Entamoeba histolytica	15-25	Progressive directional	*Finely granular *May contain ingested red blood cells	Small, central Karyosome Fine , evenly distributed peripheral chromatin
Entamoebal coli	15-50	Nondirectionl	*Vacuolated *Ingested bacteria	*Large , eccentric Karyosomes * Coarse uneven peripheral chromatin
Endolimax nana	5-12	Nondirectionl	*Vacuolated *May containIngested bacteria	* Large , irregularly shaped karyosome * No peripheral chromatin
Iodamoeba buetschlii	6-20	Nondirectional	*Vacuolated *May containIngested bacteria	*Large karyosome surrounded by achromatic granules * No peripheral chromatin

E



F

E, **F:** Trophozoites of *E. histolytica*/*E. dispar* in a direct wet mount stained with iodine.



I

I: Trophozoite of *E. histolytica* with ingested erythrocytes stained with trichrome. The ingested erythrocyte appears as a dark inclusion. Erythrophagocytosis is the only characteristic that can be used to differentiate morphologically *E. histolytica* from the nonpathogenic *E. dispar*.