

Isospora belli

Basic guidelines

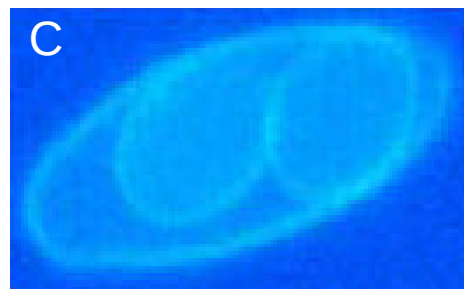
- A. Multiple stool samples (at least 3) should be tested before a negative result is reported.
- B. To maximize recovery of oocysts, stool samples in formalin should be concentrated prior to microscopic examination (e.g., 10 min at $500 \times g$ when using the formalin-ethyl acetate concentration procedure).
- C. Choice of diagnostic techniques depends on available equipment and reagents, experience, and considerations of time and cost.

1. Wet mount

Under bright-field microscopy, the oocysts are large (25 to 30 by 10 to 19 μm) and have a typical ellipsoidal shape (Figures A and B). Sporulated oocysts (Figure B) can be observed if the stool specimen is kept in potassium dichromate at room temperature for more than two days.

Under UV fluorescence, the oocyst wall autofluoresces as illustrated in Figure C. An intense blue fluorescence is obtained with the preferred UV excitation filter set (330 to 365 nm). If this filter set is not available, a less intense green fluorescence can be obtained with blue excitation (450 to 490 nm). Other objects, however, can also autofluoresce.

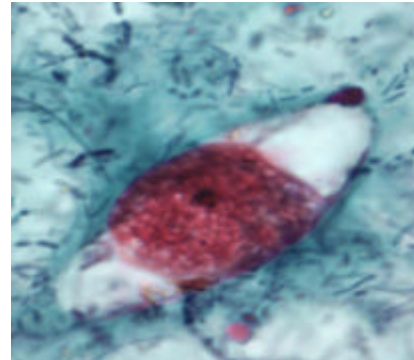
Utilization of both DIC and autofluorescence is an efficient and reliable approach for identification of this coccidian (where objects found by autofluorescence are checked by examination under DIC and vice versa) However, it requires a fluorescence microscope and does not provide a stained slide that can be archived.



Key points for laboratory diagnosis of isosporiasis

2. Modified acid-fast stain

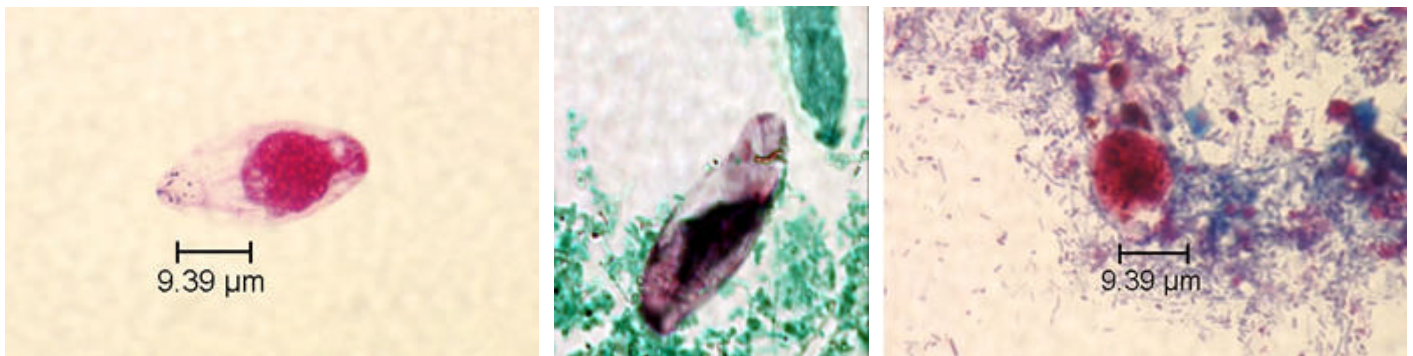
A blue-green background, or contrasting counterstain, of fecal debris allows the oocysts to stand out. They are variably stained: some will stain light pink to deep purple, while others may be unstained. The oocysts (25 to 30 μm) have the typical ellipsoidal shape as shown in the wet mount; their internal structure will not be seen. Some oocysts may appear collapsed or distorted on one side. This staining method is the easiest and most practical, and provides a permanent record.



An *Isospora belli* oocyst stained with the modified acid-fast technique.

3. Safranin stain

Oocysts stain uniformly, red to reddish-orange. The oocysts (25 to 30 μm) will have the typical ellipsoidal shape as in the wet mount; their internal structure may not be seen. Some oocysts may appear collapsed or distorted to one side. However, this technique requires heating, therefore additional equipment is necessary (e.g., microwave oven).



Isospora belli oocyst stained with safranin stain technique.



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