

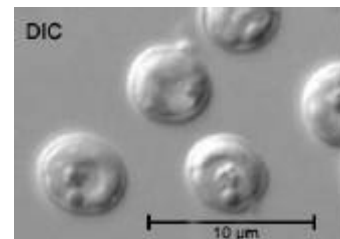
Cryptosporidium spp.

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.

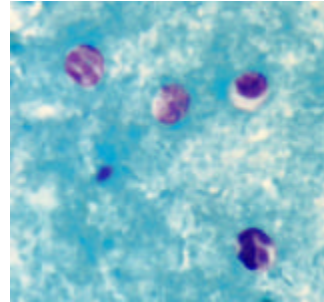
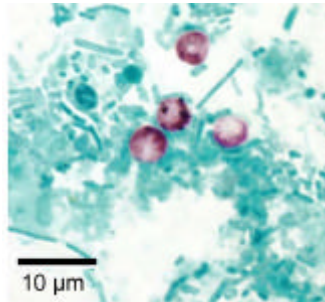
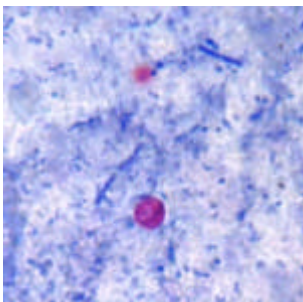
1. Wet mount

In bright-field microscopy using differential interference contrast (DIC), oocysts appear as small round structures (4 to 6 μm) similar to yeasts. They do not autofluoresce. This method is less useful for *Cryptosporidium* than it is for *Cyclospora*, especially when low numbers of oocysts can be obscured by other fecal elements.



2. Modified acid-fast stain

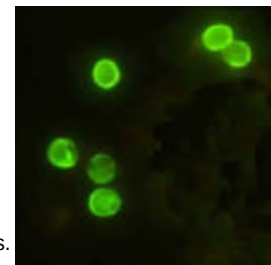
Oocysts (4 to 6 μm) often have distinct oocyst walls and stain from light pink to bright red. However, staining may be variable. In particular, infections that are resolving can have colorless oocyst "ghosts." Mature oocysts may have discernible sporozoites (up to 4). This method is the easiest, and most practical, and provides a permanent record. Misdiagnosis may result, however, due to confusion with artifacts.



Stool smear containing *Cryptosporidium parvum* oocysts stained with modified acid-fast technique.

3. Direct fluorescent antibody (DFA) assay

This technique offers the highest combination of sensitivity and specificity and is considered the gold standard by many laboratories. However, it does not provide a stained slide that can be archived. It requires special equipment (fluorescence microscope) and commercially available test kits.



C. parvum oocysts labeled with immunofluorescent antibodies.

Laboratory diagnosis of cryptosporidiosis

4. Enzyme immunoassay (EIA)

The EIA does not rely on microscopy skills, is highly sensitive and specific, and is useful for screening large numbers of specimens. However, it may require special equipment (microplate reader) and commercially available test kits. Antigens of *Cryptosporidium* in stool are detected using this method; therefore, specimens should not be concentrated prior to testing. Borderline positives and questionable negatives should be confirmed by an FA assay.

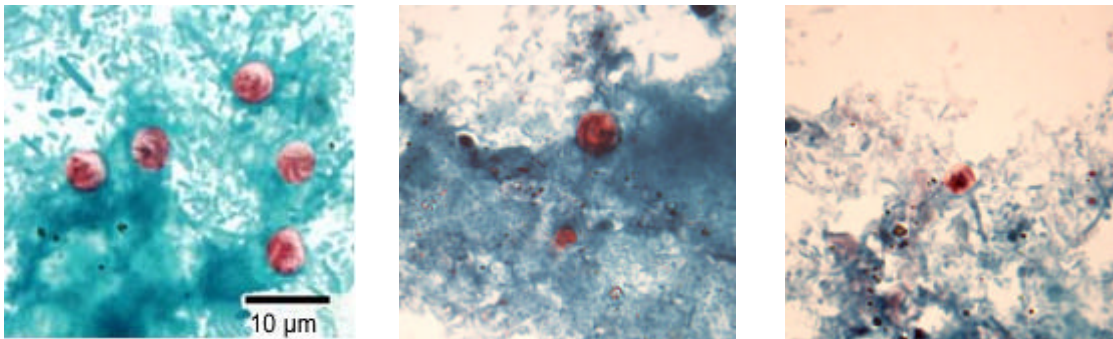
The quality of reagents in commercially available kits may be variable, or deteriorate under storage conditions; for that reason external controls are necessary to determine whether the kit is properly performing.

Methods for detection (but not confirmation) of *Cryptosporidium*

Oocysts may be detected by the following methods, but should be confirmed by the diagnostic techniques listed above.

A. Safranin stain

Oocysts of *Cryptosporidium* often (but not always) stain a bright reddish-orange color. This method, advocated for *Cyclospora*, is not widely used for *Cryptosporidium* because the *Cryptosporidium* oocysts may not always properly stain.



Stool smear containing *Cryptosporidium parvum* oocysts stained with safranin stain technique.

B. Trichrome stain

Oocysts may be detected, but should not be confirmed, by this method. Because trichrome stain is the routine staining technique for stool specimens in most laboratories, laboratorians should be familiar with the appearance of *Cryptosporidium* stained with trichrome so that oocysts may be detected during routine examinations. However, this staining method is inadequate for definitive diagnosis because all oocysts will appear unstained. Oocysts appear as small round structures measuring 4 to 6 µm. The diagnostic techniques listed above should be used to confirm *Cryptosporidium* when the presence of this coccidian is suspected in a trichrome stained smear.



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