Laboratory diagnosis of cryptosporidiosis

Cryptosporidium spp.



Basic guidelines

- A. Multiple stool specimens (at least 3) should be tested before a negative result is reported.
- B. To maximize recovery of oocysts, stool specimens in formalin, or other fixatives, should be concentrated prior to microscopic examination (e.g.,10 min at 500 × g when using the formalinethyl-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

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.







2. Modified acid-fast stain

Oocysts (4 to 6 μ m) 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).







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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.



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.

4. 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.





5. Trichrome stain

Oocysts may be detected, but should **not** be confirmed, by this method. This staining method is inadequate for definitive diagnosis because all oocysts will appear unstained. Oocysts appear as small round structures measuring 4 to $6 \mu m$.





Other methods for detecting Cryptosporidium in stool.

6. 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.

7. Rapid immunochromatographic cartridge assays

The rapid cartridge assays may be used with preserved specimens and are quick and easy to perform.



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