

Cryptosporidium & Giardia Testing

Cryptosporidium and Giardia are microscopic protozoan parasites found in surface waters of rivers and lakes used for drinking water supplies. These parasites are shed in either oocyst form (Cryptosporidium) or cyst form (Giardia) in feces. During this protective state (i.e. oocyst / cyst), Cryptosporidium and Giardia are difficult to remove from water systems as ordinary water disinfection techniques do not kill oocysts / cysts. The best filtration systems occasionally allow a few organisms to pass through. These protozoans can remain dormant for long periods in the oocyst / cyst form. However, they become active upon entering a host affecting the gastrointestinal tract of humans and animals.

Cryptosporidium and Giardia cause medical conditions of Cryptosporidiosis / Giardiasis. Infections may be asymptomatic or may cause diarrhea, nausea, abdominal cramps, fever, vomiting and headaches. While healthy individuals usually recover from the illness within several weeks, immunocompromised individuals may be unable to recover and as a consequence, suffer debilitating illness or death.

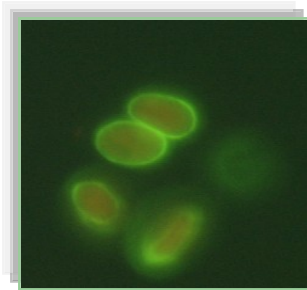
Several waterborne disease outbreaks have been attributed to Cryptosporidium. In 1993 the Milwaukee, Wisconsin municipal water system suffered a severe Cryptosporidium outbreak. Over 400,000 people fell ill, while 70 individuals died as a result of this outbreak.

After the 1993 incident, the EPA began requiring municipal water systems serving over 100,000 people to test their source waters for Cryptosporidium. However, methods at that time proved inadequate to detect and enumerate Cryptosporidium. Therefore in 1996, EPA initiated development of a new method. This effort created Method 1623, the most sensitive analytical technique approved for detection and enumeration of both Cryptosporidium and Giardia.

Interpretation of Cryptosporidium and Giardia positive results is difficult since the protozoans detected may be dead, the Cryptosporidium or Giardia detected may not be infective to humans, or the monitoring recoveries of the parasites are low. Only intact cysts or oocysts have the potential to be infective, however, the presence of even an empty Cryptosporidium or Giardia indicates infiltration into the water supply.



Cryptosporidium stained with immunofluorescent antibody (IFA) at 1000X magnification



Giardia stained with immunofluorescent antibody (IFA) at 1000X magnification

Cryptosporidium and Giardia are first detected by the apple-green fluorescence of IFA. Though other objects may fluoresce similarly, the analyst must also look for other identifying characteristics under light microscopy, such as appropriate size (3 to 7 μm in length for Cryptosporidium and 8 to 18 μm in length for Giardia) and internal morphology.



SUMMARY OF METHOD 1623

A ten (10) liter volume of water is collected in a carboy in the field and shipped to the laboratory. The sample is filtered in the laboratory and the oocysts, cysts, and extraneous materials are retained on the filter. Materials on the filter are eluted with an aqueous buffered salt and detergent solution. The eluate is centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated.



Attaching magnetic beads conjugated to anti-Cryptosporidium and anti-Giardia antibodies magnetizes oocysts and cysts. Magnetized oocysts and cysts are separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts.


Oocysts and cysts are then stained on well slides with fluorescently labeled monoclonal antibodies and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (D.I.C.) microscopy.



Qualitative analysis consists of scanning slides for objects that meet size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts or *Giardia* cysts.

Potential oocysts or cysts are then confirmed through DAPI staining characteristics and D.I.C. microscopy. Oocysts and cysts are identified when size, shape, color, and morphology agree with specified criteria and examples in a photographic library.

Quantitative analysis consists of counting the total number of objects on a slide confirmed as oocysts or cysts. Reproducible calibration and testing of the filtration, immunomagnetic separation (IMS), staining, and microscopy systems provide quality assurance.

McCoy & McCoy Laboratories is working to become certified for testing *Cryptosporidium* and *Giardia* by EPA Method 1623. Please contact us to discuss your monitoring and source water assessment needs. 



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