A PRACTICAL GUIDANCE DOCUMENT FOR THE DETECTION OF CYCLOSPORA
August 2013

THE HIGHLIGHTS

- *Cyclospora* is spread by the fecal-oral route due to contamination of food and water.
- This outbreak is believed to be linked to bagged salad mix.
- *Cyclospora* is best detected using a modified acid-fast or modified safranin stain of concentrated fecal smears.
- Inform your clinicians that the routine “O&P” will not detect *Cyclospora*. Add a note to your routine O&P result that this test will not detect *Cyclospora*, *Cryptosporidium*, or *Cystoisospora* species. Report any detected/suspected organisms to your public health laboratory/department.

The Organism

*Cyclospora cayetanensis* is a cyst-forming protozoan parasite of humans that normally causes a self-limiting diarrhea. The oocysts of *C. cayetanensis* are spherical in shape and range in size between 8-10 μm in diameter (larger than *Cryptosporidium* species which are 4-6 μm). Infection is transmitted when a person ingests sporulated oocysts in food or water contaminated with feces. The oocysts excyst in the intestine and release 2 sporozoites which then invade the epithelium. The organism undergoes both asexual and sexual reproduction before developing into unsporulated oocysts which are then passed in the feces. The unsporulated oocysts are not immediately infective. For details of this life cycle, see the following CDC website: [http://dpd.cdc.gov/dpdx/HTML/Cyclosporiasis.htm](http://dpd.cdc.gov/dpdx/HTML/Cyclosporiasis.htm).

*Cyclospora* is endemic in tropical and subtropical regions around the world. In the US *Cyclospora* infections are not common; however, foodborne outbreaks of cyclosporiasis have been linked to various types of imported fresh produce.

The Current Outbreak

This outbreak was first reported in Iowa on June 28, 2013. As of August 12, 2013, there were 539 cases (32 hospitalizations, 0 deaths) in 19 states that have been reported to the CDC with more cases being investigated (Figure 1). The source of the outbreak is believed to be linked to commercially prepared, bagged salad mix; however, the investigation is ongoing.

Case inclusion for the current outbreak includes laboratory evidence of *Cyclospora* and no travel outside of the US/Canada during the 2 weeks prior to illness.
Detection

Stool should be collected and immediately processed or appropriately fixed (10% formalin or other fixative) and a concentration method must be employed. At least 3 specimens (collected ≥ 24 hours apart) should be examined.

*Cyclospora* species are not usually detected by a conventional O&P examination (*i.e.*, concentrated wet-mount & Trichrome stain) but are best detected using a modified acid-fast (MAF) or modified safranin (MS) stain. Oocysts can also be detected using light microscopy of wet-mounts using differential interference contrast (DIC) microscopy (to provide structural details) or using UV fluorescent microscopy. Anything suspicious for *Cyclospora* oocysts should be reported to your public health officials.

Using MAF stain, oocysts are round, measure 8-10 μm, stain any shade of pink/purple or as ‘ghost cells’ (no stain), and may be slightly wrinkled or collapsed on one or more sides (Figures 2A, 2B, 2C). With the MS stain, oocysts stain more consistent and are red/orange (Figure 2D). Note: The MS stain requires boiling the safranin during staining. Detailed staining procedures for both of these stains can be found under the laboratory diagnosis tab at: [http://dpd.cdc.gov/dpdx/HTML/Cyclosporiasis.htm](http://dpd.cdc.gov/dpdx/HTML/Cyclosporiasis.htm).

With wet-mount/UV microscopy, oocysts appear as round/refractile structures with well-defined walls (Figure 3A & 3B). As these can be difficult to tell from stool artifacts, autofluorescence
should be used for confirmation. Blue fluorescence is seen with UV light (330-365nm filter) (Figure 3C) and green fluorescence can be seen with blue excitation (450-490nm filter).

**Reporting**

Inform your clinicians that the routine “O&P” will not detect *Cyclospora* species. Add a note to your routine O&P result that this test will not detect *Cyclospora, Cryptosporidium, or Cystoisospora* (formerly *Isospora*) species and offer additional testing for *Cyclospora* organisms as above when clinically warranted.

Cyclosporiasis is a nationally notifiable disease. Laboratories must communicate all positive results to their health department to determine if any additional or specific action is required.

**Figure 2.** MAF (A-C) and MS stains (D). Red arrows indicate oocysts. Images A-C @ 400X magnification.

![Figure 2](image1)


**Figure 3.** Wet-mount microscopy. A. DIC, B. transmitted light, C. UV. Red arrows indicate oocysts. Images B, C @ 400X magnification.

![Figure 3](image2)

Panel A is courtesy of DPDx: Laboratory Identification of Parasites of Public Health Concern. Panels B-C are courtesy of M.R. Couturier.

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