THE HIGHLIGHTS

— *Cyclospora cayetanensis* is spread by indirect fecal-oral route due to contamination of food and water.

— *C. cayetanensis* stains poorly with both wet-mount and trichrome (permanent) stains used commonly in routine Ova & Parasite (O&P) examinations.

— Modified acid-fast or modified safranin stains of concentrated fecal smears can detect these poorly staining organisms; however UV microscopy is significantly more reliable for detection of the oocysts and should be performed when possible.

— The FilmArray® gastrointestinal panel and *C. cayetanensis*-specific laboratory developed Nucleic Acid Amplification Tests (NAAT) can detect *C. cayetanensis* in stool. Other commercially available NAAT gastrointestinal panels do not detect *C. cayetanensis*.

— UV microscopy of concentrated stool wet-mounts is recommended for routine identification of *Cyclospora* in clinical specimens.

— Inform your clinicians that the routine “O&P” will not readily detect *Cyclospora*. Add a note to your routine O&P result that this test will not readily detect *Cyclospora, Cryptosporidium,* or *Cystoisospora*.

— Report any detected/suspected organisms to your public health laboratory/department.

The Organism

*Cyclospora cayetanensis* is a protozoal parasite of humans that normally causes a self-limiting diarrhea. The oocysts of *C. cayetanensis* are spherical in shape and are 8-10 \( \mu \text{m} \) in diameter (larger than *Cryptosporidium* which is 4-6 \( \mu \text{m} \)). Infection is acquired when a person ingests food or water that has been contaminated with sporulated oocysts. For details of this life cycle, see the following CDC website: https://www.cdc.gov/dpdx/cyclosporiasis/index.html.

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

The Current Outbreak

A *Cyclospora* outbreak first made news headlines in July, 2017 based on the increased number of cases reported in Texas. Cyclosporiasis has been reported in patients with no reported travel
preceding illness in >70% of U.S. states. This season’s case count has already surpassed recent past outbreak years (2013-2015) in the number of U.S.-acquired cases and states affected. For current data on outbreaks and updates, see the following CDC website: https://www.cdc.gov/parasites/cyclosporiasis/outbreaks/index.html.

Microscopic Detection

Stool should be collected and fixed immediately (10% formalin or other appropriate single vial fixative). Specimens must be concentrated before examination. At least 3 specimens (each collected ≥ 24 hours apart) should be examined for achieving the highest sensitivity.

*Cyclospora* is not usually detected by a conventional O&P examination (*i.e.*, concentrated wet-mount & trichrome stain) but can be detected using a modified acid-fast (MAF) or modified safranin (MS) stains. 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.

Using MAF, oocysts are round, measure 8-10 µm, stain pink (Figure 1A) or as ‘ghost cells’ (no stain, Figure 1B), and may be slightly wrinkled or collapsed on one or more sides. With MS, oocysts stain more consistently and are red/orange (Figure 1C). The MS stain requires boiling the safranin during staining and may be cumbersome for many laboratories. Detailed staining procedures for both of these stains can be found at: https://www.cdc.gov/dpdx/diagnosticprocedures/stool/staining.html

With wet-mount/UV microscopy, oocysts appear as round, refractile structures with well-defined walls (Figure 1D). UV microscopy is more reliable than MAF due to the fact that all oocysts will naturally fluoresce, which mitigates the loss of sensitivity that can occur with a high abundance of “ghost cells” on MAF. Wet-mounts for UV microscopy should be prepared from the concentrated sample (without iodine). Blue fluorescence is seen from oocysts with UV light (330-365 nm filter) and green fluorescence can be seen with blue excitation (450-490 nm filter).

During this current outbreak, a significant number of positive samples identified in commercial laboratories have been readily identifiable with UV microscopy but barely detectable or entirely missed by MAF. At one reference laboratory, despite extensive experience with O&P and MAF, less than 20% of samples that were readily identified as *Cyclospora*-positive by UV microscopy wet-mount were readily identified by MAF (M. Couturier, unpublished data). In cases where physicians did not order the MAF stain, rare oocysts seen on trichrome stain were abundantly detectable on the corresponding UV preparation, (example in Figure 2). The decreased sensitivity of MAF compared to UV microscopy has been observed in samples fixed in 10% formalin as well as single vial fixatives, demonstrating this is not a fixative-specific limitation.

Key Points for UV Identification of *Cyclospora*:

1. Oocyst size and shape are very consistent (range only between 8-10 µM; round or slightly flattened on one side).
2. Oocyst color is very consistent (blue at 330-365 nm or green at 450-490 nm filters).
3. Do not prepare wet-mounts with iodine for UV microscopy. Oocyst autofluorescence is impaired in the presence of iodine.
4. Potentially positive samples should be sent to a public health laboratory or reference laboratory for confirmation of *Cyclospora*.

### Molecular Detection

Molecular tests are another sensitive method to detect *Cyclospora* in stool. *C. cayetanensis* can be detected by the FilmArray® Gastrointestinal panel (BioFire, bioMérieux) and select laboratory developed tests. However, at the time of writing, most other common gastrointestinal panels do not target *C. cayetanensis*, including xTag® gastrointestinal pathogen panel (Luminex Corporation), BD MAX™ enteric parasite panel (BD Molecular Diagnostics), and Verigene® enteric pathogens test (Luminex Corporation). Clinicians should consult with their primary clinical laboratory to determine which molecular test is utilized and whether *C. cayetanensis* can be detected.

### Testing Recommendations

Routine O&P, MAF or MS stains, and/or molecular detection should be performed when clinically indicated. In addition to routine testing, we recommend increased utilization of the UV microscopy of stool wet-mounts:

1. Perform UV microscopy of concentrated stool wet-mounts with all test orders that include a MAF or MS stain.
2. If routine UV microscopy of wet-mounts is not feasible due to limited resources, laboratories should make every effort to include UV microscopy during known *C. cayetanensis* outbreaks.
3. When *C. cayetanensis* suspicion is noted in the order requisition, laboratorians should ensure that appropriate detection methods have been ordered and UV microscopy should be included in the stool work-up.

### Reporting

Inform your clinicians that the routine “O&P” examination will not detect *Cyclospora*. If possible, consider including a comment with routine O&P results that this test will not readily detect *Cyclospora, Cryptosporidium*, or *Cystoisospora* species and offer additional testing for these organisms 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 1.** *Cyclospora* oocysts visualized by microscopy. (A) MAF stained oocyst (B) oocysts without stain retention (‘ghost cells’) using MAF, (C) oocyst stained with MS, and (D) wet-mount under UV (330-365 nm). Images A-C captured at 1000X magnification, image D captured at 400X magnification.

Panels A, B, and D are courtesy of M.R. Couturier, ARUP Laboratories/University of Utah. Panel C is courtesy of DPdx: Laboratory Identification of Parasites of Public Health Concern. 

**Figure 2.** A representative 2017 *Cyclospora*-positive stool sample. (A) Rare ‘ghost cell’ oocyst with a slightly wrinkled center detected on trichrome stain at 1,000X magnification. (B) Abundant oocysts of the corresponding wet preparation at 400X magnification. Red arrows indicate example oocysts (NOTE: not all oocysts have been indicated).

Images are courtesy of M.R. Couturier, ARUP Laboratories/University of Utah.

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