Update to EPA Method 1623.1 for Detection of Cryptosporidium: Tips and Common Deficiencies in Preparation for Round Two of LT2 Testing

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Background

- Jan. 2012: Method 1623 is revised to become 1623.1
- Included approvals for irradiated oocysts and cysts
- Addition of minimum QC requirements and updated acceptance criteria (MS/MSD, OPR and IPR)
- Dispersant addition and temperature monitoring
- Bead pellet wash during IMS and adjustment of pellet volume
- Clarification of control chart procedures and reporting requirements
- Enhanced recommendations for complex sample testing

Supplement 2
Fifth Edition of the EPA LC Manual

- Effective November 2012
- Requirements in addition to method
- Clarification of requirements for certification
- Clarification of analyst and principal analyst requirements
- Inclusion of requirements for support equipment not previously included in the methods
- Inclusion of information about stains, mounting medium and spiking suspensions

Supplement 2
Fifth Edition of the EPA LC Manual

- Additional information about microscope use
- Updates to the analytical methodology including examination
- Information regarding sample collection and protection
- Additional information about the analysis of complex samples
- Updates to required QC protocols and procedures
- Updates to record requirements and the reporting of data
- Used in conjunction with the requirements of the 40CFR and the methods (and TNI where applicable)

What is the dispersant, sodium hexametaphosphate (NaHMP)?

- Dispersant cation used to facilitate flocculation of insoluble particles in suspension, especially colloidal size particles like clay and organic matter that have negative surface charges
- Used as a water softener (i.e. Calgon)
- Found in toothpaste (i.e. Crest Pro-Health) and other food products
- Online link to video demonstrating its use:

http://www.youtube.com/watch?v=U-pBHvBeazs

NaHMP and 1623.1

- 5% w/v NaHMP solution (1632.1, 7.6.1.1)
- Improves the removal of debris during analysis
- Improves the quality of the slides due to the reduced debris
- Enhances removal of debris particularly for samples with low recovery using 1623.
- Added during the elution step
- Cannot be added to capsules that have for example, clogged. The dispersant step is by-passed and a note must be retained regarding this problem in the raw data records (bench sheet).

IMS Bead Pellet Wash

- Required by 1623.1, 13.3.2.17
- Formerly a recommendation in 1623 (13.3.4) as a tip in minimizing carry-over debris onto the slides
- 1X phosphate saline solution is added to the microcentrifuge tube with the magnet in place
- Magnet is removed and the tube is rocked gently until the beads are re-suspended
- The magnet is replaced and the process is repeated
- Tube stands undisturbed for 1 min to allow residual liquid to flow to the bottom of the tube.
- Pipette mixed and aspirated. Tube is not shaken or removed from the MPC-S during the step.

Potential NELAP Assessment Findings

Clarification and Note:

This part of the presentation makes the assumption that all samples are handled properly throughout the analysis. It also assumes that all analysts and principal analysts are performing the microscopic examination with appropriate magnifications, that Koehler illumination has been established and that all characterizations are performed correctly.

Potential NELAP Assessment Findings

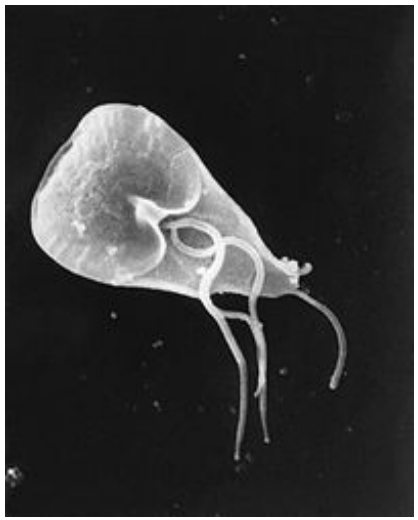
- Not recording the lot number of the HCL and NaOH standard solutions in the raw data.
- Not recording the volume of sample to the nearest ¼ liter
- Not recording the size to the nearest 0.5 µm
- Not reporting the time of analysis for all parts of the procedure that have a holding time of <72 hours
- Not matching the procedures in the SOP to the practices at the bench
- Balance calibrations not performed daily

Potential NELAP Assessment Findings

- Support equipment not marked with the current calibration status
- Not maintaining the cycle of two PT passes per year. (Cryptosporidium and Giardia now on the NELAP PT Fields of Testing tables)
- Missing information on the slide examination forms and sample bench sheets
- Not characterizing all oocysts (and cysts) found on real world sample slides or not maintaining a record of the characterizations

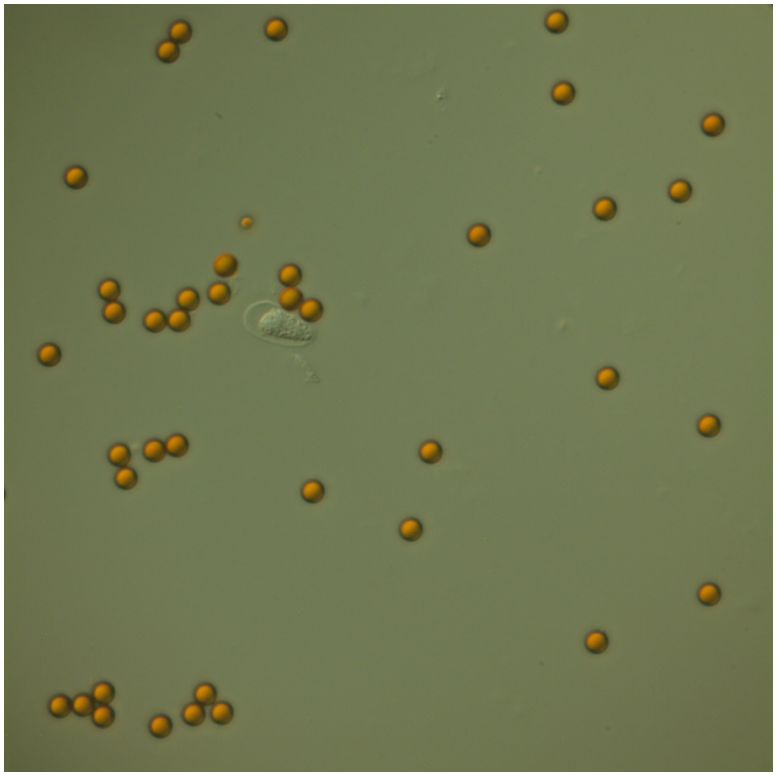
Microscopic Targets

Cryptosporidium –typical sporozoite banana shaped feature



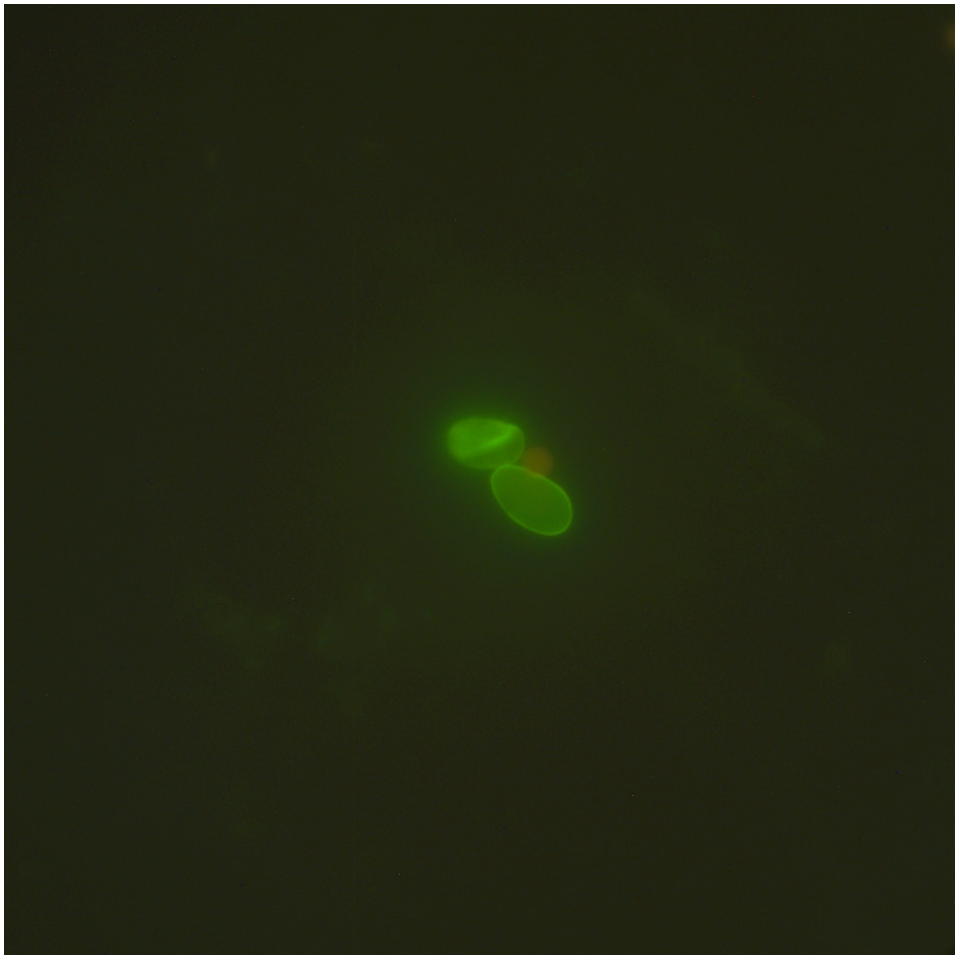
Giardia

Examinations



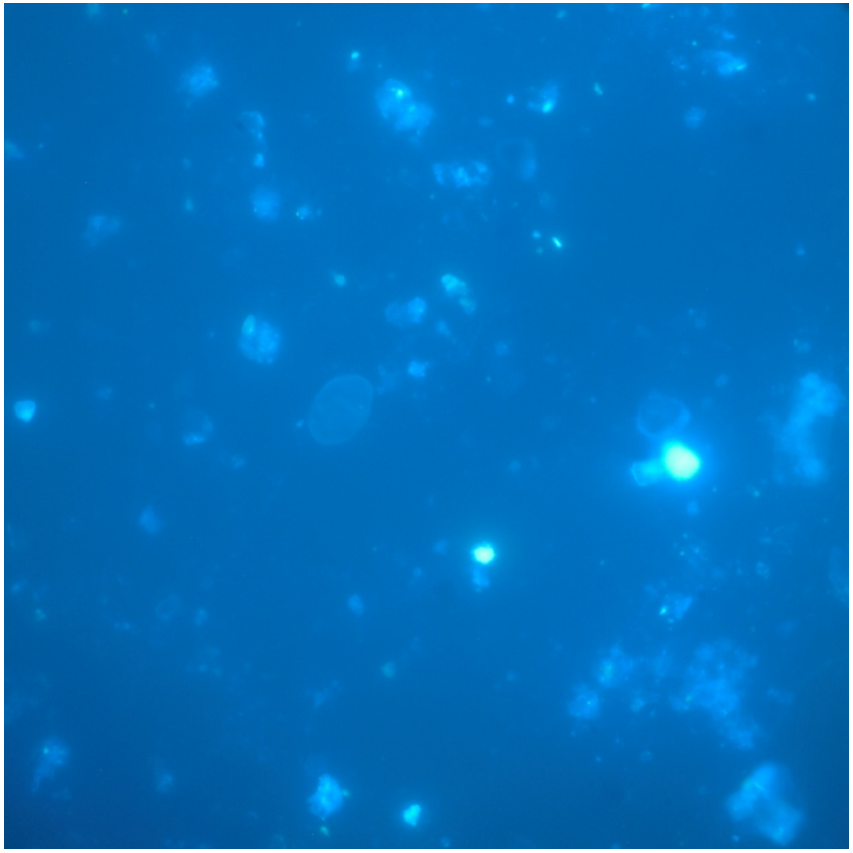
Beads on slides: Magnets may not be working as they should.
Tip: Try storing magnets apart from each other to maintain magnetic strength.

Natural water sample-Giardia-FITC
Minimum 200X



Looking for apple-green fluorescence
Brightly highlighted edges
Defined cell wall
Round to ovoid
8-18 µm long by 5-15 µm wide

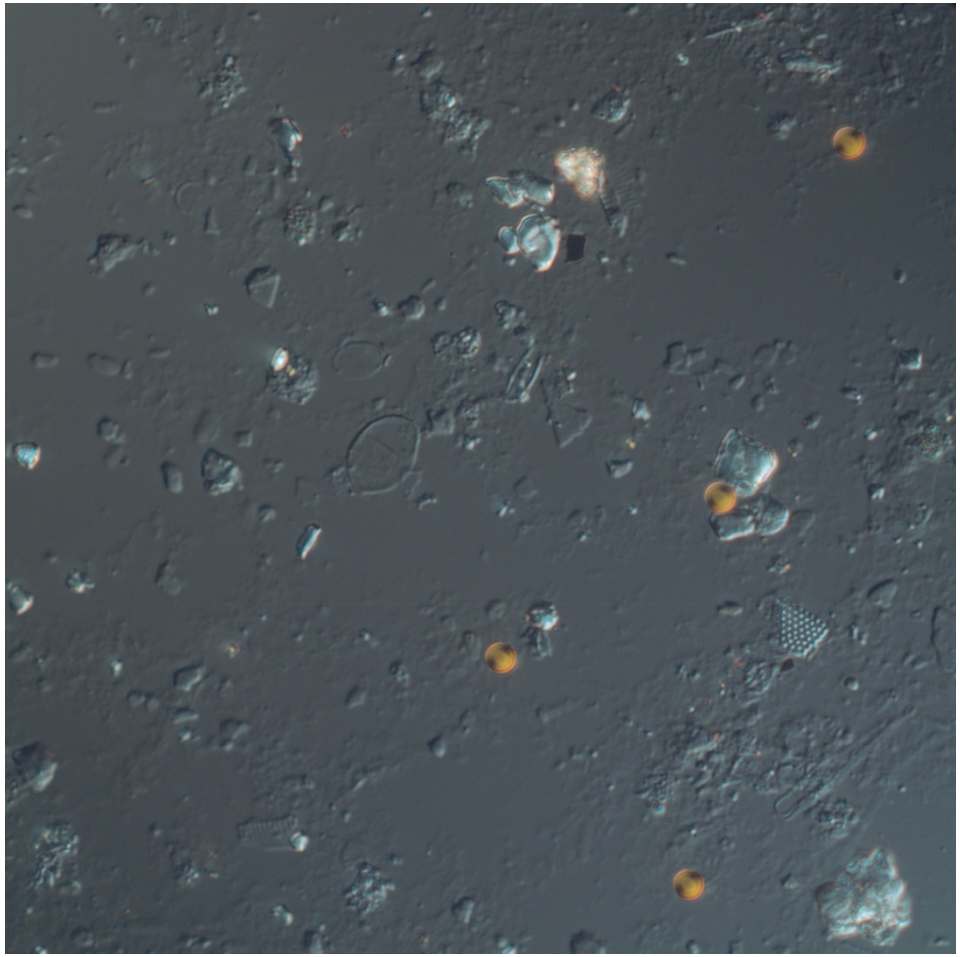
Natural water sample-Giardia-DAPI 400X



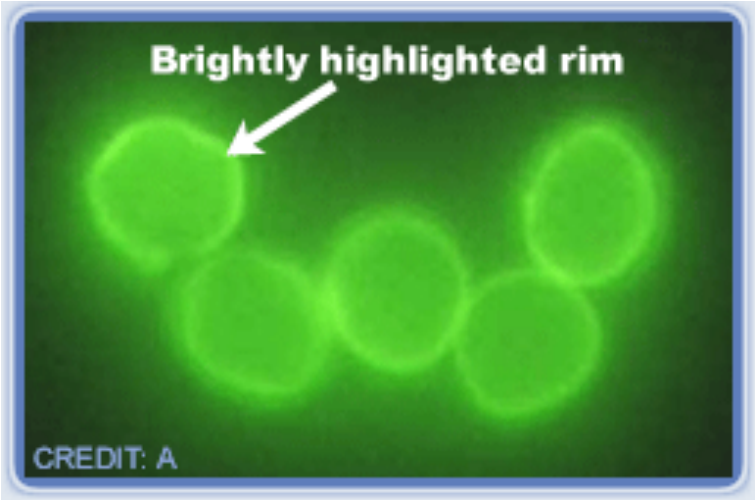
DAPI positive
Can be: light blue internal (no distinct nuclei) and a green rim
Intense blue internal staining
Up to four distinct nuclei
No blue-DAPI negative

*DAPI concentration can be increased to enhance the microscopy

Natural water sample-Giardia-DIC 1000X-Oil

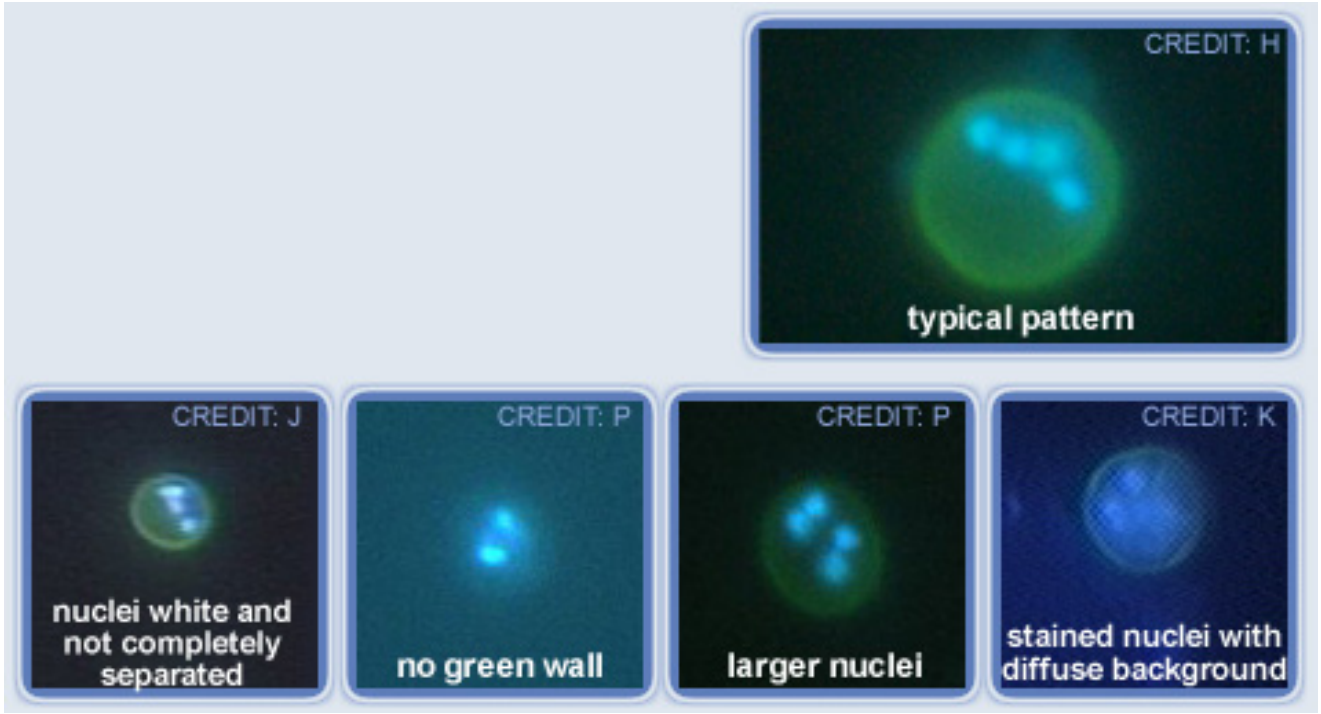


Cryptosporidium FITC

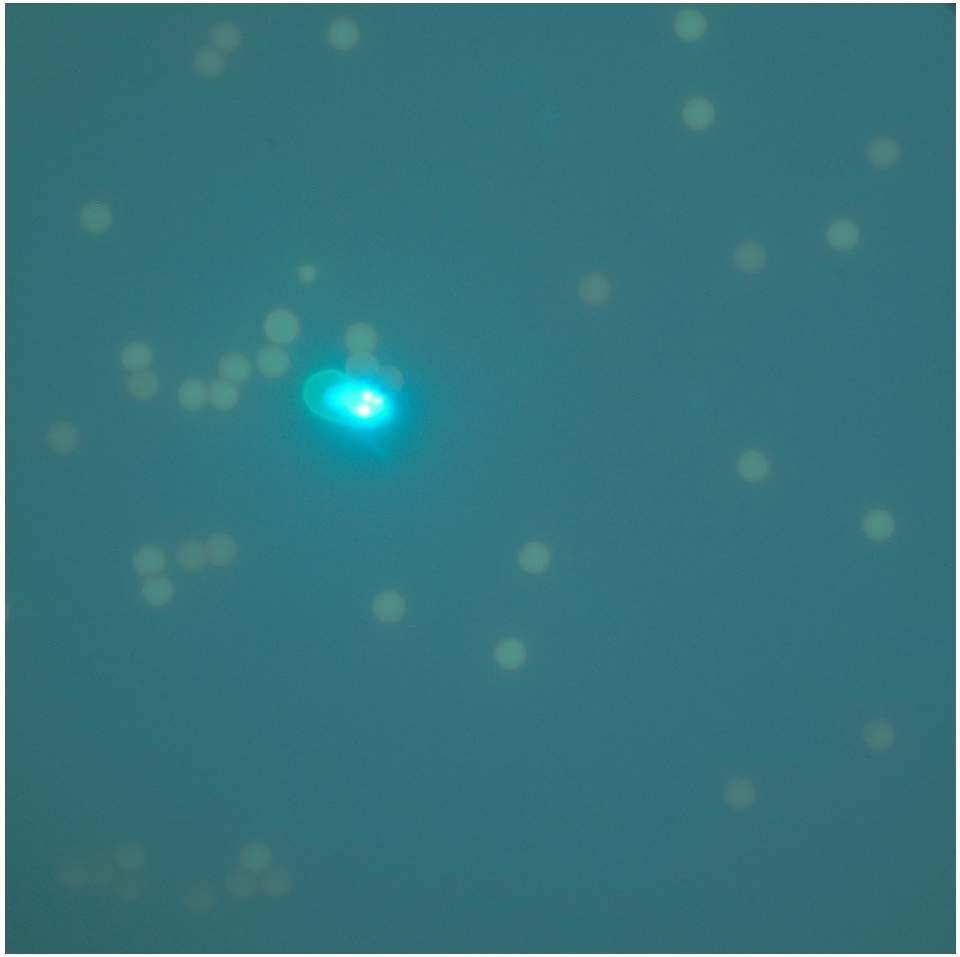


Looking for ovoid or spherical shape
4-6 µm in diameter
Brightly highlighted edges
Inner green is lighter than outer wall

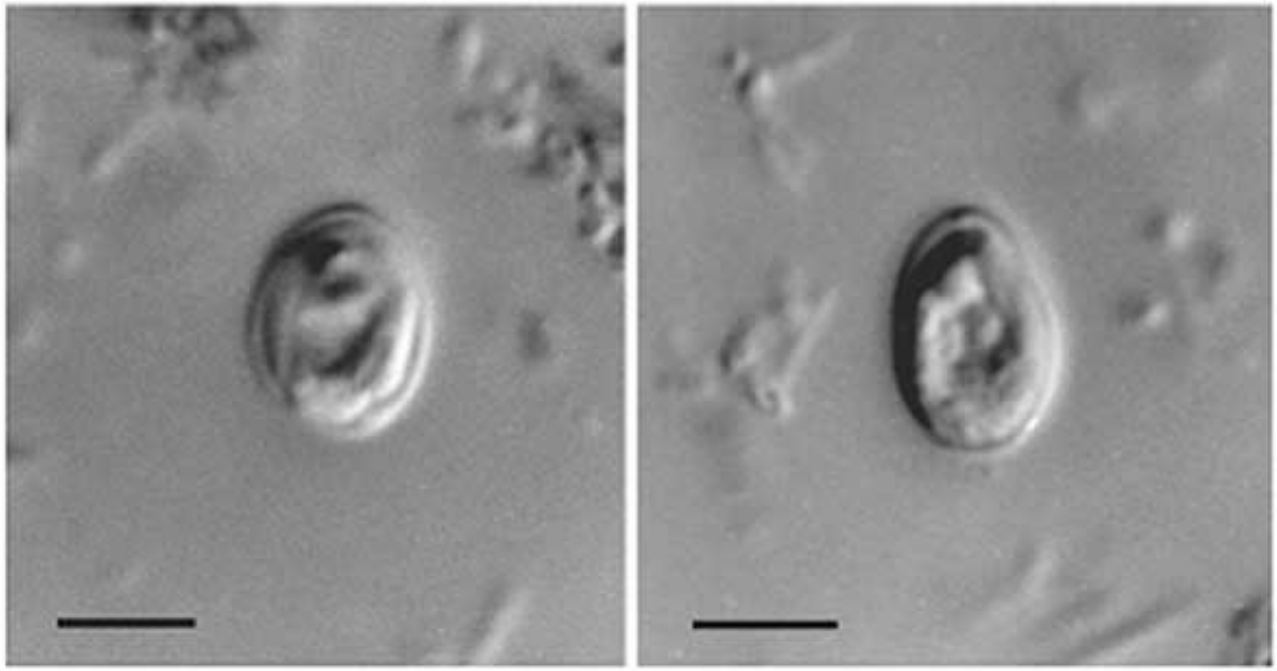
DAPI Examinations



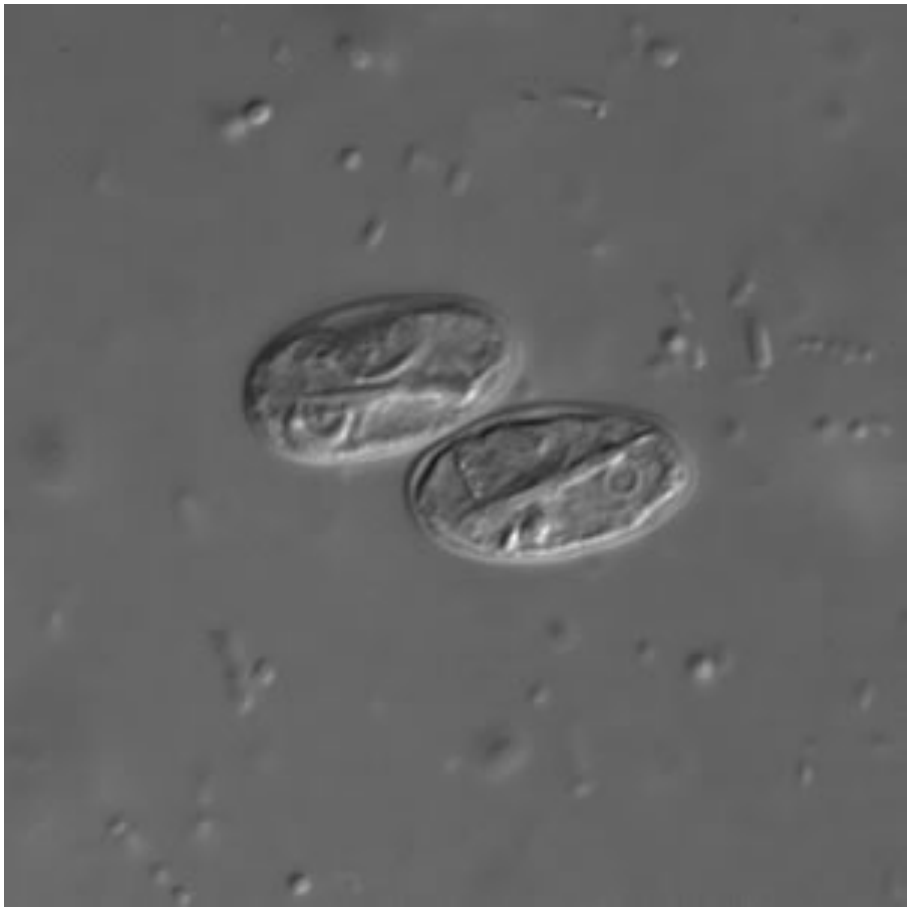
Nuclei: DAPI “dots”



Cryptosporidium-DIC-1000x-Oil



Giardia-DIC-1000X-Oil



Typical morphological features:
axonemes, median bodies and nuclei

Important Links

- http://www.youtube.com/watch?v=akXNxM94qkU&feature=youtu.be (overall method video)
- http://www.youtube.com/watch?v=SEIH9wblGjo (reviewing data video)
- http://water.epa.gov/scitech/drinkingwater/labcert/upload/epa815f12006.pdf (link to Supplement 2)
- http://water.epa.gov/lawsregs/rulesregs/sdwa/lt2/lab_home.cfm (EPA link to all things C/G and LT2)