Ongoing Quality Assurance for *Cryptosporidium* Analysis

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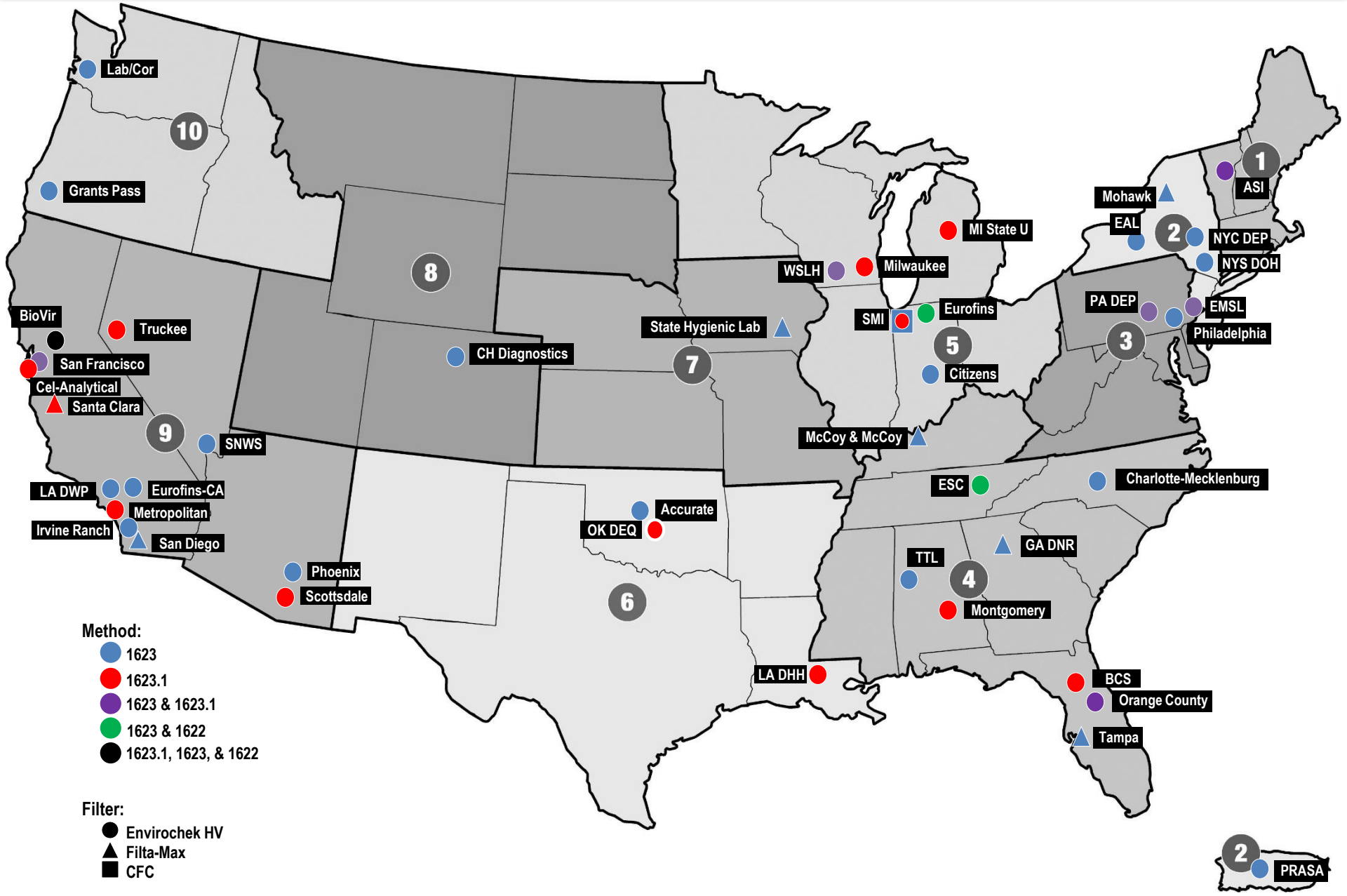
Cryptosporidium Lab Certification History

- 2002: EPA *Cryptosporidium* Lab Quality Assurance Program
  - *Cryptosporidium* Lab evaluations initiated by the EPA
- 2006: Beginning of LT2 Compliance Monitoring for Public Water Systems
- 2009: PTs provided by ACLASS certified PT provider
  - TNI FoPT table
- 2010: Training State Certification Officers for *Cryptosporidium*
- 2015: State Certification Programs for *Cryptosporidium*

44 Certified *Cryptosporidium* Labs in EPA Regions



Approved Methods for Cryptosporidium Detection

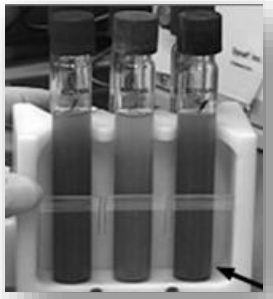
- Method 1622
  - *Cryptosporidium Only*
- Method 1623
  - *Cryptosporidium* and *Giardia*
- Method 1623.1
  - *Cryptosporidium* & *Giardia*
  - Modification of 1623

Cryptosporidium Detection Methods Overview

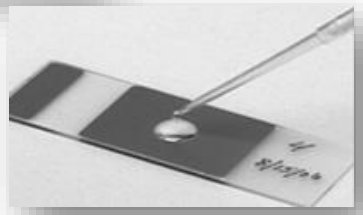
Sample

Filter

Centrifuge Tubes



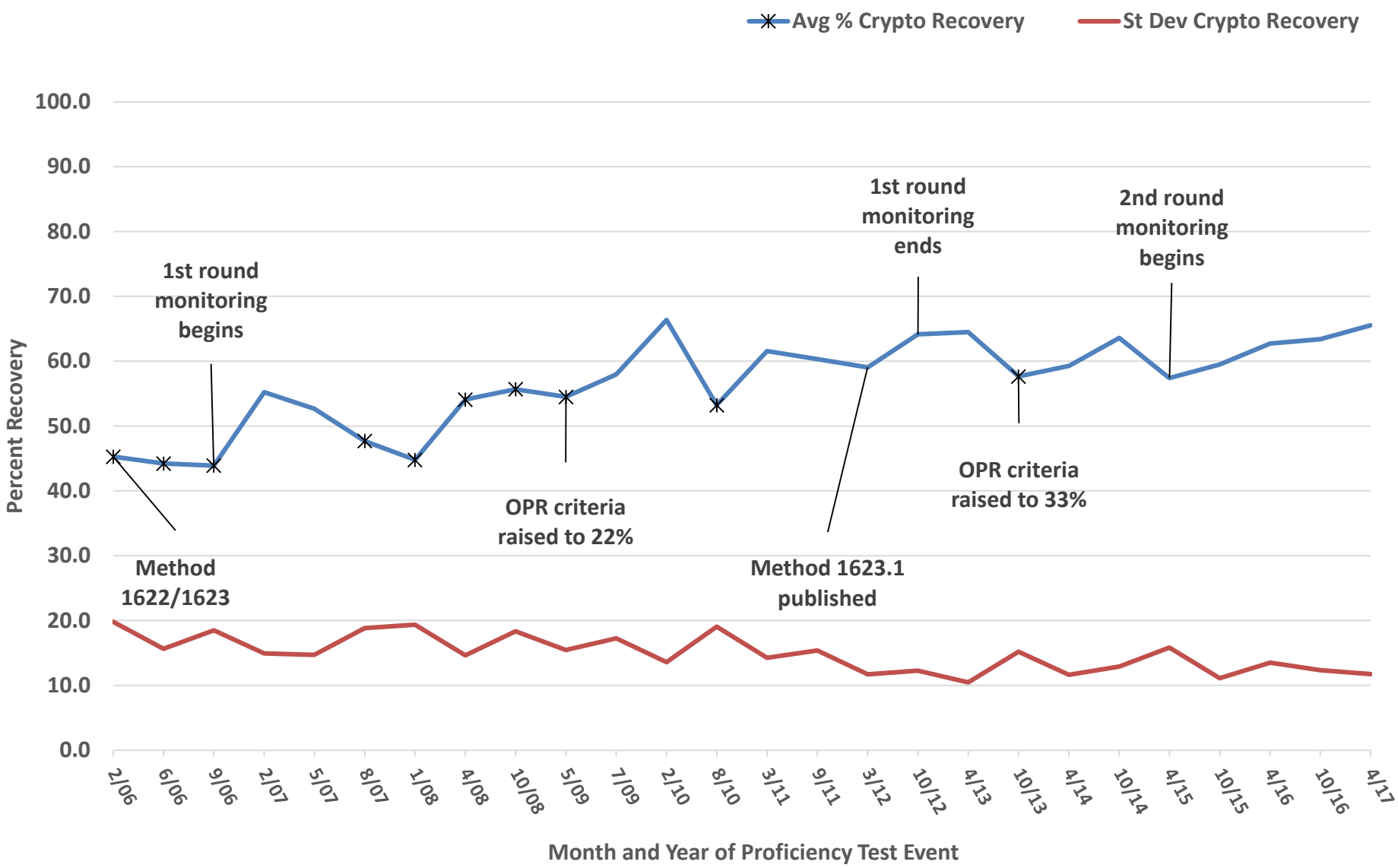
Immunomagnetic Separation



Slide

10 L → → → → 100 µL

Cryptosporidium PT Recoveries for Approved Laboratories



∗ indicates PT samples contain a standard matrix

Acceptance limit increased to 33%

	Method 1623 2005 Version	Federal Register Feb. 25, 2009	Federal Register Sept. 5,2013
Cryptosporidium OPR Minimum Acceptance Criteria	11%	22%	33%
Number of samples	293	333	753
Number of Labs	6	58	56
Blind vs. Unblind	Unblind	Blind	Blind

State Oversight for *Cryptosporidium* Laboratories

State Program Equivalence

- Conformity with EPA Drinking Water Laboratory Certification Manual
- Certification Officer passed EPA *Cryptosporidium* CO Training Course

Technical Support Center

- Train certification officers
- Provide written guidance
- Provide technical support for states, regions and laboratories
- Review/assist regional programs
- Maintain a list of links to state websites naming certified labs

- Federal Register Vol. 78, No. 172, September 5, 2013. 54643

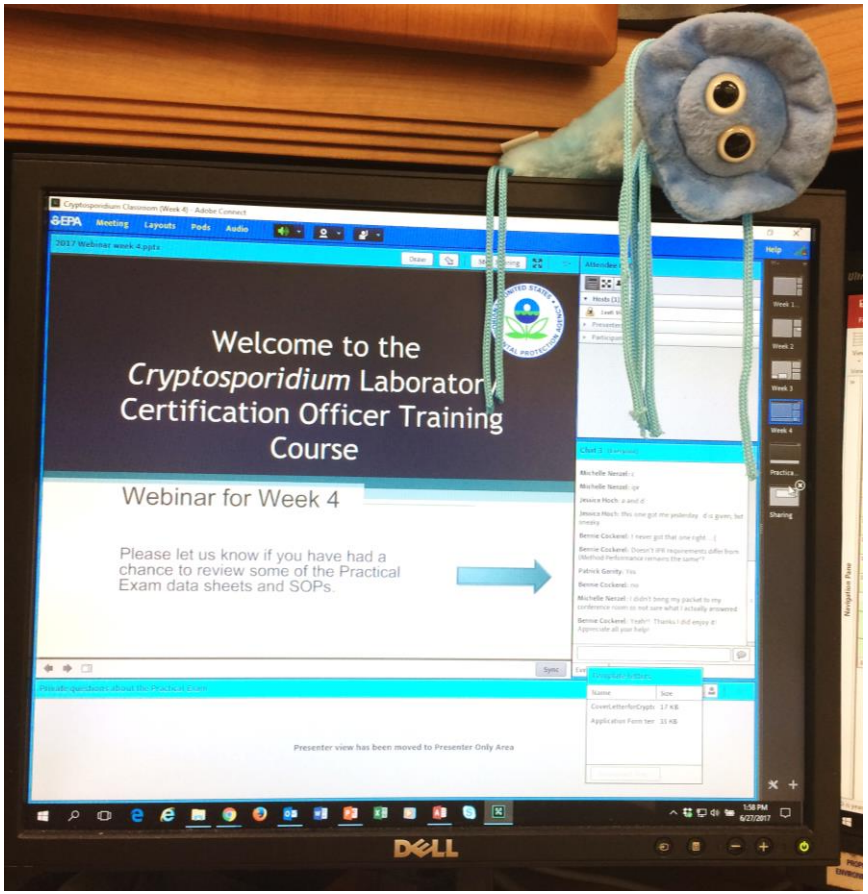
Substantial Conformity

- Checklists A, B, & C
- Positive Staining Control and OPR slide review by experienced microscopist
- Blind spikes proved by 3rd party vendor
- Microscopy Skills Evaluation by 3rd party expert

- Federal Register Vol. 74, No. 36, February 25, 2009. 8531

Cryptosporidium Certification Officer Training

- Courses offered in Cincinnati
  - 2010
  - 2011
  - 2013
- Courses offered online
  - 2013
  - 2014
  - 2015 (twice)
  - 2016
  - 2017
  - Next offering September 2017



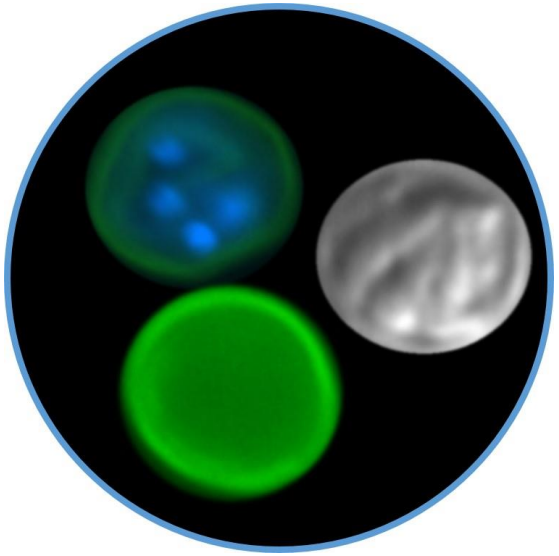
What can I learn at the *Cryptosporidium* CO Course?

- Audit a lab's ability to follow the method consistently
- Identify discrepancies between lab practices and written procedures
- Recognize proper lab techniques that reduce the loss of organisms

What can I expect from the Cryptosporidium CO Course?

- Course is 4 weeks of online lessons
  - 8-10 hours study/week
- Weekly webinars
- Online open-book exam and mock laboratory

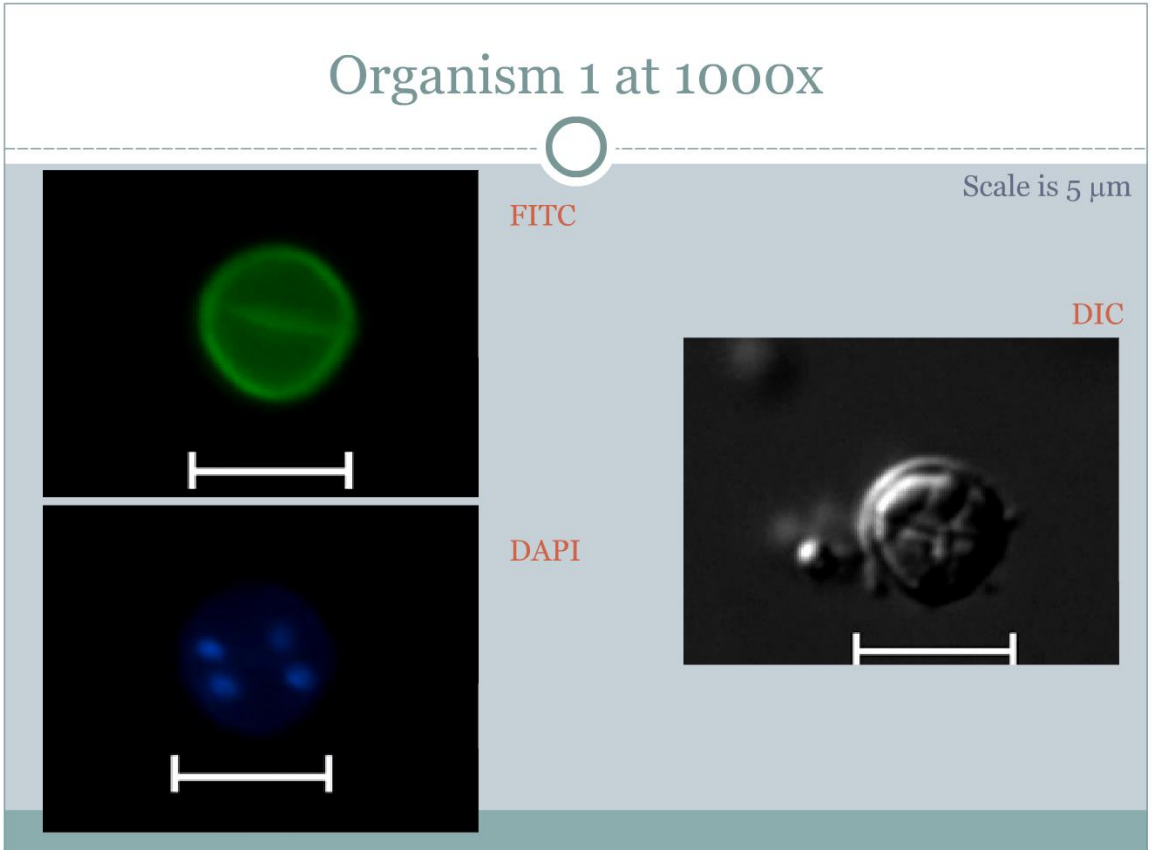
Website updates for continued training



- Continuous access to CO Course materials
- Videos of Method and modifications
- Training tools for microscopy, IMS, and sampling
  - Note reviewing this material is not a substitute for completing or auditing the course

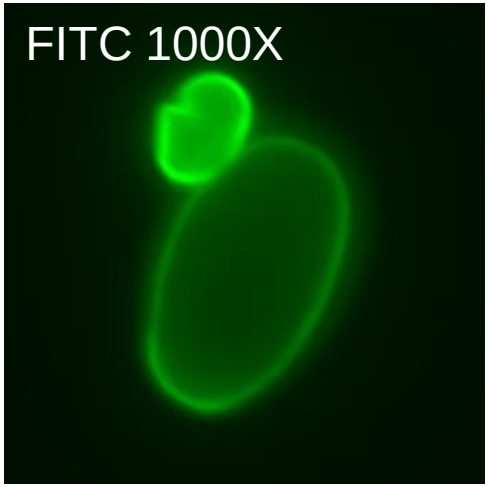
Audit Support: Analyst Skills Evaluation

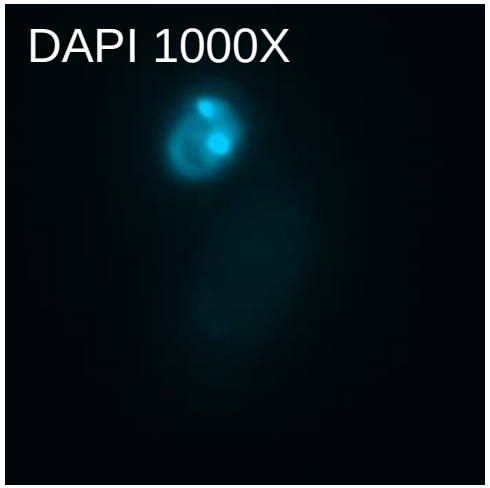
- Online Analyst Skills Evaluation
  - Every Analyst in the country has completed the online assessment



Audit Support: Slide Review

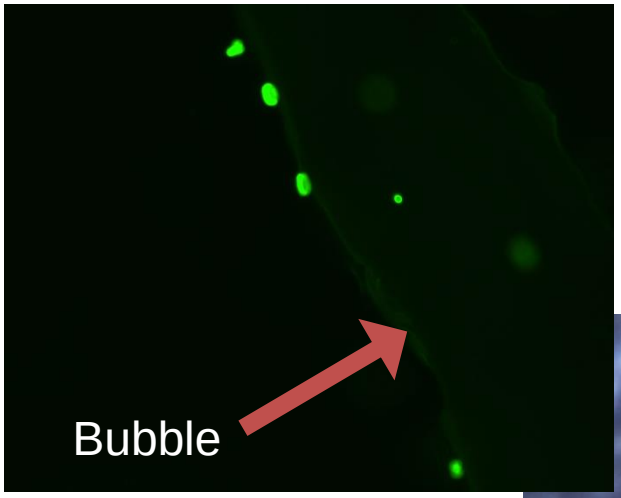
- Evaluation of Microscope Slide Preparation
- Subject mater expert evaluates the OPR and positive staining control using a defined rubric, and scores 1 to 4.

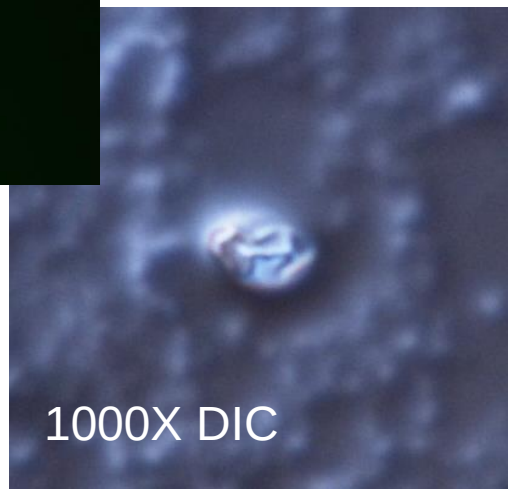


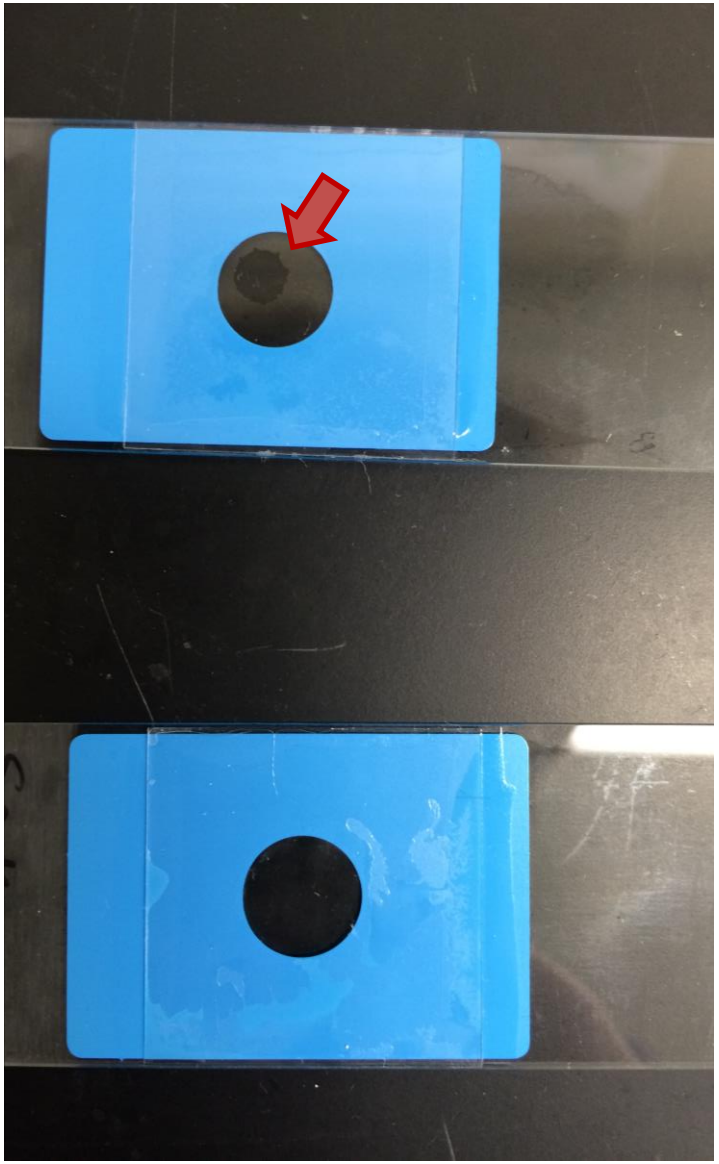


Common concerns with slides: Bubbles

- Bubbles can be seen with the naked eye
- DAPI and DIC do not work correctly in a bubble

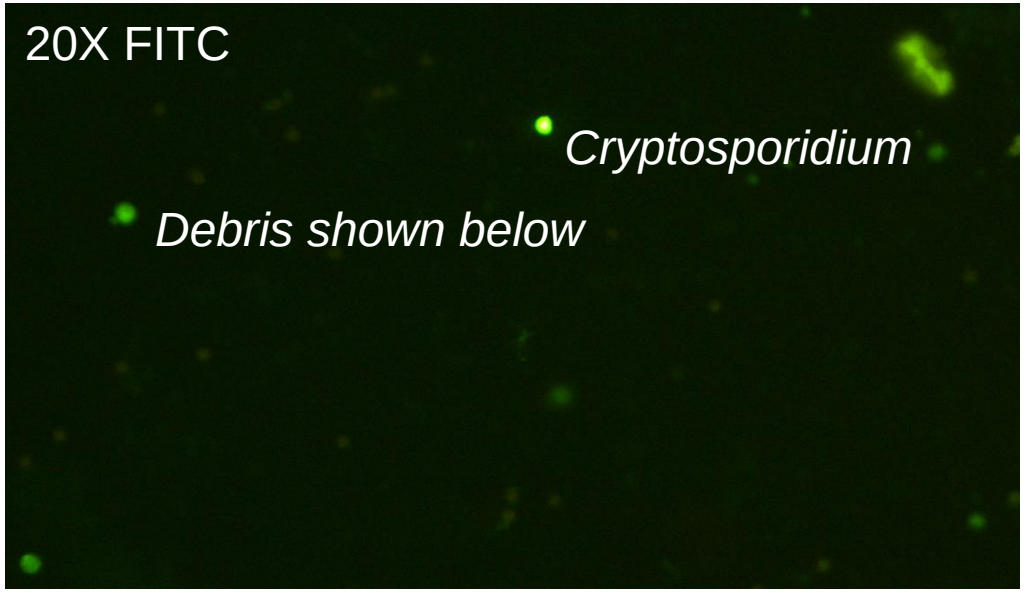


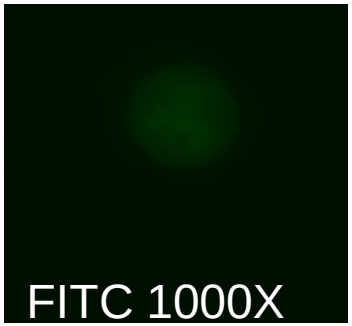




Common concerns with slides: Debris

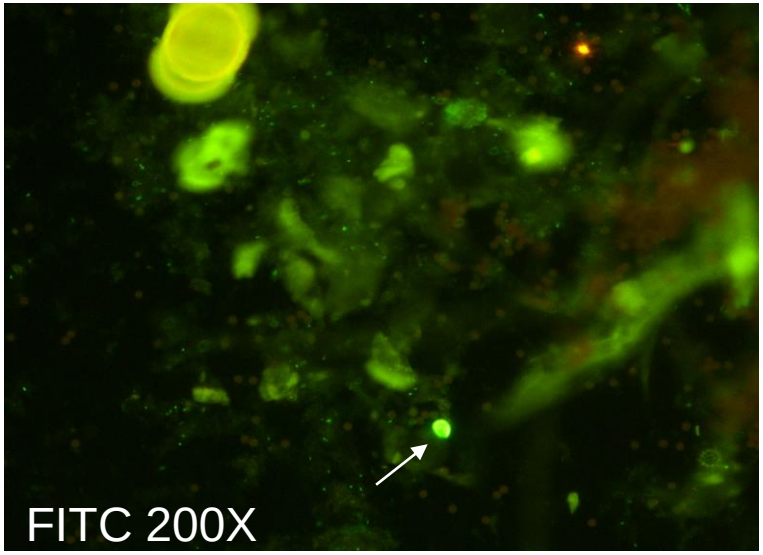
- Positive Staining Controls and OPRs should have minimal debris
- No debris should fluoresce like a *Cryptosporidium* oocyst or *Giardia* cyst

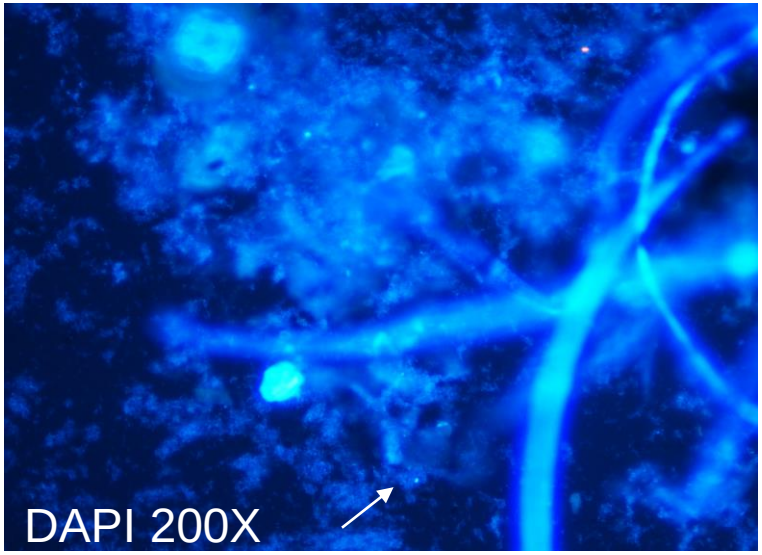


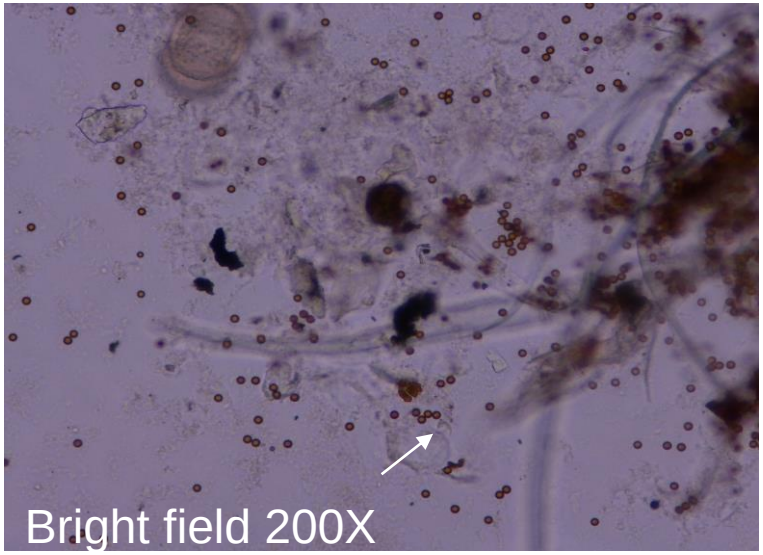


Common concerns with slides: Debris

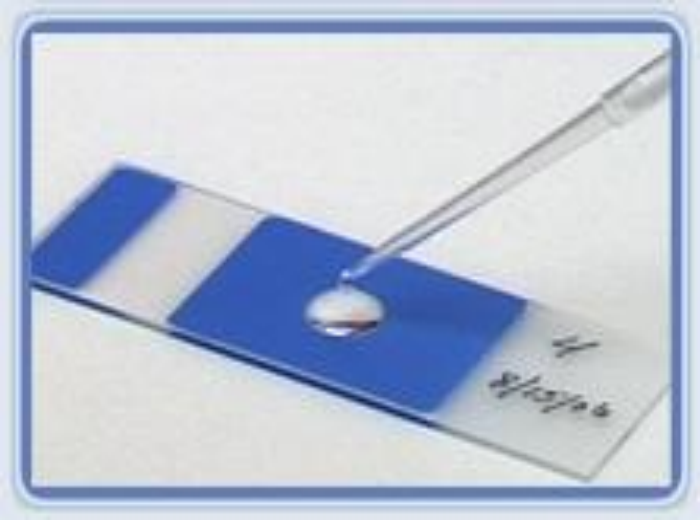
- Reagent Water OPRs should have little to no debris

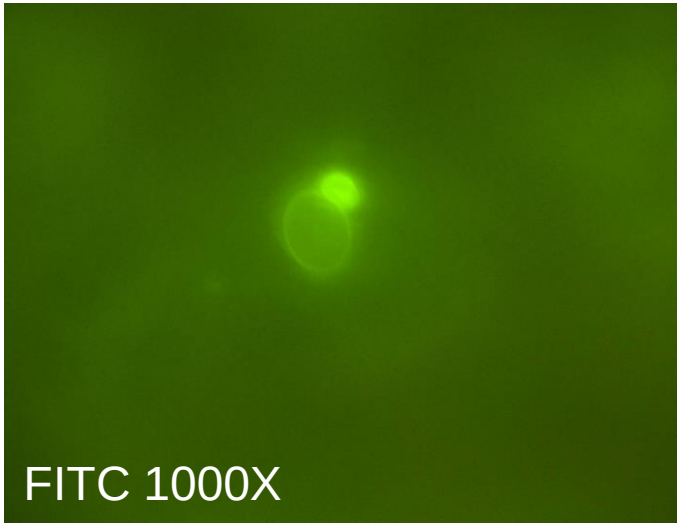


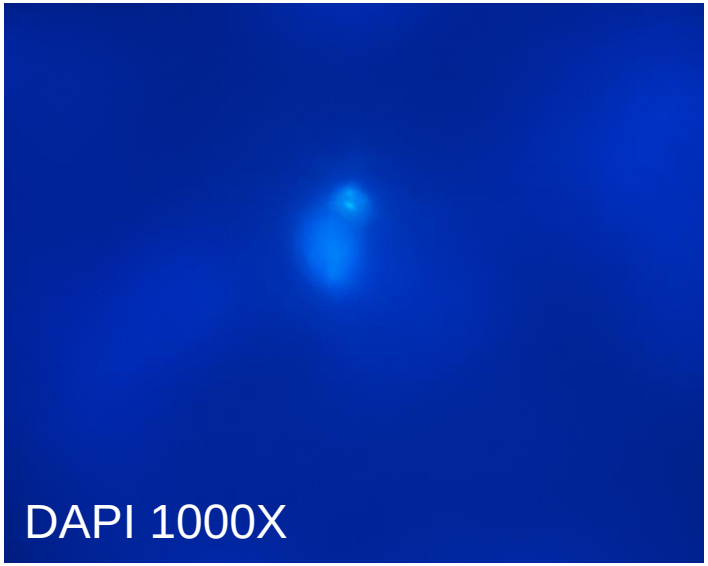


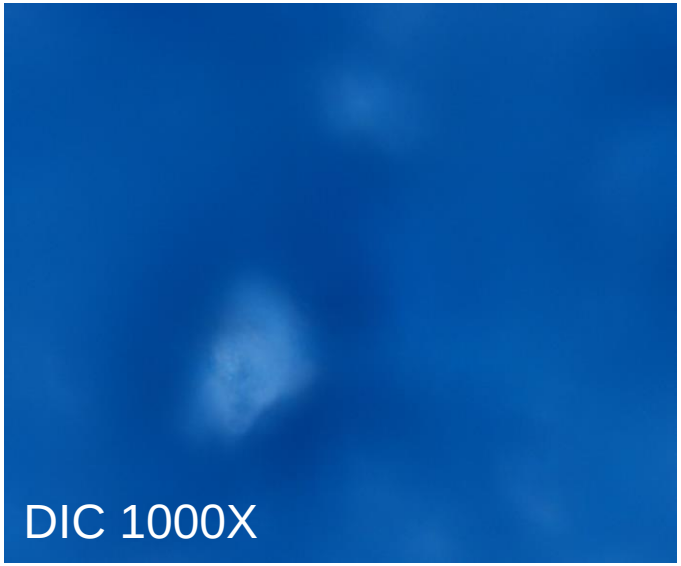


Organisms Outside of the Well









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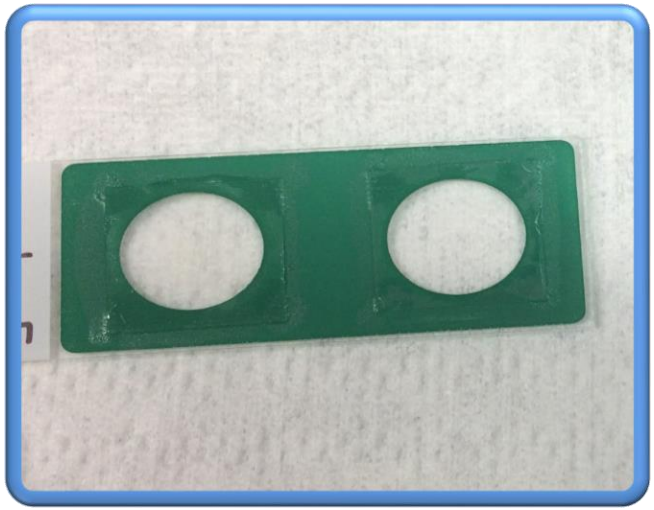
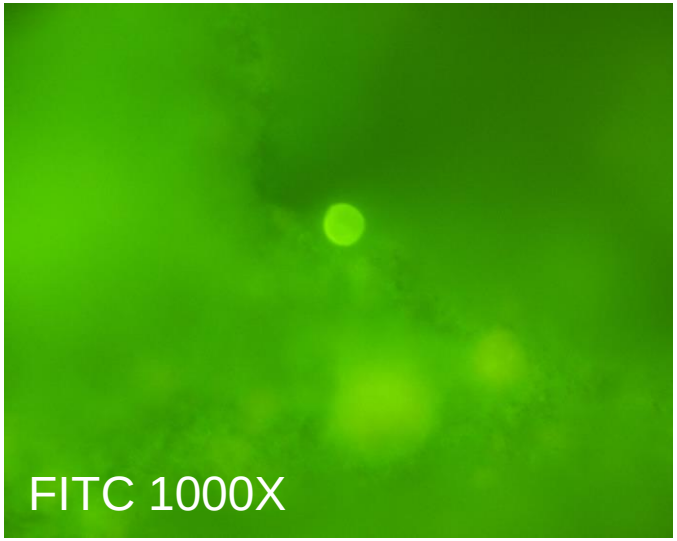
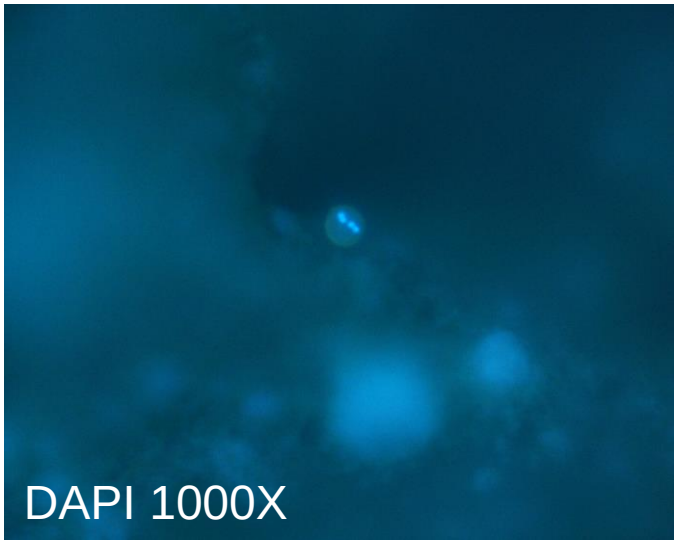
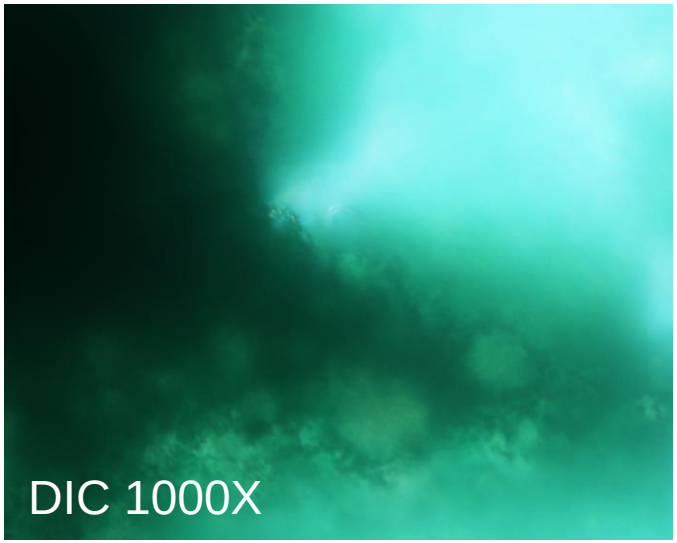






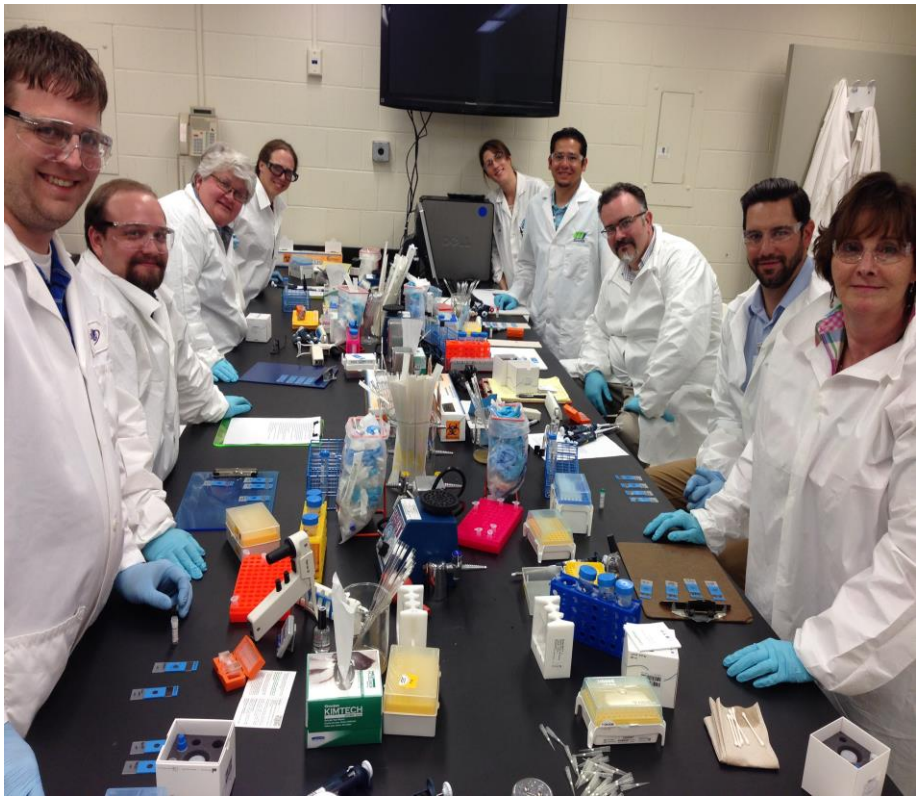


Laboratory Support

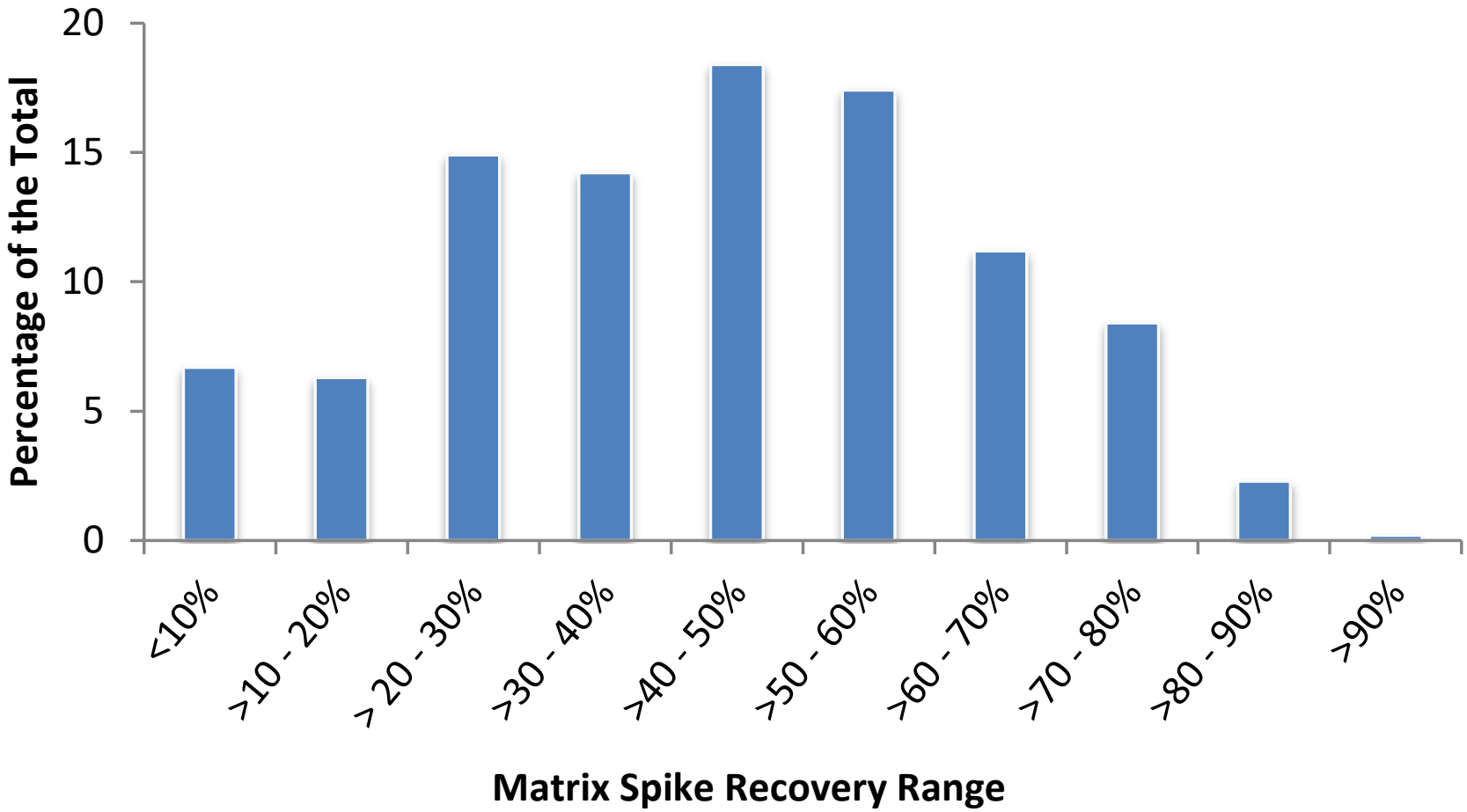
- Call log for reported laboratory concerns or issues
  - Recovery drops when we run multiple samples?
  - Presence of magnetic particles interfering with recovery.
  - Product concerns or issues
- Technical Support
  - Method questions
  - Tips and Tricks
  - Research
  - Webinars
  - Microscope assistance

Analyst Training Workshops and Forums

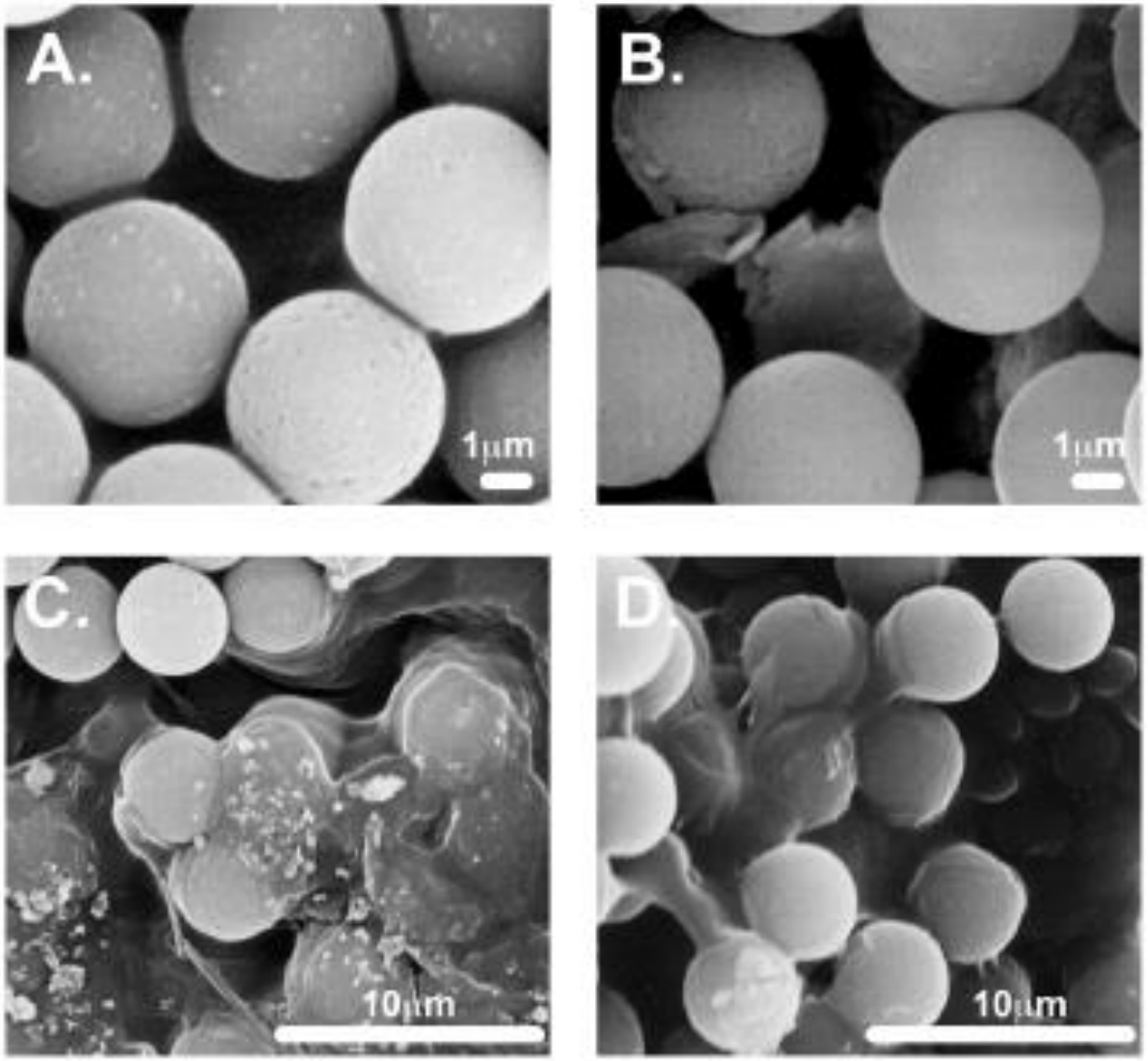
- Skill sharing forums with experienced analysts
- Hands-on workshops for newer analysts
  - Microscopy technique
  - IMS tips and tricks
  - Productivity
  - Recovery
- Workshops offered
  - 2015
  - 2016



Cryptosporidium and *Giardia* Recoveries can be matrix dependent

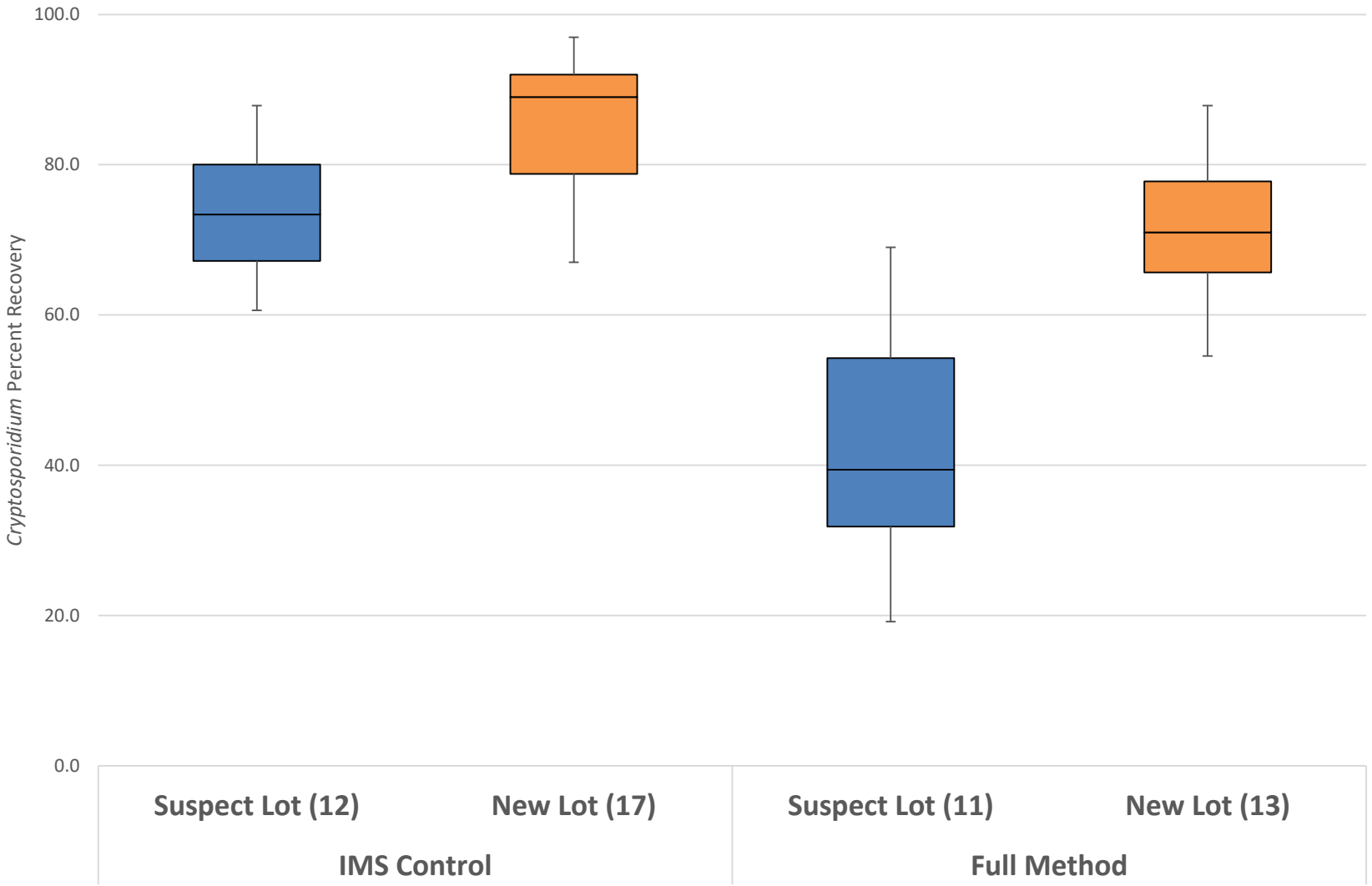


Matrix Effects Can Impact IMS Bead Steps

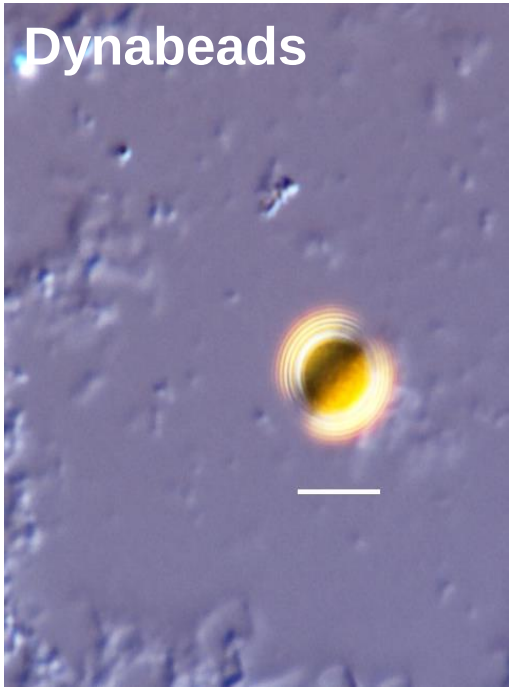


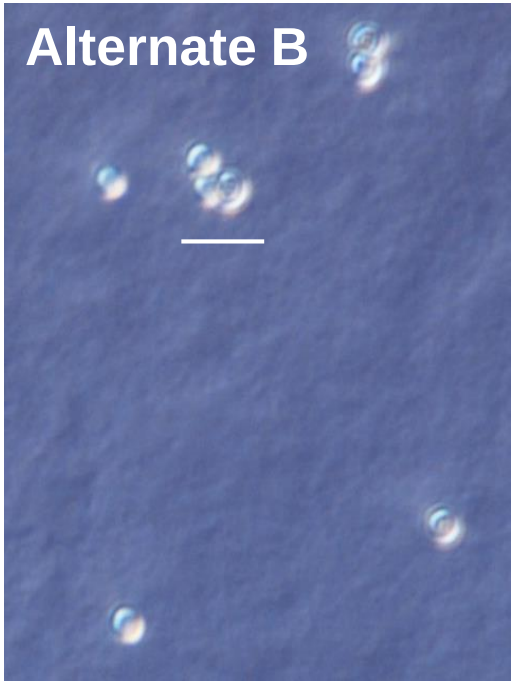
N.J. Shaw et al. / Journal of Microbiological Methods 75 (2008) 445–448

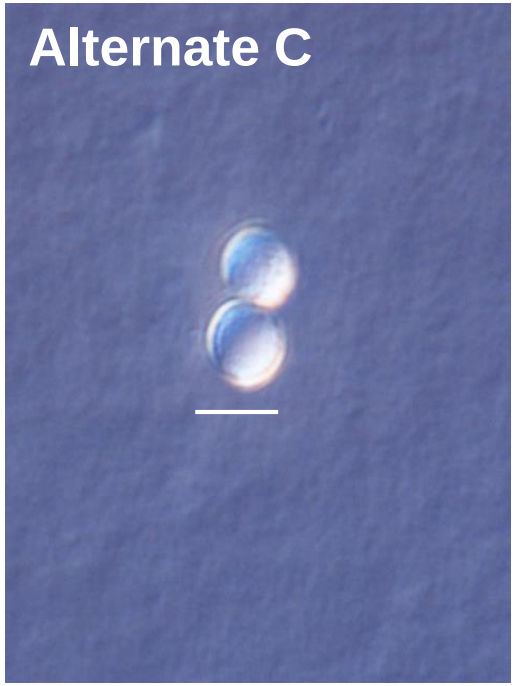
Lot to lot variation has been reported



Three Beads Evaluated



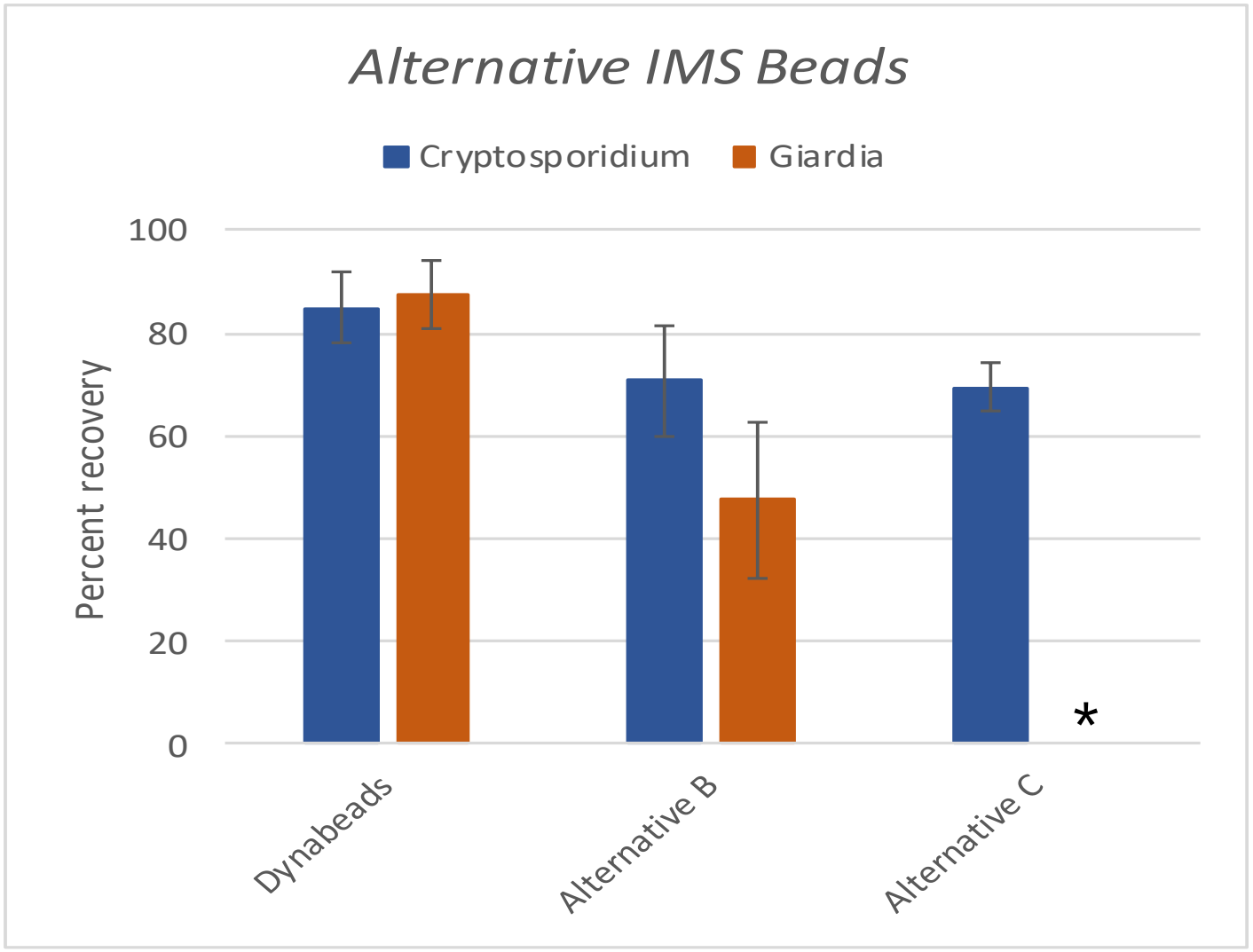




**Scale bar is 5 microns for each image*

Reagent Water IMS Control Recoveries

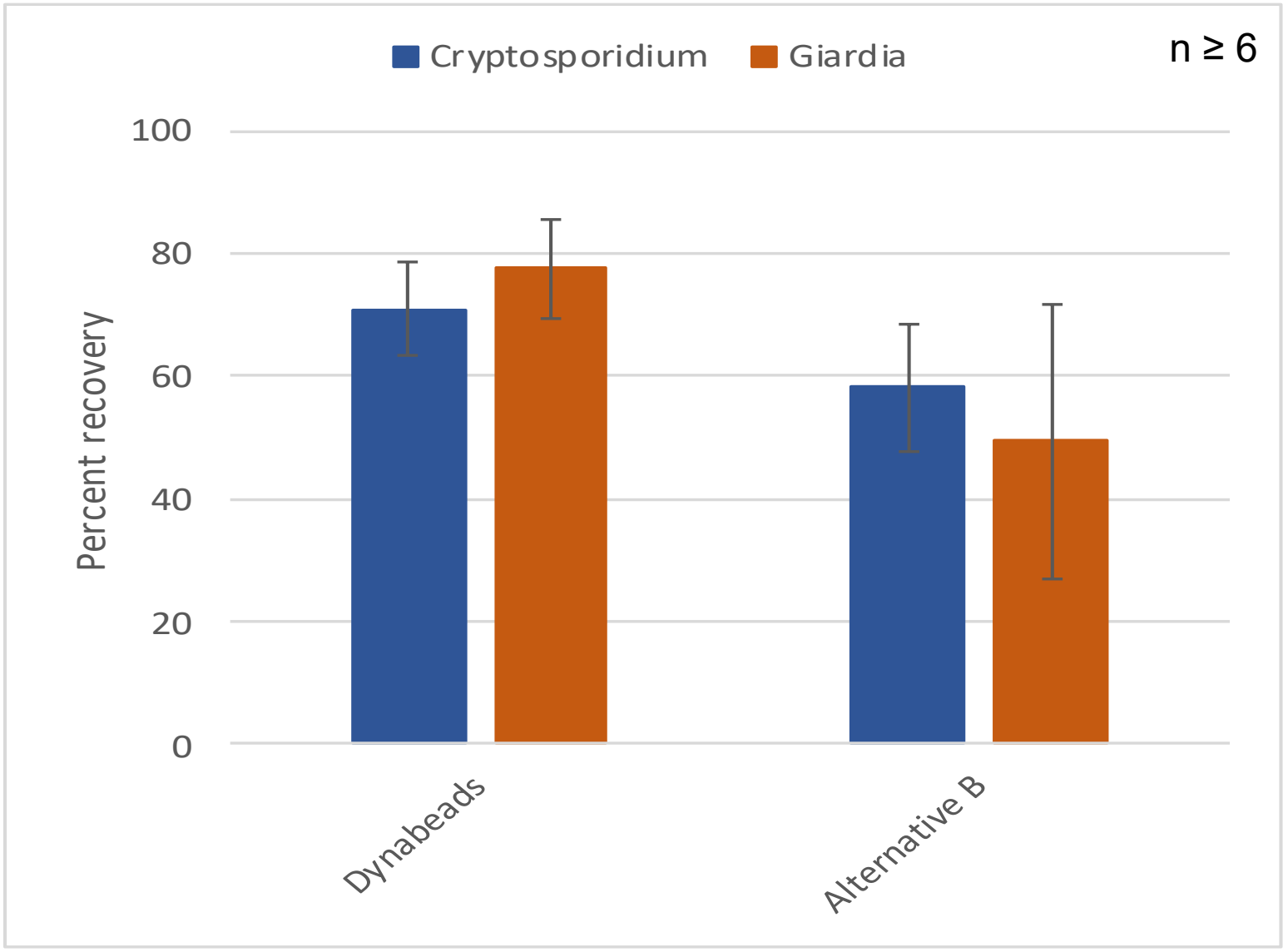
n ≥ 4



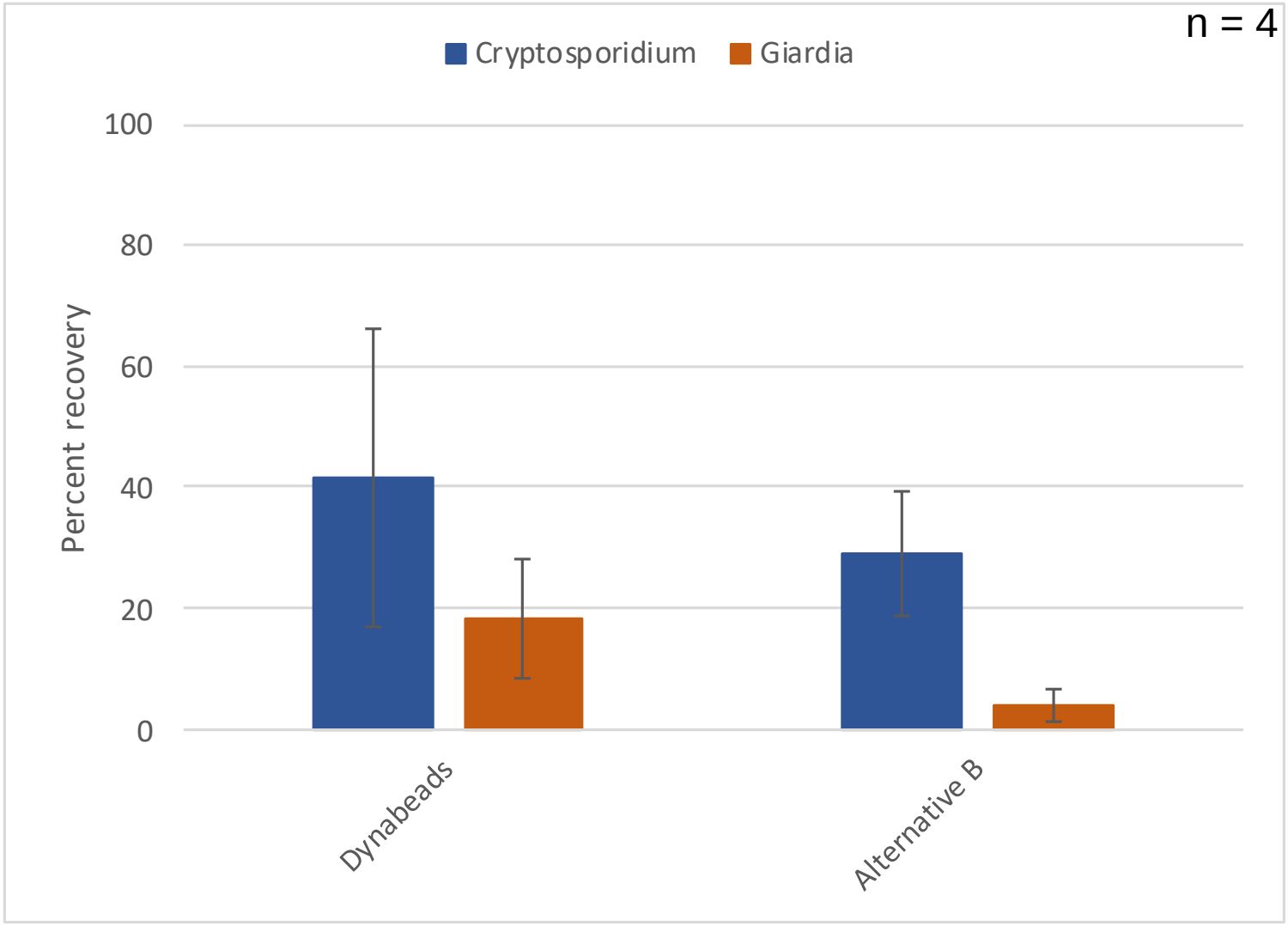
* *Giardia* IMS beads are not available with this product

Change from IMS Controls to Full Method

Method 1623.1 Recovery in Reagent Water



Method 1623.1 Recovery in Ohio River Water



Result of Laboratory Support Study

- The Dynabeads resulted in higher average *Cryptosporidium* recoveries compared to both alternative beads in all conditions.
- The alternative products either resulted in significantly lower *Giardia* recoveries in all matrices or did not have a *Giardia* bead.
- Overall, tests of the alternate IMS beads did not demonstrate that they were comparable to results obtained using Dynabeads

Continuing Certification Officer and Laboratory Support

- TSC provides technical assistance to laboratories and certification officers
  - Audit support
    - Slide reviews
    - Microscopy skills evaluation
- Frequent communication with vendors
- Certification Officer training offered online






Thank You!!

- For more information contact:
  - LT2LabCert@epa.gov