Detection of *Cryptosporidium* by EPA Method 1623: A Summary of the Method and Method Validation

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Workshop on Developing a Plan to Identify *Cryptosporidium* Sources in the Potomac River

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Objectives

- Method Evaluation Criteria
- Method 1623 Lab Validation Criteria
- Overview of EPA Method 1623
- Summary of Results with Method 1623
- Conclusions

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Challenges in Detecting Environmental Parasites

- Tend to occur in low numbers
- Tend to occur intermittently
- Mix of human and animal pathogens
- Mix of viable, stressed, and dead organisms
- Debris and water chemistry

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Environmental Methods and Laboratory Analysis

- Multiple analytical approaches
  - Information gathered
  - Performance efficiencies
  - Multiple analysts and labs
- Performance-based Methods
  - Similar approach, different protocols
- Different water matrices
- QC/ QA

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Method Evaluation Criteria

Lindquist, et al, 1999

- Statistical Performance
  - % Recovery
  - Detection limit
  - Precision
  - Confidence intervals
  - Specificity
  - False positive rate
- Collaborative Testing

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Method Evaluation Criteria, cont.

- Data Generated
  - Genus/ species
  - Viability
- Method Description
  - Completeness
    - Scope and interferences
    - Materials, supplies, and equipment
    - Data reporting, interpretation, analysis
  - Sampling
    - Range of volumes
    - Holding times and preservation
  - Managerial Criteria
    - Training, personnel, etc.

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US EPA Method 1623

- Benchmark for detecting and quantifying *Giardia* and *Cryptosporidium* in water
- http://www.epa.gov/microbes/
- Performance-based method
- Genus level analysis
- Viability not determined
- Multi-lab validated

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Spikes for Recovery Studies

- Live oocysts
  - Age (<6 weeks)
  - Enumeration method
    - Flow cytometry
    - Serial dilution
    - Time and storage effects
- Gamma-irradiated oocysts
  - EasySeed
  - Warnecke, et al, 2003
- Differentiated oocysts
  - ColorSeed
  - Francy, et al, 2004

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Single Lab Validation and QA Criteria for *Cryptosporidium*

- Initial Performance and Recovery (IPR)
  - Spiked reagent water
  - Mean of 4 tests (>24% recovery, RSD 55)
- Ongoing Precision and Recovery (OPR)
  - 1 spiked reagent water/ week or 20 samples
  - >11% recovery
- Method Blanks
  - 1 with IPR and 1 weekly
- Matrix spike (MS)
  - Spiked field sample and reference sample
  - 1/ 20 field samples, over at least 12 months per water type
  - 15-118% recovery

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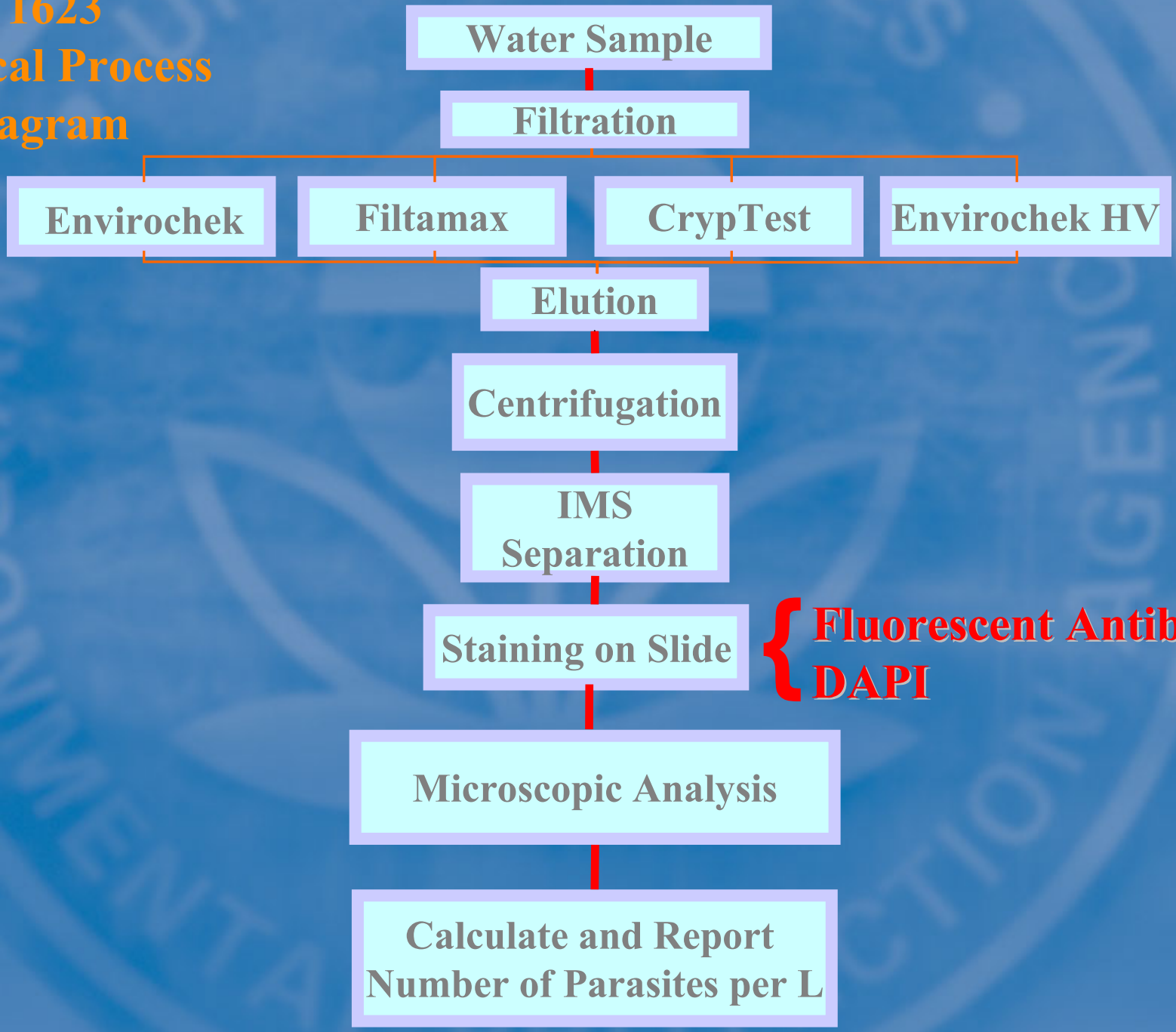
Multi-lab Validation Criteria

- 3 labs must meet single lab's IPR, OPR, and Method blanks
- MS/ MS duplicate
  - 2 spiked field samples with reference samples
  - 1/ 20 samples, over at least 12 months per water type
  - 15-118% recovery average, RSD 30

Method 1623 Analytical Process Flow Diagram



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Filtration

- Raw water 10-50 L
- Finished water up to 1000 L
- Flow rates 1 to 4 L/ min

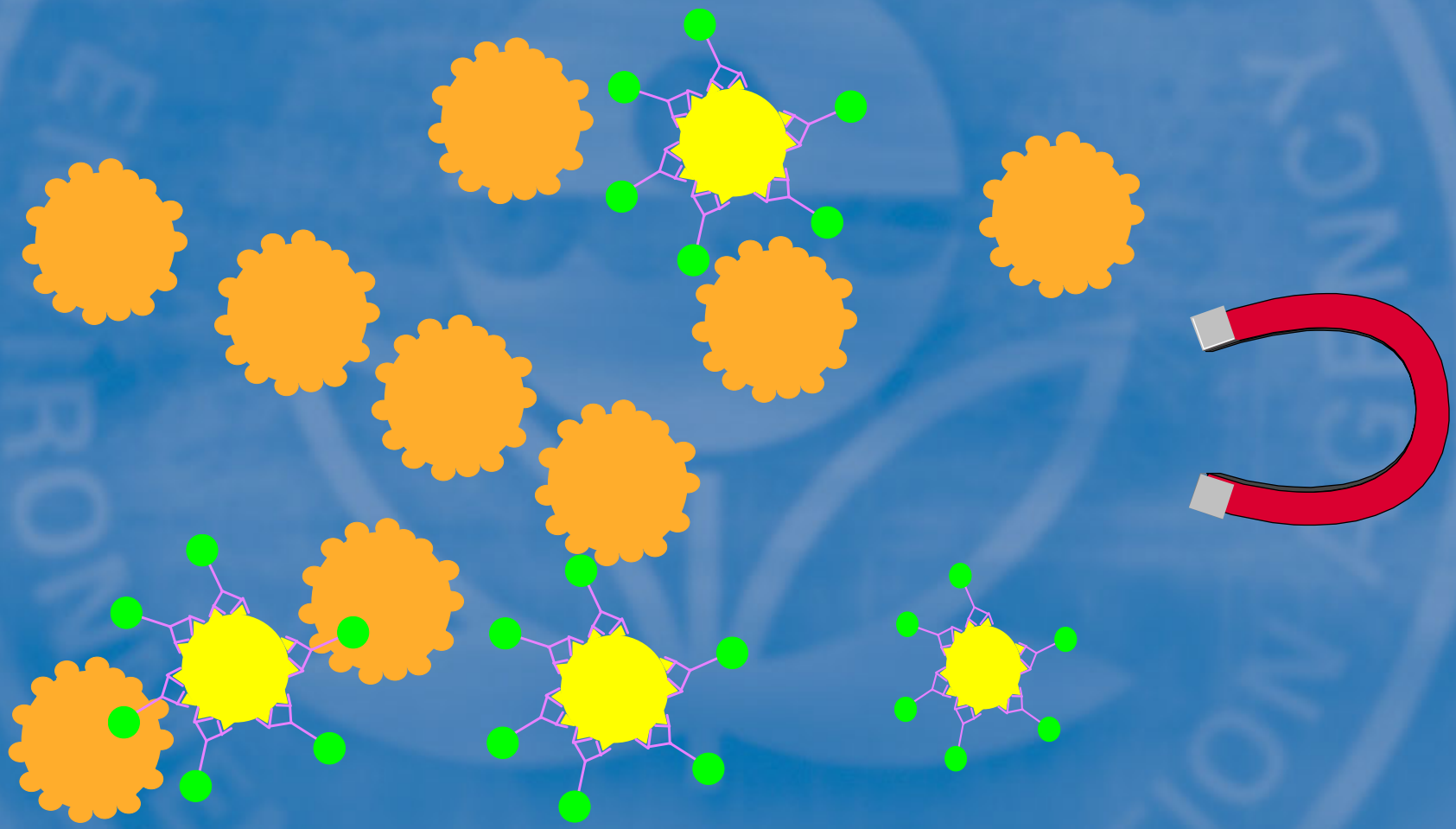
4 validated filters

- Envirochek, Envirochek HV, and Cryptest all 1 µm porosity
- Filtamax nominal 1 µm porosity

Immunomagnetic Separation (IMS)

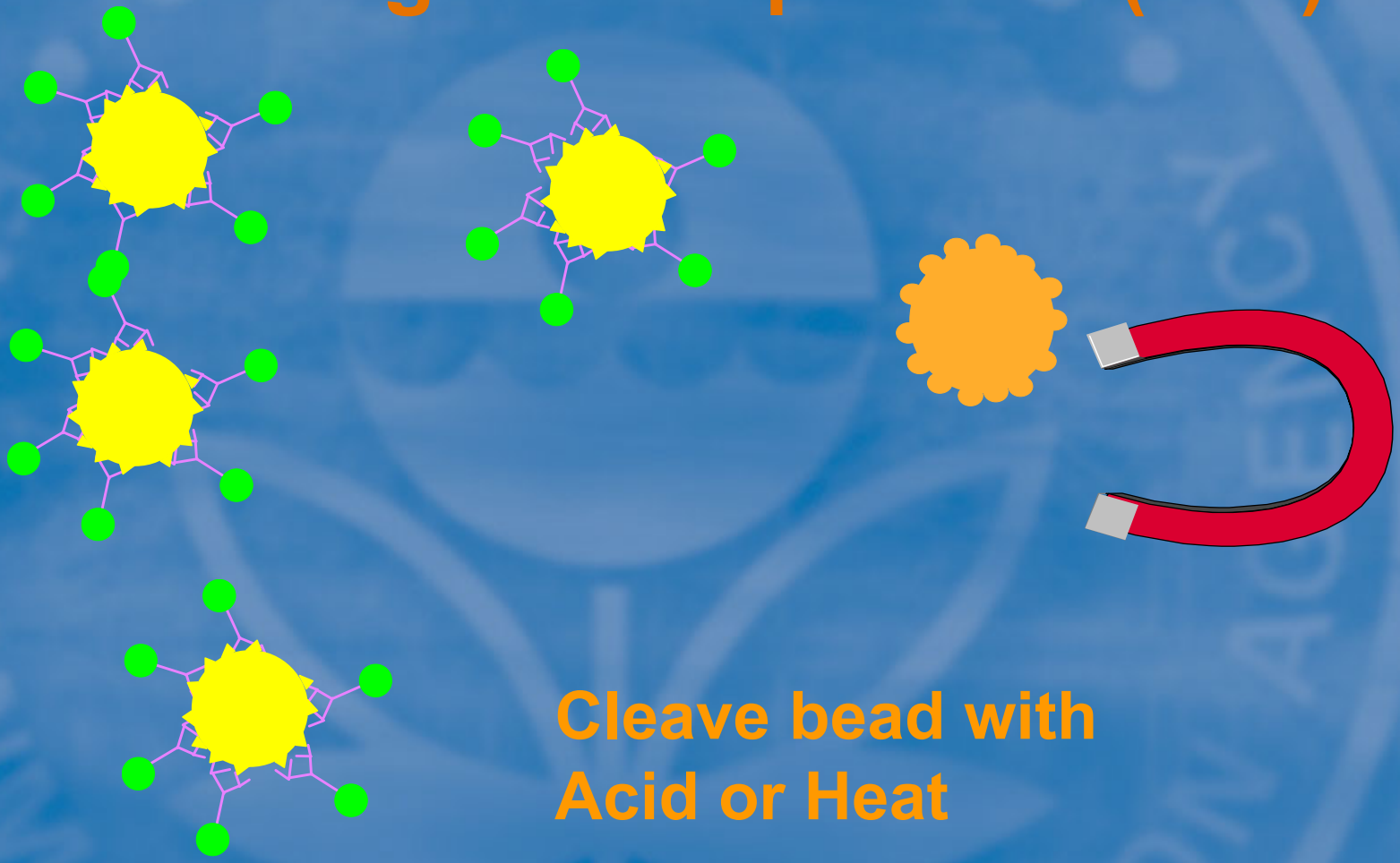
Label with antibody conjugated to paramagnetic bead

Immunomagnetic Separation (IMS)



Capture with magnetic field,
remove debris

Immunomagnetic Separation (IMS)



Cleave bead with
Acid or Heat

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Staining and Microscopy

- Oocysts and cysts dried onto slide
- Stain with FITC conjugated monoclonal antibody (mAb) and DAPI
- Primary detection by mAb
- Confirm by detecting DAPI stained nuclei and/or internal structures

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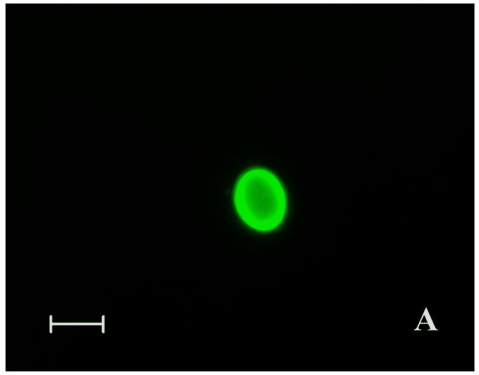
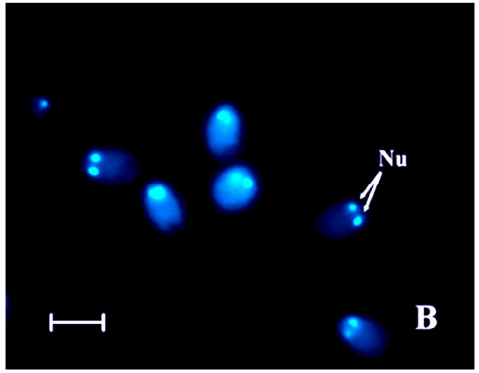
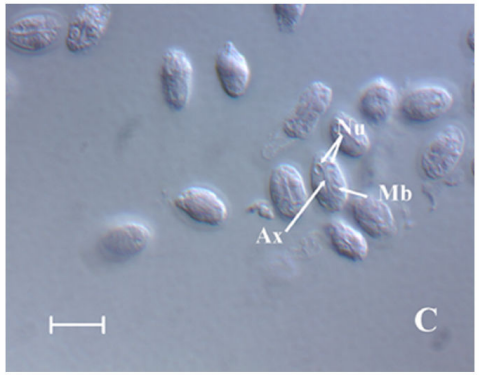
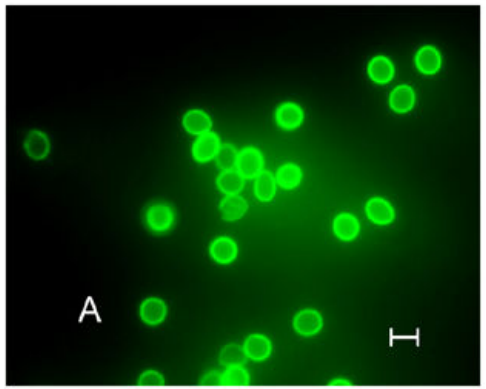
Giardia

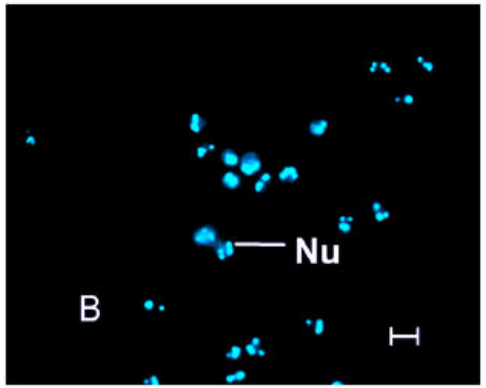


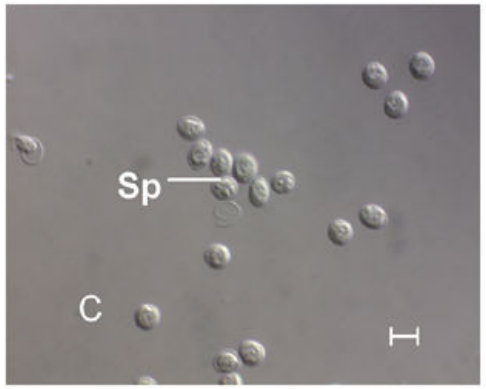




Cryptosporidium







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Results from Studies Evaluating Method 1623

- Most evaluated performance in surface, ground, or drinking water.
- Adversely affected by turbidity
- Wide range of recoveries reported (0% to 80%+)
- Prone to false negative and false positive results

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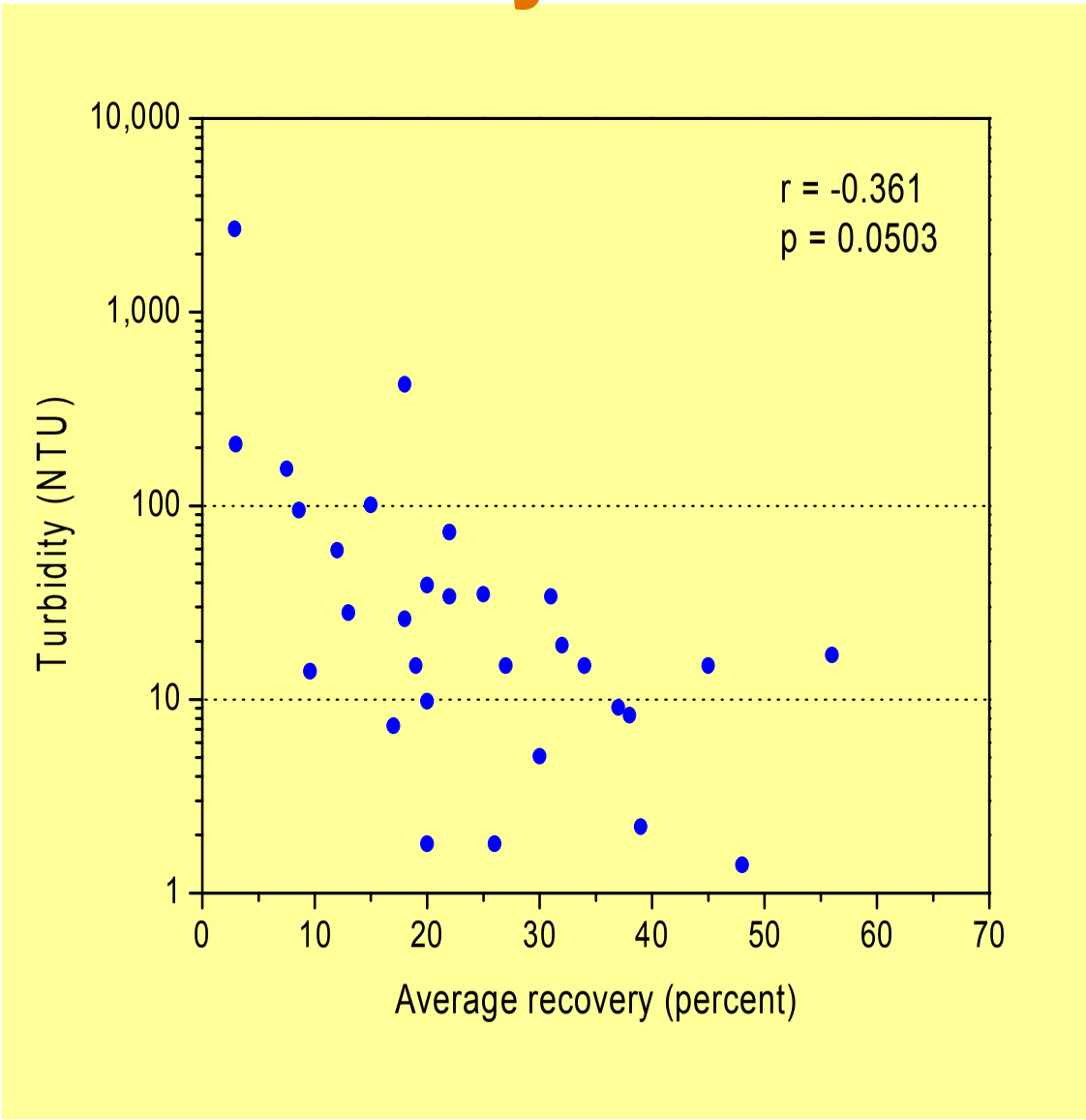
Turbidity Effect



Francy, et al, 2004

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Conclusions

- Method 1623
  - Matrix effects
  - Variable performance
- How do you interpret results?
  - Good QA program
  - Performance criteria
- How do compare results from different labs?
  - Lab validation
  - Objective comparison criteria