Method 1623 Improvements

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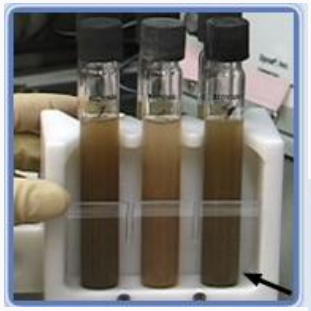
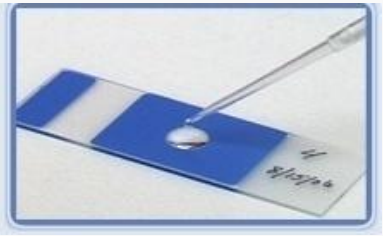
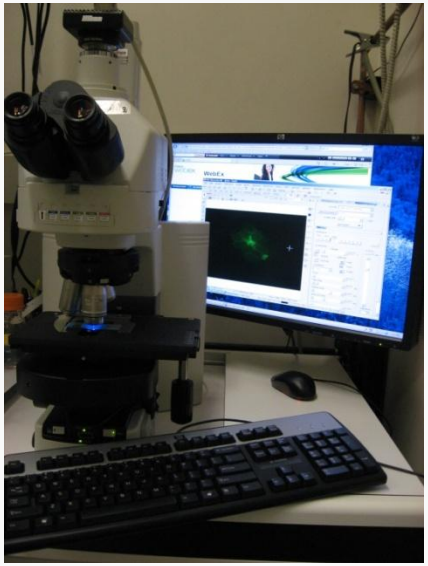
Can We Enhance Program-wide Data Quality and Consistency?

- Challenging matrices
- Improve accuracy and precision
  - Method components
  - Laboratory performance



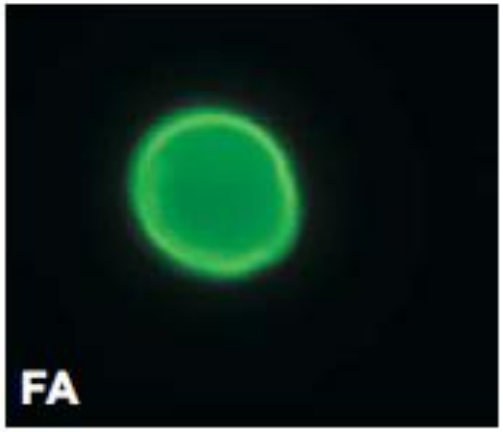


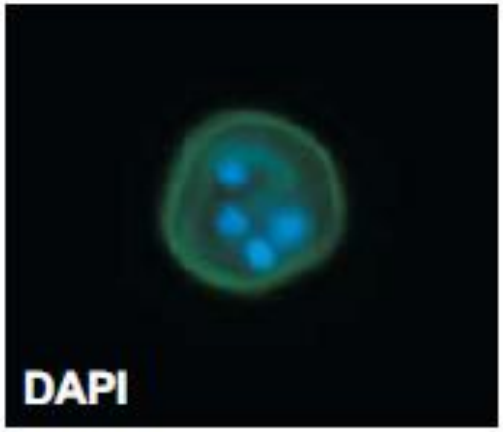
10 L → → → → 100 µl

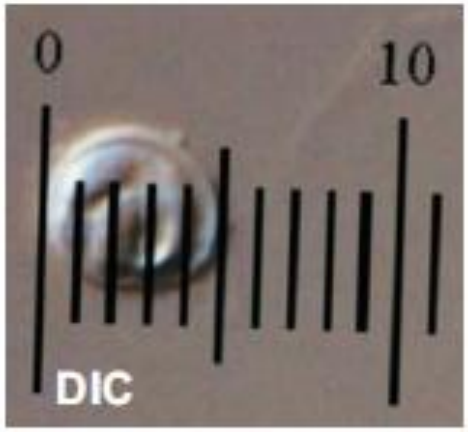


Determinative Assay

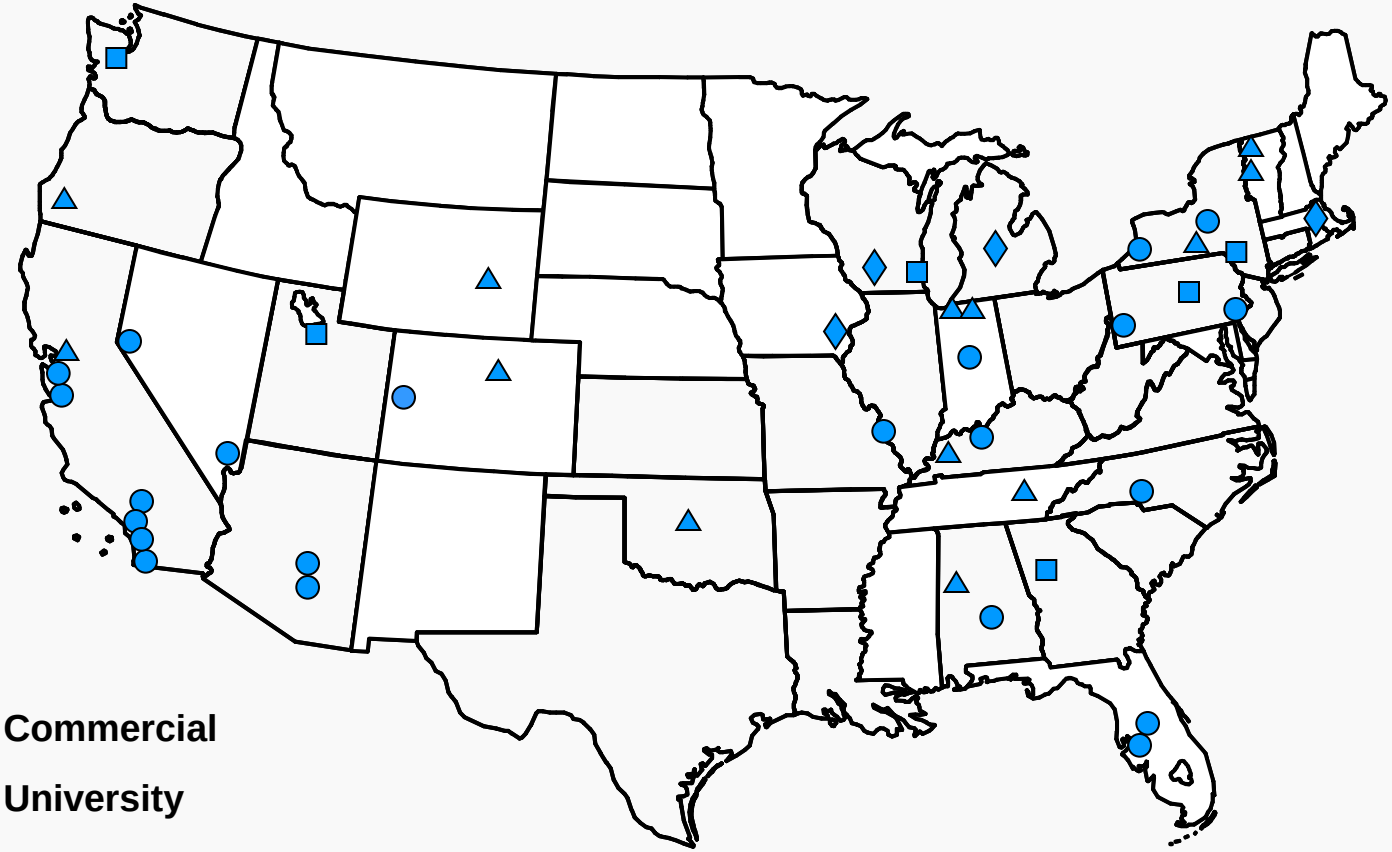
- Fluorescence
- Size and shape
- Nuclei and Sporozoites
  - DAPI
  - DIC





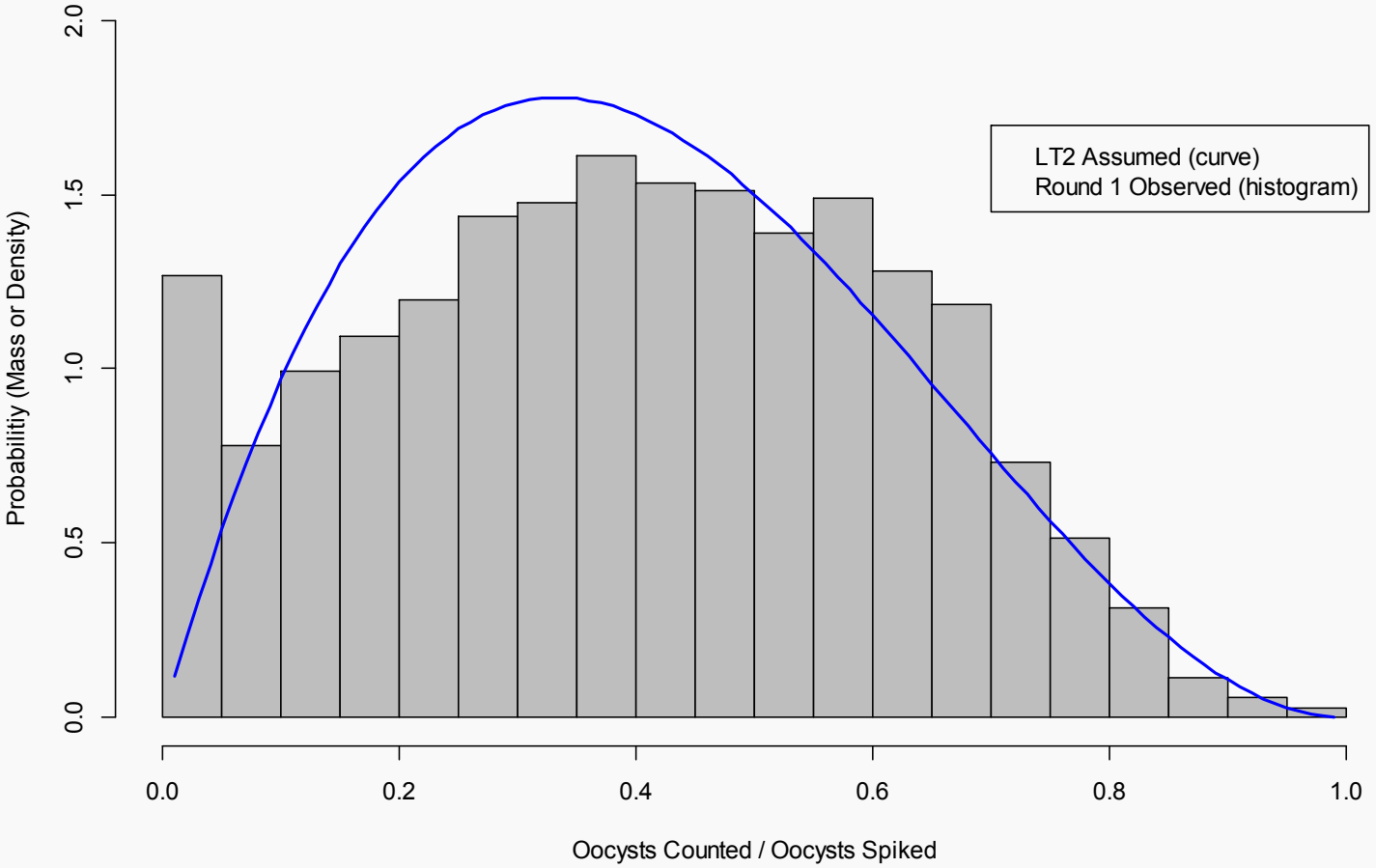


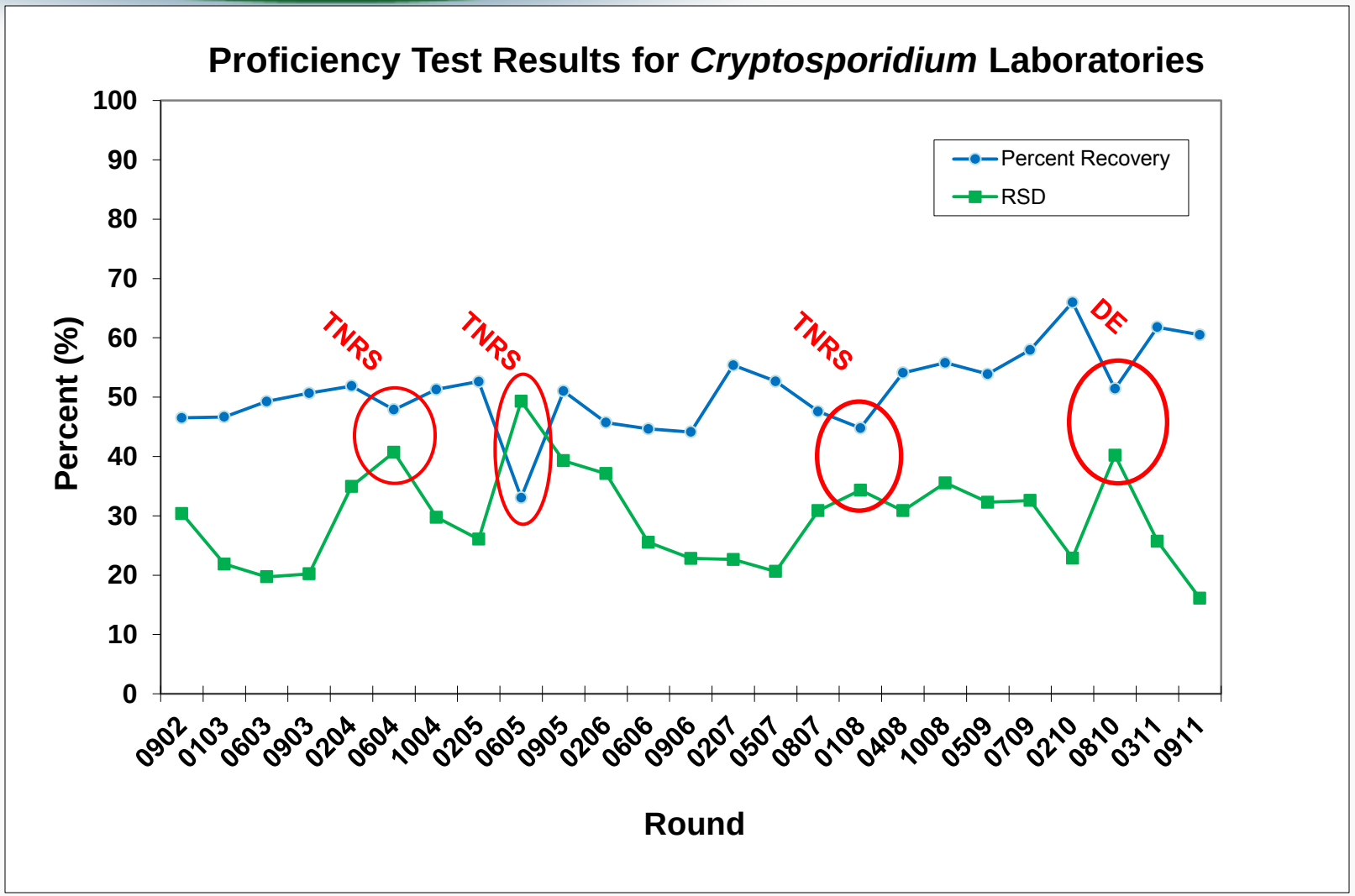
47 Approved *Cryptosporidium* U.S. Laboratories

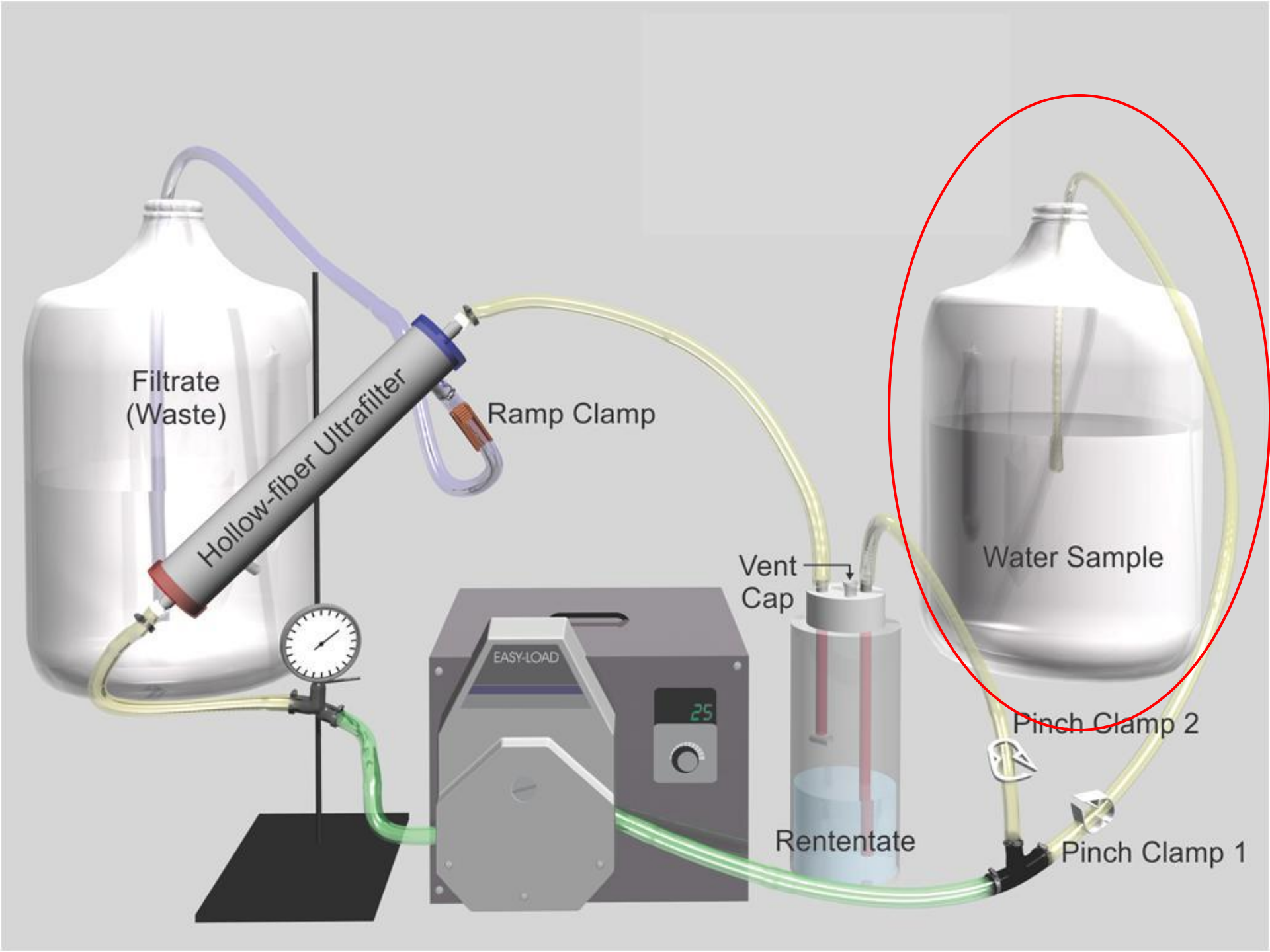


- Commercial
- University
- Public Water System
- State/Regional Lab

Observed and LT2-Projected Recovery Distributions




Proficiency Test Results for *Cryptosporidium* Laboratories
Percent (%)
100
90
80
70
60
50
40
30
20
10
0
Percent Recovery
RSD
TNRS
TNRS
TNRS
DE
0902
0103
0603
0903
0204
0604
1004
0205
0605
0905
0206
0606
0906
0207
0507
0807
0108
0408
1008
0509
0709
0210
0810
0311
0911
Round


Filtrate
(Waste)
Hollow-fiber Ultrafilter
Ramp Clamp
Vent
Cap
Water Sample
EASY-LOAD
25
Pinch Clamp 2
Rententate
Pinch Clamp 1

Sodium hexametaphosphate (NaHMP)

- Reduces filter fouling during ultrafiltration
- Improves detection in waste and finished waters
- Improves the dispersion efficacy of surfactants by
  - sequestering ions associated with water hardness
  - lowering the surface tension
  - increasing the zeta potential of particles

LT2 Rule *Cryptosporidium* & *E. coli* Sample Collection Recommendations

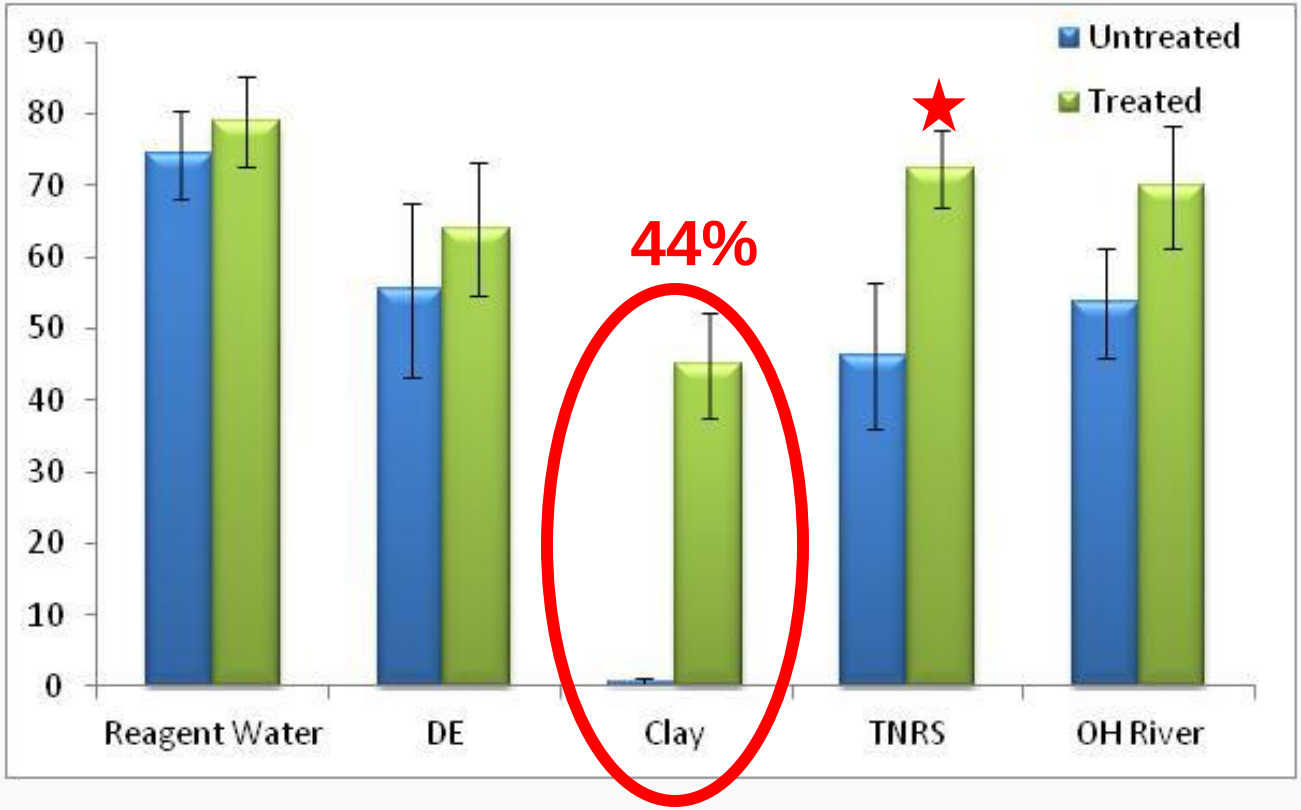
Welcome

This is the United States Environmental Protection Agency Long Term 2 Enhanced Surface Water Treatment Rule Module on recommended procedures for *Cryptosporidium* and *E. coli* sample collection. This module was developed to help train public water system personnel to collect source water samples for analysis.

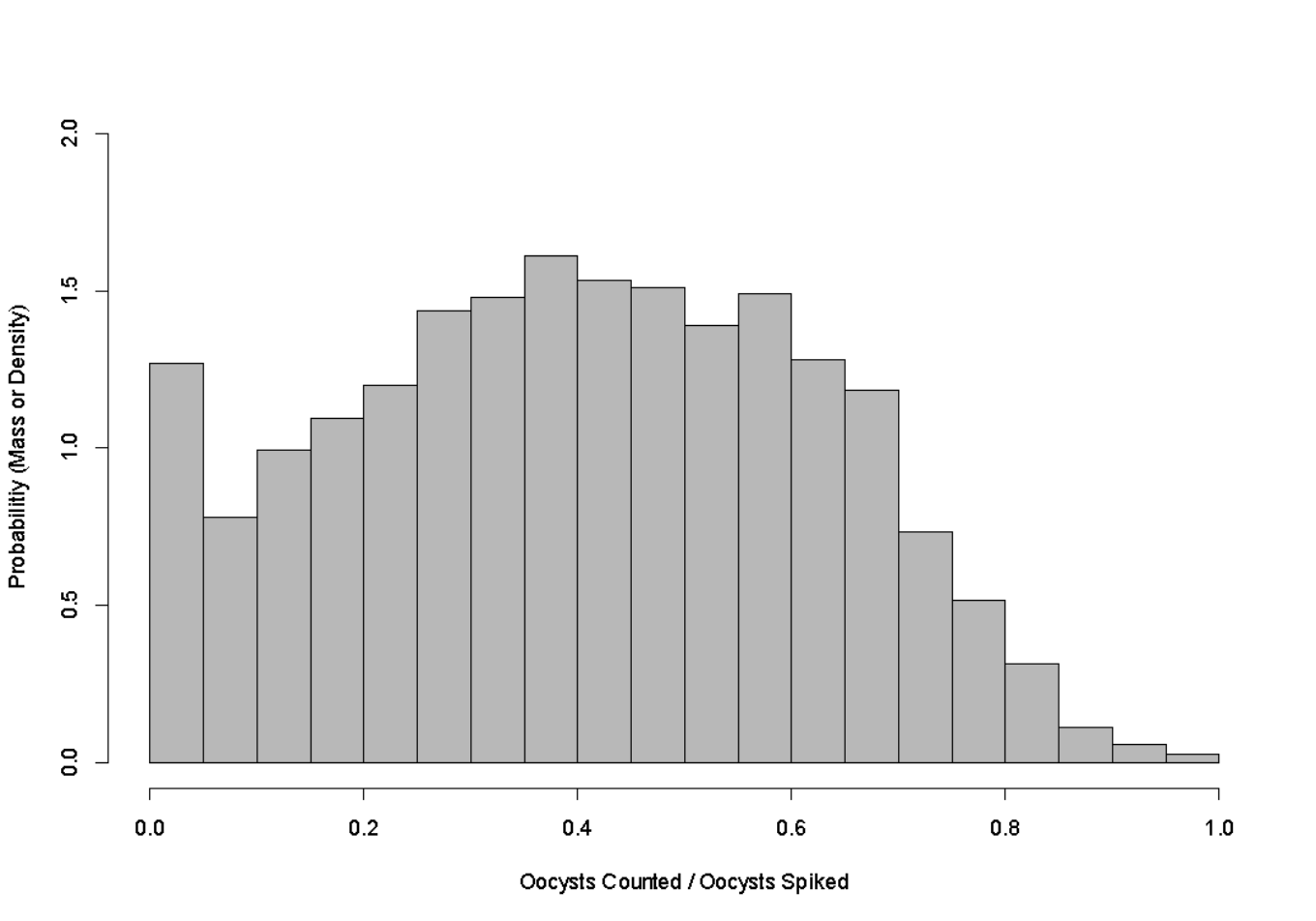
Select the link for detailed information on the Long Term 2 Enhanced Surface Water Treatment Rule.

Recovery using 5.0% NaHMP

n≥8



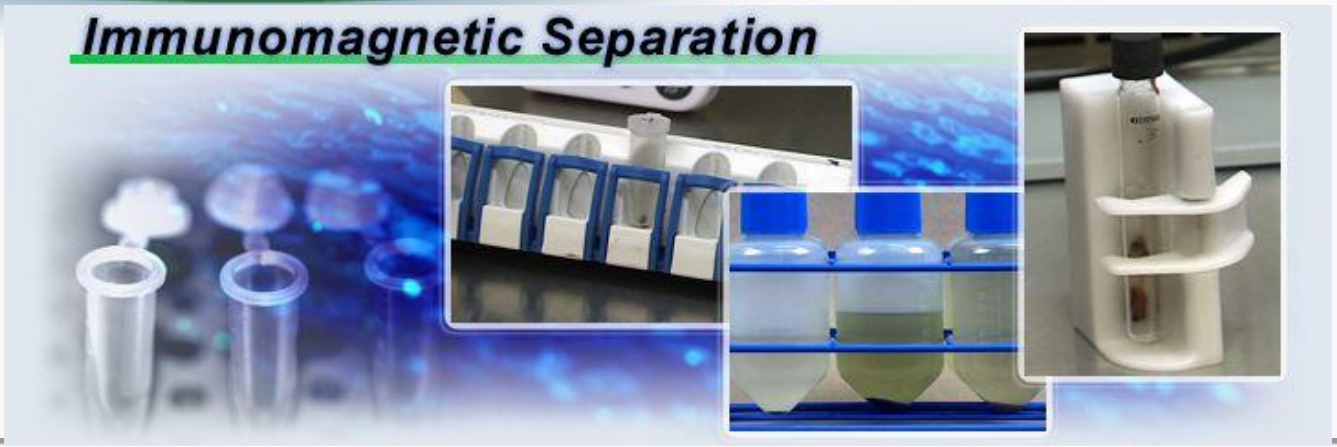
Distribution of Observed Recovery

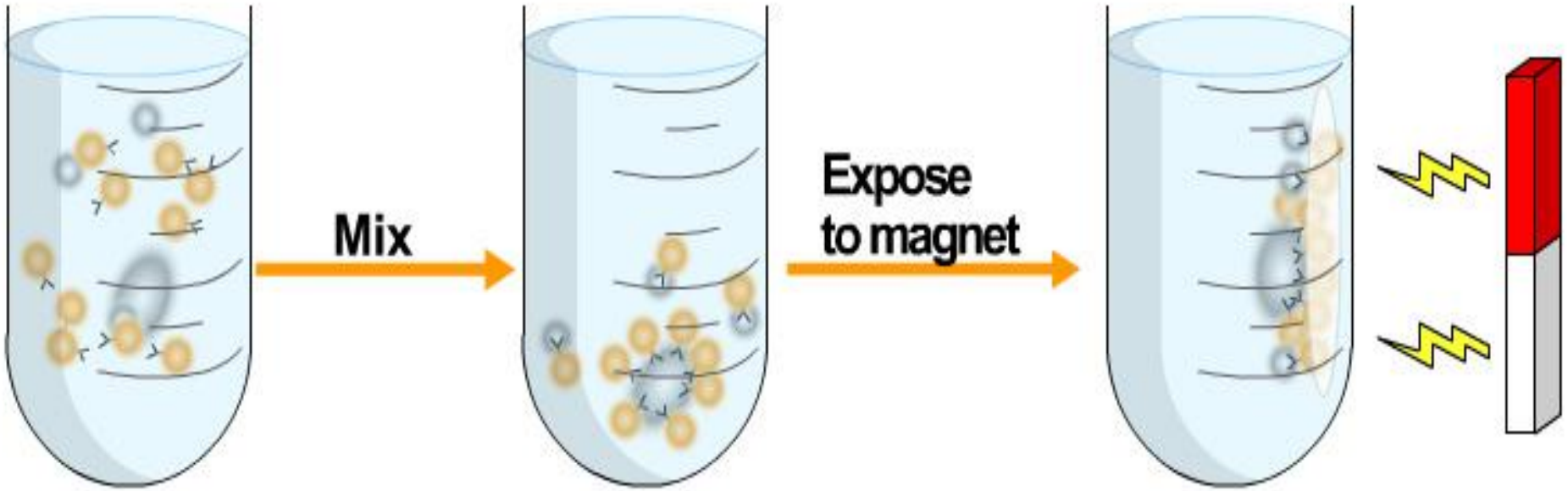


Review of Laboratory Practice

- Laboratories with a low frequency of low recovery
- Same laboratories also had high accuracy and precision for PTs in matrix and reagent water
- Five of eight did an IMS rinse step to remove debris

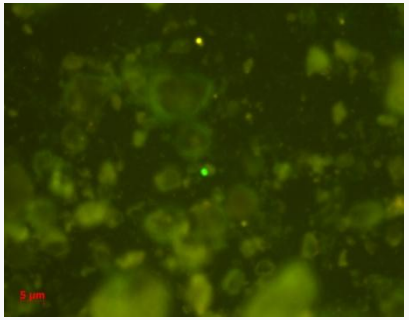
Immunomagnetic Separation

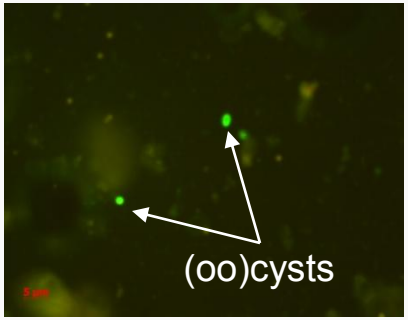




No Wash

Wash





Pellet Wash in Microcentrifuge Tube

remove visible obstructions in samples with extraneous debris and microbiota

2008

Expense for Method Modification

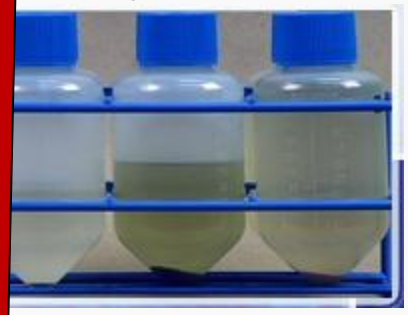
- Cost increase 25¢ per sample
- Processing Time increases ~20 minutes per batch
- Microscopy may decrease ~10 minutes per slide
- Theoretically it's possible to save time
  - E.g. 8 samples in a batch for 20 minutes – 10 minutes/slide (80m) would yield an hour saved at the microscope

Method 1623 Modification

Sample

Filter

NaHMP Added



Centrifuge Tubes

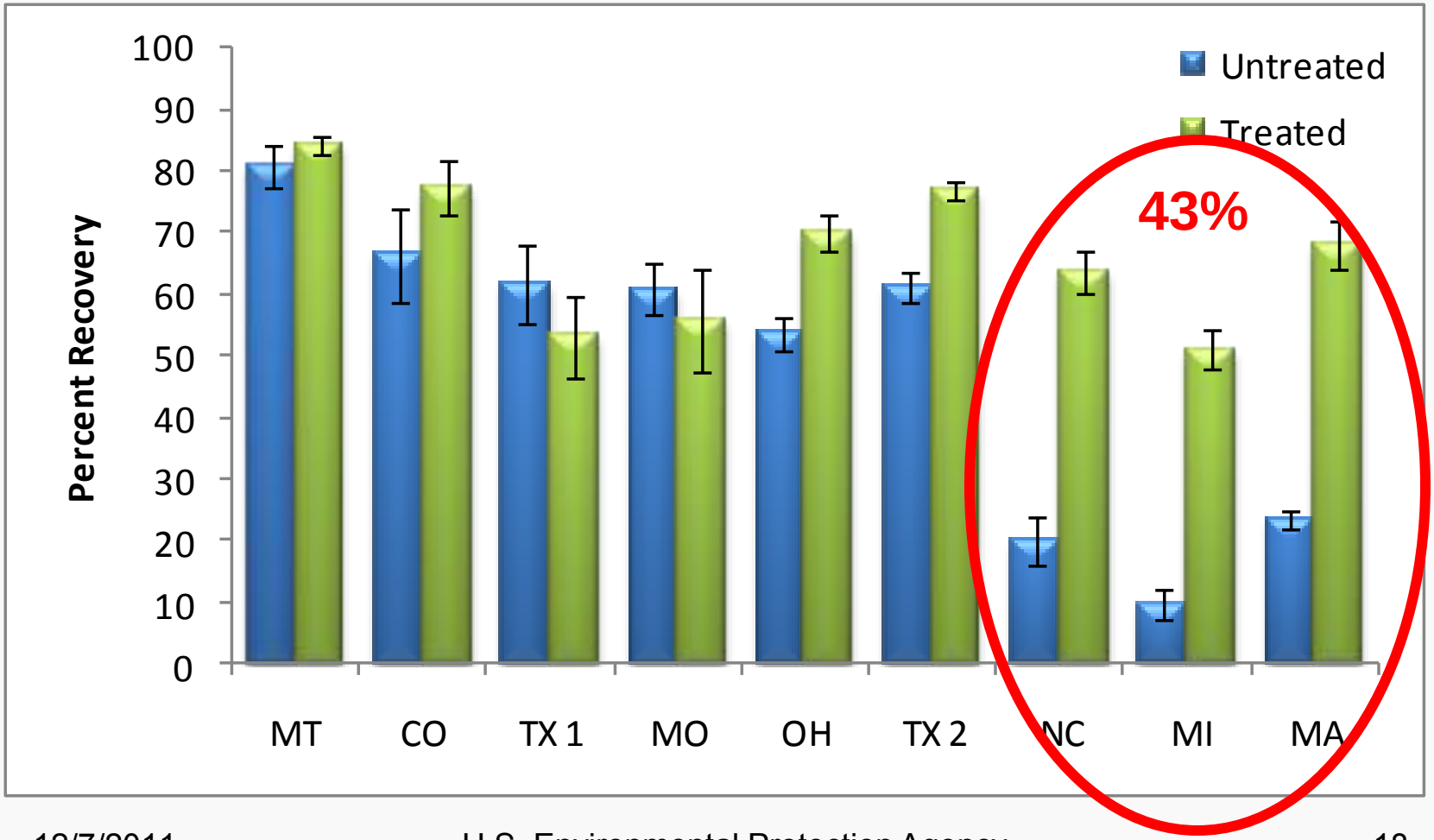
IMS

Bead Pellet Wash

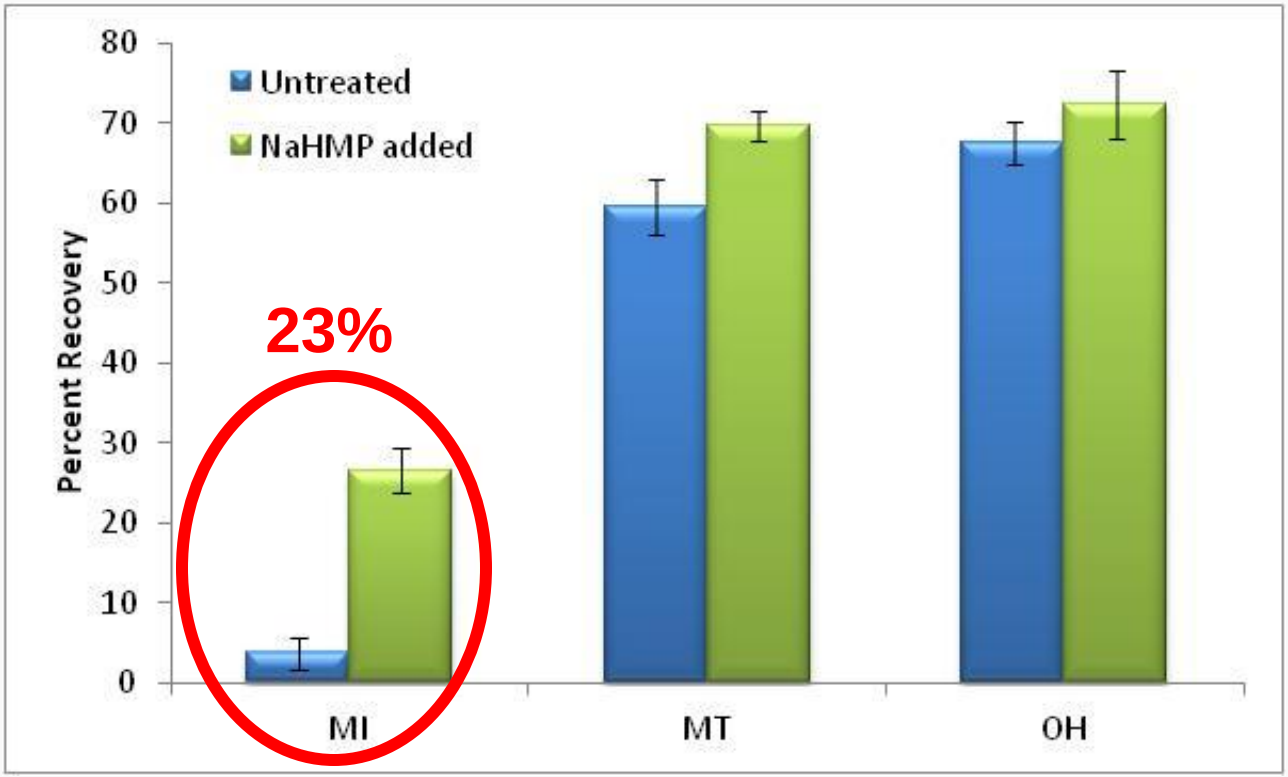
Slide

10 L → → → → 100 µl

Side by Side Comparison in Sources from Nine Public Water Systems



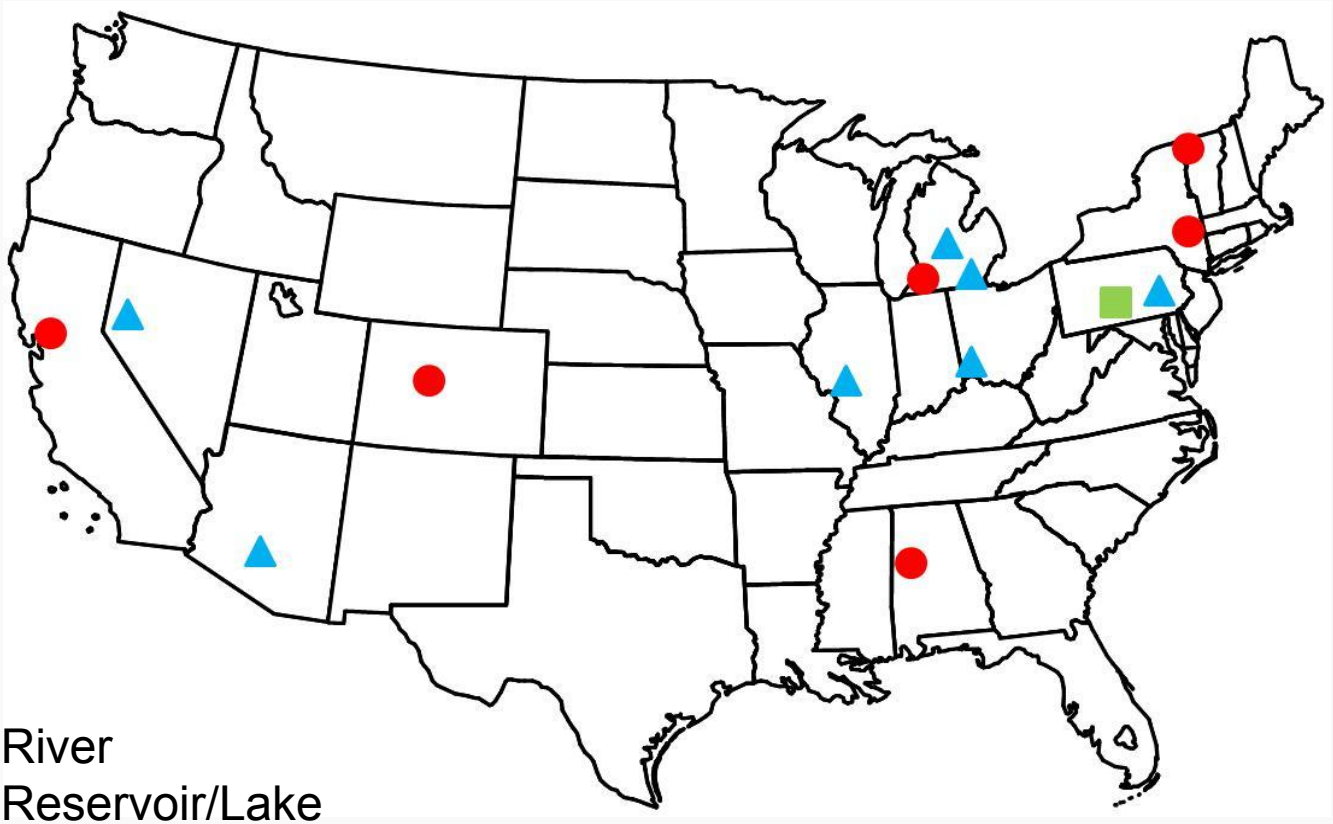
Side by Side Comparison of 3 Source Waters in 4 Laboratories



Experimental Design for Multi-Laboratory Evaluation of the Modifications

- Live Harley Moon isolate of *C. parvum*
- Same lot of sampling capsules and reagents
- 14 Sources used by public water systems
- 140 samples analyzed
  - 70 source water
  - 70 reagent water

Distribution of Source Waters



River
Reservoir/Lake
Ground

Variation in Source Water Samples

- Turbidity: 1 to 53 NTU
- Conductivity: 33 to 885 µS
- pH: 6.5 to 8.5

Good Quality Control Practices

- Trip control
- Positive and negative controls
- Data verification and validation
  - no outliers were detected

Results: Reagent Water (n=56)

- 60% Mean recovery
- Range of recovery was 34% to 73%
- 16.2% average within lab RSD
- No oocysts in negative control samples

Results: Source Water (n=53)

- 61% Mean recovery
- Range of recovery was 26% to 80%
- 12.7% average within lab RSD
- No oocysts found in un-spiked samples.

Accuracy Increased 27 Percentage Points in Source Water

		Method 1623 1999	Modified Method 2011
Reagent Water	Mean % Recovery	**40** (n=29)	**60** (n=56)
	Mean RSD (%)	**24**	**16**
	Standard Deviation	**9**	**9**
Source Water		8 sources	14 sources
	Mean % Recovery	**34** (n=14)	**61** (n=53)
	Mean RSD (%)	**25**	**13**
	Standard Deviation	**9**	**7**

Method Flexibility

- Select from options for procedural components
  - Multi-lab validated
  - Historical standardized procedure
- Additional alternate test procedures
  - Side-by-side method comparisons
  - 2010 EPA Guidance for conducting method studies

Examples of Procedural Options

- Spiking Suspensions
  - WI State Lab of Hygiene
  - EasySeed™
  - AccuSpike™
- Stain
  - Aqua-Glo™
  - Crypt-a-Glo™
  - EasyStain™
  - MeriFluor®

Method Modification Reduces the "Matrix Effect"

- Side by side data showed as much as 43% improvement in source water with low recovery
- Accuracy increased 27% in 14 source waters compared with validation data from Method 1623

We Have an Opportunity to Enhance Data Quality for the LT2