Evaluation of a *Cryptosporidium* Internal Standard for Determining Recovery with Environmental Protection Agency Method 1623

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Scientific Question

A new product, ColorSeed, has been designed as an internal standard for determining the recovery of *Cryptosporidium;* however, it has not been validated. Are the recoveries achieved with ColorSeed equivalent to those of live oocysts in reagent and stream water samples?

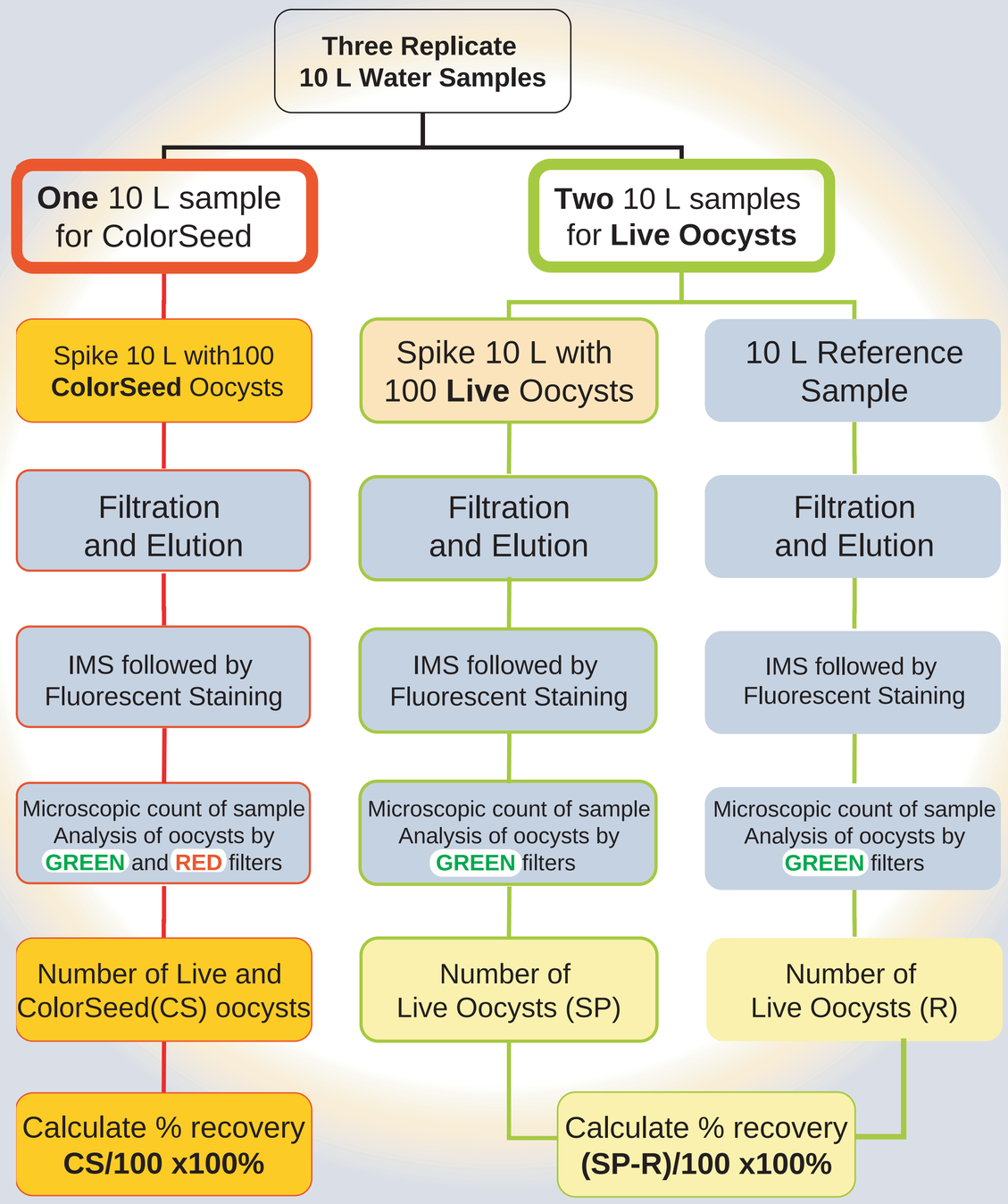
Cryptosporidium

- Parasite with infective oocyst stage
- Causes human gastroenteritis
- Caused 1993 Milwaukee waterborne outbreak, affecting over 400,000 people
- EPA Method 1623 is the baseline detection method for water samples

US EPA Method 1623

- A 10 L water sample is filtered
- The oocysts are eluted and purified by immunomagnetic separation (IMS)
- The oocysts are detected by microscopy after fluorescent antibody staining
- Oocyst recovery is variable and must be evaluated with controls to interpret results
- Method performance is linked to water quality
- Commercial costs average $400/ sample

Study Design for Analysis of Samples



Method 1623 QA Program

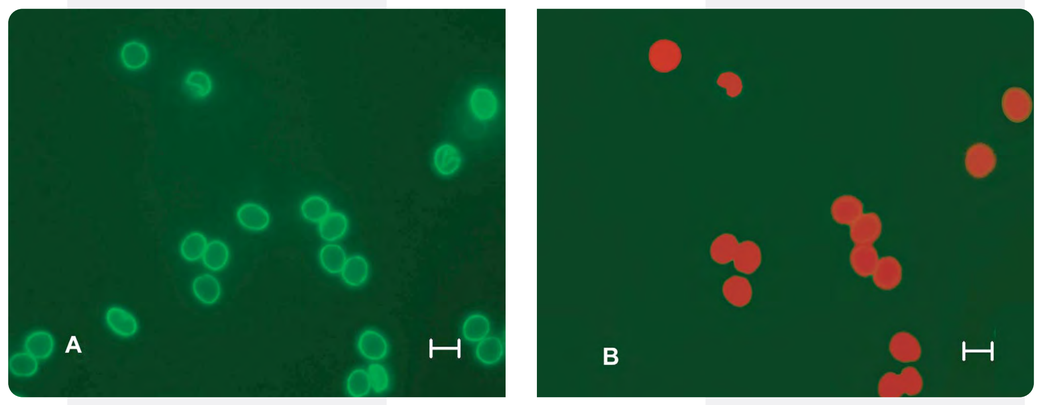
- Determine recovery of spiked oocysts in reagent and raw waters
- Two spiking options evaluated

Live oocysts

- Spiked and natural oocysts appear the same
- Requires analysis of 2 samples
  - 1 spiked sample
  - 1 reference sample

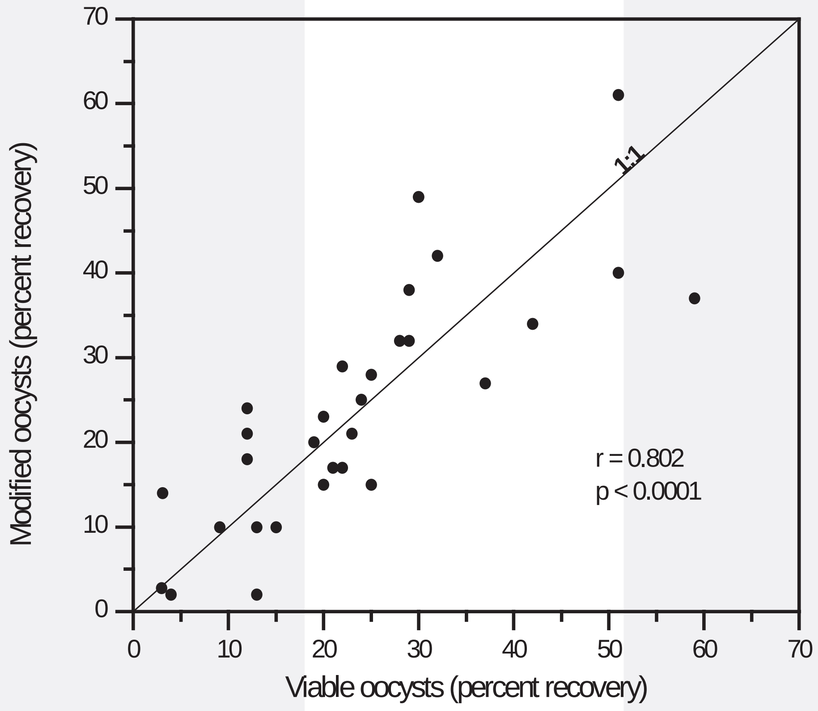
ColorSeed

- Gamma irradiated oocysts
- Permanently stained with Texas Red
- Spiked and natural oocysts look different
- Requires analysis of only 1 sample



Same field images of mixed live and ColorSeed oocysts when viewed by green (A) and red (B) filters

Results



Summary and Conclusions

- No statistical difference in recovery between live and ColorSeed oocysts was observed in reagent or stream waters.
- ColorSeed is an appropriate internal standard for Method 1623.
- The use of ColorSeed allows spiking studies to be in only one sample, reducing the costs by $400 per control.

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