# Validation of novel DNA isolation procedures from limited numbers of *Cyclospora* oocysts


## Summary

*Cyclospora cayetanensis* is a highly infectious foodborne parasite that causes gastrointestinal illness in humans and is typically acquired through consumption of contaminated water, produce, or berries. *Cyclospora* infections are characterized by sporadic shedding of oocysts in feces, which, combined with the need to ingest only a few oocysts for full infection, make diagnostics difficult. Since <10 oocysts suffice to cause infection, detection methods must be sensitive and robust. We subdivide this problem into two key aims: 1) developing a simple, efficient method for DNA isolation enabling downstream molecular diagnostics, and 2) concentrating oocysts from produce washes. Success would improve the ability to collect, concentrate, and isolate DNA from *Cyclospora*. The aim is to develop these methods for deployment in production and processing facilities, and to obviate the need for specialized instrumentation or costly reagents. The primary goal of this proposal is to develop rapid methods for DNA isolation from limited numbers of *Cyclospora* oocysts to support surveillance efforts.


## Objectives

1. Evaluate >10 methods for efficient DNA extraction from *Cyclospora* oocysts. [focus of this poster]
2. Validate methods for concentrating oocysts from bulk water samples, as would be used in produce washes or wastewater analysis.

## Methods

*Eimeria acervulina* oocysts were obtained from Dr. Mark Jenkins, USDA, and exposed to various chemical compounds targeting oocyst wall moieties: lipids, carbohydrates, and proteins. Oocysts were washed to remove dichromate, following which they were added to 500µL of test compound on an Epoch Bioscience DNA column. To remove residual test compound before PCR amplification, the suspension was spun through the filter and a brief column cleanup using Qiagen buffers AW1 and AW2 was performed. Isolations were analyzed using the TapeStation bioanalyzer to determine quality and quantity of nucleic acid isolated. PCR amplification was performed using primers against the β-tubulin locus. Resulting amplicons or fluorescence curves were compared to amplification performed following isolation using the UNEX method.

## Results to Date

A workflow for chemical nucleic acid isolation from the surrogate *Eimeria acervulina* was established (**Figure 1**). Selected compounds were applied to *E. acervulina* oocysts over a range of concentrations and contact times. DNA concentrations were compared to the UNEX method of isolation (**Figure 2**). PCR amplification of the β-tubulin gene, used as a readout for DNA isolation efficiency, was performed using conventional and realtime PCR. TapeStation bioanalyzer results for all isolations indicate low yields, but this does not hinder PCR (example, **Figure 3**). Following PCR amplification, singlet reactants that facilitated β-tubulin amplification include sodium periodate (PI), Qiagen buffer P2 (SDS and NaOH), lactic acid, ethyenediamine, KOH, and NaOH (**Figure 3**). Combinatorial testing is underway using the Opentrons 2 robotic liquid handling platform.

## Benefits to the Industry

By exploiting novel chemistries to disrupt oocyst walls, this work seeks to provide alternative methods for coccidian detection and diagnosis that reduce hands-on time, reagent and equipment costs, or both. An ideal scenario consists of a single cocktail that lyses oocysts, but which does not damage nucleic acids, combined with a rapid on-site diagnostic.

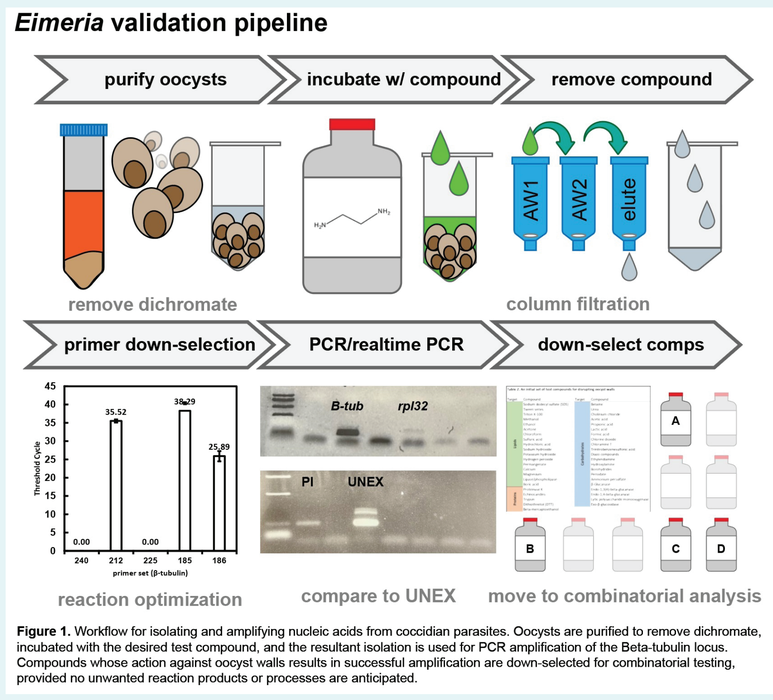


**Figure 1.** Workflow for isolating and amplifying nucleic acids from coccidian parasites. Oocysts are purified to remove dichromate, incubated with the desired test compound, and the resultant isolation is used for PCR amplification of the Beta-tubulin locus. Compounds whose action against oocyst walls results in successful amplification are down-selected for combinatorial testing, provided no unwanted reaction products or processes are anticipated.

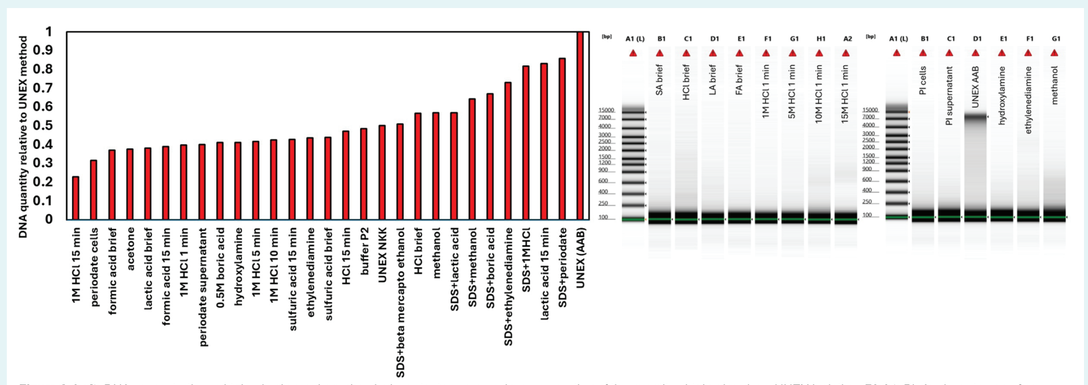


**Figure 2. Left:** DNA concentrations obtained using various chemical reactants expressed as a proportion of the quantity obtained under a UNEX isolation. **Right:** BioAnalyzer traces of some representative isolations using the indicated compounds. Despite low concentrations, PCR amplification was successful after treatment with several of the above compounds.

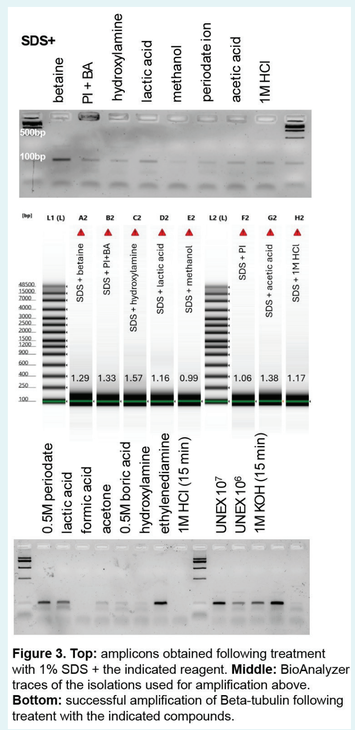


**Figure 3. Top:** amplicons obtained following treatment with 1% SDS + the indicated reagent. **Middle:** BioAnalyzer traces of the isolations used for amplification above. **Bottom:** successful amplification of Beta-tubulin following treatent with the indicated compounds.


### Contact

Aaron A. Baumann, PhD (Co-PI)
University of Tennessee
Center for Agricultural Synthetic Biology (CASB)
abaumann@utk.edu

### Authors

Aaron A. Baumann (Co-PI), Lezlee Dice, Mark Jenkins, Benjamin Rosenthal (Co-PI), Scott C. Lenaghan (PI)



### Project funding dates

January 1, 2025 – December 31, 2025

### Acknowledgements

We are grateful to the following for intellectual or financial contributions to this work: Lorraine Fuller, Dr. Christy Yeager, Dr. Richard Gerhold, Dr. John Schaefer, and the Center for Produce Safety.