Simultaneous Detection of *Entamoeba histolytica*, *Cryptosporidium parvum* and *Giardia lamblia* in fecal samples using a single enzyme immunoassay

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Abstract

Introduction: Diarrheal infections account for 21% of all deaths of children under the age of 5 worldwide despite large public health efforts for the improvement of sanitation, clean water supplies and introduction of oral rehydration solution. The traditional screening test, the microscopic ova and parasite (O&P) exam of stool, suffers from poor sensitivity and specificity, and requires expertly trained personnel to interpret results. Improved diagnostic methods specific for enteric pathogens that could be easily applied in resource poor countries would be valuable in the management diarrheal illnesses. The detection of parasite antigen in stool by enzyme-linked immunoassay (ELISA) is the current diagnostic method of choice. However, real-time PCR tests (RT-PCR) for the enteric parasites are not yet practical or cost effective as screening assays. In this study, a prototype of a fecal ELISA screening test designed to simultaneously detect *Entamoeba histolytica, Cryptosporidium spp, and Giardia sp.* was field tested against the gold standard of individual ELISA to evaluate the feasibility of a single ELISA screening test having comparable or greater sensitivity in the detection of these enteric parasites.

Methods: Stool specimens were obtained from a cohort of children and adults from Dhaka, Bangladesh where *E. histolytica* is endemic. The Tri-Combo ELISA provided by TechLab, Inc (Blacksburg, VA) is a conventional two-step ELISA format with HRP-conjugated detecting antibodies for colorimetric development was designed to simultaneously screen stool specimens for *Giardia lamblia*, *Cryptosporidium* spp. and *E. histolytica* using a single assay well. For those samples with discrepancy results between the Tri-Combo versus individual specific antigen test, RT-PCR analysis was conducted.

Results: Based upon 235 stool specimens tested, the Tri-Combo ELISA has 99%. Sensitivity and 96% Specificity, Positive Predictive Value 95% and Negative Predictive Value 99.2%. The Tri-Combo ELISA has a 97% correlation to individual parasite ELISA and RT-PCR.

Conclusion: This preliminary data of the Tri-Combo ELISA for simultaneous detection of *Giardia sp.*, *Cryptosporidium* spp. and *E. histolytica* reveals similar test characterestics as the currently FDA approved individual parasite ELISAs. This diagnostic method represents a potential cost savings tool in the detection of enteric parasite infections.

Introduction

- A fecal screening test is needed to detect the three most common enteric protozoan parasites in the US.
- The enteric protozoa *Giardia lamblia, Cryptosporidium* spp., and *Entamoeba histolytica* share the characteristic of food and water-borne transmission, a low infectious dose (<100 organisms), and environmental stability.
  - *E. histolytica* infections may be lethal,
  - *G. lamblia* is most common
  - *Cryptosporidium* spp. is an important cause of chronic diarrhea without effective treatment in AIDS patients.
- Traditional screening test, the microscopic ova and parasite (O&P) exam of stool, suffers from poor sensitivity and specificity and requires expertly trained personnel to conduct and interpret results.
- Detection of parasite antigen in stool by enzyme immunoassay (ELISA) is the current diagnostic method of choice, but it requires the performance of three separate ELISAs for the individual parasites (1)
- Real-time PCR tests (RT-PCR) for the enteric parasites are not yet practical or cost effective as screening assays.(2,3)
- The Tri-Combo ELISA will allow for the rapid screening of patients' stool samples for the 3 most common intestinal parasites in the United States and can be applied internationally where these enteric infections are more prevalent.
- The purpose of this study is to conduct a clinical evaluation of the Tri-Combo ELISA performance characteristics in an endemic setting.

Materials & Methods

- Fecal samples were collected from 2 study sites in Dhaka, Bangladesh. 234 samples used to test the prototype Tri-Combo ELISA test.
- One hundred forty fecal samples (60 %) represent acute diarrheal illness.
- The Tri-Combo parasite ELISA test is a conventional two-step ELISA format with HRP-conjugated detecting antibodies for colorimetric development specifically designed to simultaneously detect *Entamoeba histolytica, Giardia lamblia,* and *Cryptosporidium parvum* specific antigens in unpreserved human fecal specimens.
- Each well of the 96-well ELISA plate is coated with antibodies specific for the three parasites and the conjugate contains antibodies that are specific for all three parasites. A positive result indicates the presence of one or more of the three parasites in the specimen.
- Each fecal sample was also tested on FDA approved individual ELISA assay for *G. Lamblia* , *E. histolytica*, and *Cryptosporidium* for comparison to the Tri-Combo assay.
- Fecal samples with discrepant results between Tri-Combo and Individual ELISA testing underwent RT- PCR Testing.

Results

- TABLE 1 outlines the comparison data following ELISA discrepant resolution as determined by RT-PCR analysis.
- In total, 14 specimens (6.0%) were found to be discrepant between the Tri-Combo ELISA and the individual ELISAs.
  - RT-PCR analysis found 3 specimens to be negative that were identified as negative by the Tri-Combo ELISA, but positive by the individual ELISAs.
  - RT-PCR confirmed 4 specimens to be positive that were identified as positive by the Tri-Combo ELISA, but negative by the individual ELISAs.
  - The 7 remaining discrepant specimens showed agreement between the individual ELISAs and RT-PCR, but not the Tri-Combo ELISA (as represented by the final analysis in TABLE 1).
- Of the 97 parasite-positive specimens utilized for the study, 59 were *Giardia lamblia* positive, 25 were *Cryptosporidium* spp. positive and 13 were *E. histolytica* positive.
- The sensitivity of the Tri-Combo ELISA for each parasite was 100%, 100%, and 92.3%, respectively.
- The Tri-Combo ELISA demonstrates a 97% correlation to current individual ELISA assay for *G.lamblia, E.histolytica,* and *Cryptosporidium spp.*

TriCombo ELISA results	Individual ELISA and RT-PCR Results		
	Positive	Negative	Total
Positive	95*	5	100
Negative	1	133	134
Total	96	138	234

1 stool specimen was excluded secondary to insufficient quantity for further testing

Table 1: Clinical evaluation of the Tri-Combo ELISA's ability to detect *Giardia*, *Cryptosporidium* and *E. histolytica* in fecal samples

	Result	95% Confidence Interval
Sensitivity	99.0%	93.6% - 99.9%
Specificity	96.4%	90.4% - 98.2%
Positive Predictive Value	95.0%	87.1% - 97.6%
Negative Predictive Value	99.2%	95.3% - 99.9%
Correlation	97.0%	96.2% - 97.6%

Table 2: Summary of Data from Tri-Combo ELISA Stool Antigen Test ing

Conclusion

- This preliminary data of the Tri-Combo ELISA for simultaneous detection of *Giardia sp.*, *Cryptosporidium* spp. and *E. histolytica* reveals similar test characterestics as the currently FDA approved individual parasite ELISA.
- This in the field testing reveals that the Tri-Combo multiplex ELISA can represents a potential cost savings tool in the detection of enteric parasite infections for large-scale specimen screening.

Literature Cited

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