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 immunosorbent assay (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 specifically 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. 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*, Cryptosporidium spp. and *Giardia lamblia*.
- 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 ELISA and Individual ELISA testing underwent RT-PCR Testing.

### Results

- **TABLE 1** outlines the comparison data following ELISA and 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.
- Cryptosporidium 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 2**).

<table>
<thead>
<tr>
<th>Tri-Combo ELISA results</th>
<th>Individual ELISA and RT-PCR Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>95*</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
</tr>
</tbody>
</table>

1-Test result was invalid secondary to insufficient quality for testing

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

### Conclusion

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

### Literature Cited


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