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Multisite Performance Evaluation of an Enzyme-Linked Immunosorbent Assay for Detection of *Giardia*, *Cryptosporidium*, and *Entamoeba histolytica* Antigens in Human Stool

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A novel fecal antigen detection assay for fresh and frozen human samples that detects but does not differentiate *Giardia* spp, *Cryptosporidium* spp, and *Entamoeba histolytica*, the Tri-Combo parasite screen, was compared to three established enzyme-linked immunosorbent assays (ELISAs) at three international sites. It exhibited 97.9% sensitivity and 97.0% specificity, with positive and negative predictive values of 93.4% and 99.1%, respectively. The Tri-Combo test proved a reliable means to limit the use of individual parasite ELISAs to positive samples.

While death from diarrheal infections has decreased, levels of morbidity have not declined in comparison to historical levels and thus remain a significant health problem, especially in the developing world (7, 11). Three of the most common causes of protozoan-associated diarrheal infections are *Giardia* spp., *Cryptosporidium* spp., and *Entamoeba histolytica* (16). All three of these parasites are transmitted via a classical fecal-oral cycle (1). *Cryptosporidium* spp. are common throughout both the developed and developing world and can cause persistent diarrhea in HIV-infected individuals (2, 12, 19). Control of this organism can prove difficult due to its resistance to standard disinfection methods (e.g., chlorination of water sources) (3). *E. histolytica* is a single-cell ameba that is the cause of amebiasis (20). Clinical manifestations include diarrhea, dysentery, toxic megacolon, and liver abscess (5). The incidence of disease due to *Cryptosporidium* spp. and *E. histolytica*, and possibly *Giardia* spp., is increased with malnutrition (13, 14). Repeated infections are common and can cause developmental delay in children (6, 15). Because treatment regimens for these infections are available, there is a need for rapid and cost-effective diagnostic screening methods (8, 10, 16, 17).

The Tri-Combo parasite screen test (TechLab, Blacksburg, VA), intended for clearance by the U.S. Food and Drug Administration (FDA), is an enzyme-linked immunosorbent assay developed to simultaneously detect *Giardia* spp., *Cryptosporidium* spp., and *E. histolytica* antigens in human fecal specimens. The microtiter plate format allows the rapid screening of large numbers of clinical specimens. Similar to other immunoassay-type tests for these three parasites, the Tri-Combo test is not designed for use with fixed fecal specimens. When a clinical sample is positive by the Tri-Combo test, additional testing for the presence of *Giardia* spp., *Cryptosporidium* spp., and/or *E. histolytica* parasites is indicated, as the test does not distinguish between these parasites.

A panel of 618 diarrheal and nondiarrheal human fecal specimens from male and female subjects aged 4 months to 89 years were tested with the Tri-Combo test at three international sites: 87 clinical samples at the National Institute for Infectious Disease (NIID) in Tokyo, Japan; 297 clinical samples at the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B), in Dhaka, Bangladesh; and 234 clinical samples at the Bernard Nocht Institute for Tropical Medicine (BNI) in Hamburg, Germany. All samples were tested with the FDA-approved individual Giardia II, Cryptosporidium II, and E. Histolytica II ELISAs (TechLab, Blacksburg, VA) as reference standards for the detection of each parasite. Samples for which the results differed between the Tri-Combo and the reference tests were reanalyzed with all tests, and if the final results still differed, the sample was recorded as a discrepant sample. Clinical samples that were positive in both the Tri-Combo and the corresponding reference standard test were scored as positive for parasite antigen. Samples discrepantly positive and negative on the Tri-Combo test versus the reference tests were considered false positives and false negatives, respectively.

The Tri-Combo parasite screen was performed following the manufacturer's directions. All incubations were performed on the benchtop, and the results were analyzed on an ELISA-format spectrophotometer with the spectrophotometer absorbance set to read at 450 nm. The individual Giardia II, Cryptosporidium II, and E. Histolytica II tests were performed according to established protocols.

For the combined panel of samples tested at all three sites, the Tri-Combo test had a sensitivity of 97.9%, specificity of 97.0%, positive predictive value of 93.4%, and negative predictive value of 99.1% (Table 1). One hundred ninety-six samples were positive in the Tri-Combo test. According to the reference tests, the distribution of positive samples by organism was similar at the various sites; most samples were positive for *Giardia* spp. At the Tokyo site, 8 samples were *Giardia* positive, 4 were *Cryptosporidium* positive, and 3 were *E. histolytica* positive. At the Dhaka site, 68 sam-

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TABLE 1 Performance of the Tri-Combo parasite screen by study site[a]

Study site	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	No. of positive samples	No. of negative samples
NIID (Tokyo, Japan)	92.9	100	100	98.7	13	74
ICDDR,B (Dhaka, Bangladesh)	100	94.25	92.48	100	133	164
BNI (Hamburg, Germany)	94	98.4	94	98.4	50	184
Combined panel (all sites)	97.9	97.0	93.4	99.1	196	422

[a] Percentages are weighted for sample number per site. PPV, positive predictive value; NPV, negative predictive value.

ples were *Giardia* positive, 31 samples were *Cryptosporidium* positive, and 34 samples were *E. histolytica* positive. Finally, 35 samples were *Giardia* positive, 13 samples were *Cryptosporidium* positive, and 8 samples were *E. histolytica* positive at the Hamburg, Germany site. Of the four samples found to be false negatives in the Tri-Combo test, three were positive in the E. Histolytica II test and one in the Cryptosporidium II test. These results are similar to the performance of other available fecal-antigen-based assays for these protozoa. The reason for the discrepant results was unknown; the antigen detection levels between the Tri-Combo test and the reference tests do not differ significantly, and the few discrepant results we observed displayed a relatively even distribution between false positives and false negatives.

The Tri-Combo parasite screen test fills a niche in the diagnostic testing of diarrheal illness caused by protozoan infections, as there is currently no other test capable of specifically detecting in a single well *Giardia* spp., *Cryptosporidium* spp., and *E. histolytica* antigens in human clinical stool samples. There is one other rapid multiplex diagnostic test for these three parasites (the Triage parasite panel; Biosite, San Diego, CA), but unlike the Tri-Combo assay, it lacks specificity for *E. histolytica*, not being able to distinguish between this pathogen and the closely related human commensal *Entamoeba dispar* (4). A potential advantage of the Tri-Combo test over multiplex PCR-based tests for these parasites is that extensive training, equipment, and reagents needed for PCR are not required (9, 18). The Tri-Combo test demonstrated excellent positive predictive and negative predictive values when tested with a pool of clinical samples from three international sites. The format of the Tri-Combo test could be well suited to the testing of large numbers of clinical samples, as minimal laboratory expertise and reagents are necessary to run many samples simultaneously. Only samples that are positive would require further testing, and the subsequent tests could be similar ELISAs that are specific for the individual parasites (such as those used as the reference tests in this study). In conclusion, the Tri-Combo parasite screen test was demonstrated to be a useful tool in the detection of *Giardia spp.*, *Cryptosporidium spp.*, and *E. histolytica* antigens in human clinical stool samples.

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REFERENCES

1. **Adam RD.** 2001. Biology of *Giardia lamblia*. Clin. Microbiol. Rev. **14**: 447–475.
2. **Brantley RK, et al.** 2003. AIDS-associated diarrhea and wasting in Northeast Brazil is associated with subtherapeutic plasma levels of antiretroviral medications and with both bovine and human subtypes of *Cryptosporidium parvum*. Braz. J. Infect. Dis. **7**:16–22.
3. **Dillingham RA, Lima AA, Guerrant RL.** 2002. Cryptosporidiosis: epidemiology and impact. Microbes Infect. **4**:1059–1066.
4. **Garcia LS, Shimizu RY, Bernard CN.** 2000. Detection of *Giardia lamblia*, *Entamoeba histolytica*/*Entamoeba dispar*, and *Cryptosporidium parvum* antigens in human fecal specimens using the Triage parasite panel enzyme immunoassay. J. Clin. Microbiol. **38**:3337–3340.
5. **Haque R, Huston CD, Hughes M, Houpt E, Petri WA.** 2003. Amebiasis. N. Engl. J. Med. **348**:1565–1573.
6. **Haque R, et al.** 2006. *Entamoeba histolytica* infection in children and protection from subsequent amebiasis. Infect. Immun. **74**:904–909.
7. **Haque R, et al.** 2009. Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh. Clin. Infect. Dis. **48**:1191–1197.
8. **Haque R, et al.** 2003. Epidemiologic and clinical characteristics of acute diarrhea with emphasis on *Entamoeba histolytica* infections in preschool children in an urban slum of Dhaka, Bangladesh. Am. J. Trop. Med. Hyg. **69**:398–405.
9. **Haque R, et al.** 2007. Multiplex real-time PCR assay for detection of *Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium* spp. Am. J. Trop. Med. Hyg. **76**:713–717.
10. **Houpt ER, Guerrant RL.** 2008. Technology in global health: the need for essential diagnostics. Lancet **372**:873–874.
11. **Kosek M, Bern C, Guerrant RL.** 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull. World Health Organ. **81**:197–204.
12. **MacKenzie WR, et al.** 1994. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. N. Engl. J. Med. **331**:161–167.
13. **Mondal D, Haque R, Sack RB, Kirkpatrick BD, Petri WA.** 2009. Attribution of malnutrition to cause-specific diarrheal illness: evidence from a prospective study of preschool children in Mirpur, Dhaka, Bangladesh. Am. J. Trop. Med. Hyg. **80**:824–826.
14. **Opintan JA, et al.** 2010. Pediatric diarrhea in southern Ghana: etiology and association with intestinal inflammation and malnutrition. Am. J. Trop. Med. Hyg. **83**:936–943.
15. **Petri WA, Jr., et al.** 2008. Enteric infections, diarrhea, and their impact on function and development. J. Clin. Invest. **118**:1277–1290.
16. **Pierce KK, Kirkpatrick BD.** 2009. Update on human infections caused by intestinal protozoa. Curr. Opin. Gastroenterol. **25**:12–17.
17. **Ricci KA, et al.** 2006. Reducing stunting among children: the potential contribution of diagnostics. Nature **444**(Suppl. 1):29–38.
18. **Taniuchi M, et al.** 2011. High throughput multiplex PCR and probe-based detection with Luminex beads for seven intestinal parasites. Am. J. Trop. Med. Hyg. **84**:332–337.
19. **Tzipori S, Ward H.** 2002. Cryptosporidiosis: biology, pathogenesis and disease. Microbes Infect. **4**:1047–1058.
20. **World Health Organization.** 1997. WHO/PAHO/UNESCO report. A consultation with experts on amoebiasis. Mexico City, Mexico 28–29 January, 1997. Epidemiol. Bull. **18**:13–14.