ASSESSMENT OF A NEW PARASITOLOGY SCREENING DIAGNOSTIC ELISA FOR THE DETECTION OF ANTIGENS OF GIARDIA SPP., CRYPTOSPORIDIUM SPP. AND ENTAMOEBA HISTOLYTICA IN FECAL SPECIMENS

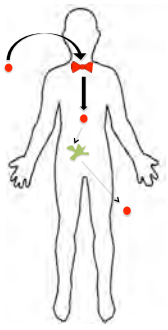
Nathaniel C. Christy[1], Janice D. Hencke[2], William A. Petri, Jr.[1], Aleyla D. Escueta[3], Forida Nazib[4], Heidrun v.Thien[5], Rashidul Haque[4], Tomoyoshi Nozaki[3], Egbert Tannich[5], Joel F. Herbein[2]

[1]University of Virginia, Charlottesville, VA, United States, [2]TechLab®, Inc, Blacksburg, VA, United States, [3]National Institutes of Infectious Disease, Tokyo, Japan, [4]International Center for Diarrheal Disease Research, Bangladesh, Dhaka, Bangladesh, [5]Bernard Nocht Institute for Tropical Medicine, Hamburg, Germany

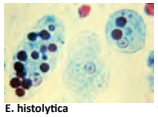
Introduction:

Episodes of diarrhea are common worldwide and 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. The three most common causes of protozoan-associated diarrheal infections are *Giardia* spp, *Cryptosporidium* spp, and *Entamoeba histolytica*. *Giardia* is a bi-nucleated parasite with a bi-phasic life cycle that causes infection following the ingestion of cysts. The parasite multiplies in the host as a flagellated trophozoite. Encystation of infectious cysts perpetuates the cycle of infection after exiting the host. *Cryptosporidium* spp. are common throughout both the developed and developing world and cause persistent diarrhea in HIV-infected populations. Infection occurs after exposure to oocysts, and control of this organism can prove difficult due to its resistance to standard disinfection methods (e.g. chlorinated water sources). *E. histolytica* is a single-cell ameba that is the cause of amebiasis. As with *Giardia*, infection occurs after ingestion of a multi-nucleated cyst and the emergence of disease-causing trophozoites that multiply in the intestine. Potentially fatal clinical manifestations include dysentery, toxic megacolon, and perforation of the intestine while some invasive cases progress to the development of deadly liver abscesses. Disease incidence caused by *Cryptosporidium* spp and *E. histolytica*, and possibly *Giardia* spp, is associated with malnutrition. Repeated infections are common and can cause developmental delay in small children. While treatment regimens for these infections are available, the surveillance for and prompt diagnosis of diarrheal illness in a population is critical for both prevention and treatment of disease. There remains a need for rapid and cost-effective diagnostic screening methods.

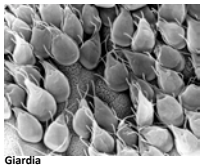
Background:



- Classic Fecal-Oral cycle of infection
- Giardiasis, Amebiasis, and Cryptosporidiosis are endemic in many third world countries and are common causes of enteric parasitic infection in the developed world.
- Repeated infections can cause malnutrition and developmental issues in children. (Mondal et al, 2009)(Petri et al, 2008)
- Inexpensive and easy-to-use diagnostic tools are needed to effectively screen for these parasites before appropriate treatment regimens can be applied.


E. histolytica


Cryptosporidium


Giardia

Study Focus and Goals:

- Evaluate the performance of a newly developed ELISA-type screen (The *Tri-Combo* parasite screen, TechLab®, Inc.) for the presence of *Giardia* spp., *Cryptosporidium* spp., and/or *E. histolytica* antigens in human stool samples under clinical laboratory conditions.
- The *Tri-Combo* parasite screen was compared to established ELISA-type tests on the market for the individual detection of *Giardia* spp., *Cryptosporidium* spp., or *E. histolytica* antigen in human stool samples

Methods:

- A total of **618** clinical samples at three international sites:

International Center for Diarrheal Disease Research Dhaka, Bangladesh:	National Institutes for Infectious Disease Tokyo, Japan:	Bernard Nocht Institute for Tropical Medicine Hamburg, Germany:
297	87	234

- All samples were run on the *Tri-Combo* test and the *Giardia II*, *Cryptosporidium II*, and *E. histolytica II* ELISA tests (TechLab®).
- Discrepant samples were re-run on the *Tri-Combo* and individual tests.

Results(1):

Comparison of the *TRI-COMBO* parasite screen by study site:

Study Site:	No. of Specimens with result			
	True Positive	False Positive	True Negative	False Negative
NIID: Tokyo, Japan	13	0	73	1
ICDDR,B: Dhaka, Bangladesh	123	10	164	0
BNI: Hamburg, Germany	47	3	181	3
Combined Panel: All Sites	**183**	**13**	**418**	**4**

Results(2):

Comparison of the *TRI-COMBO* parasite screen by study site:

Study Site:	% of Specimens with result			
	Sensitivity(%)	Specificity(%)	Positive Predictive Value(%)	Negative Predictive Value(%)
NIID: Tokyo, Japan	92.9	100	100	98.7
ICDDR,B: Dhaka, Bangladesh	100	94.25	92.48	100
BNI: Hamburg, Germany	94	98.4	94	98.4
Combined Panel: All Sites	**97.9**	**97.0**	**93.4**	**99.1**

Results(3):

Comparison of the *TRI-COMBO* parasite screen by organism:

Reference ELISA Test:	No. of Specimens with result			
	True Positive	False Positive	True Negative	False Negative
Giardia	96	13[1]	418	0
Cryptosporidium	47	13[1]	418	1
E. histolytica	42	13[1]	418	3

[1]False positive results from the entire panel of samples (a total of 13) were recorded as FP for all 3 parasites.

Conclusions:

- The Tri-Combo parasite screen effectively detected antigens from *Giardia* spp., *Cryptosporidium* spp., and *E. histolytica* parasites in clinical stool samples at three international sites as compared to three diagnostic tests specific for each individual organism.

References and Acknowledgments:

1. **Pierce, K. K., and B. D. Kirkpatrick**. 2009. Update on human infections caused by intestinal protozoa. Curr. Opin. Gastroenterol **25**:12-17.
2. **Mondal, D., R. Haque, R. B. Sack, B. D. Kirkpatrick, and W. A. Petri Jr**. 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.
3. **Petri, W. A., Jr, M. Miller, H. J. Binder, M. M. Levine, R. Dillingham, and R. L. Guerrant**. 2008. Enteric infections, diarrhea, and their impact on function and development. J. Clin. Invest **118**:1277-1290.
4. **Tzipori, S., and H. Ward**. 2002. Cryptosporidiosis: biology, pathogenesis and disease. Microbes and Infection **4**:1047-1058.
5. **Carranza, P. G., and H. D. Lujan**. 2010. New insights regarding the biology of *Giardia lamblia*. Microbes and Infection **12**:71-80.
6. **WHO/PAHO/UNESCO** report. A consultation with experts on amoebiasis. Mexico City, Mexico 28-29 January, 1997. Epidemiol Bull **18**:13-14.

This work was supported by NIH grant AI43596 to W.A.P. and NIH (NIAID) grant R42 AI77171 to J.H.
We thank TechLab®, Inc. for the contribution of the *TRI-COMBO*, *GIARDIA II*, *CRYPTOSPORIDIUM II*, and *E. HISTOLYTICA II* stool antigen detection tests.
WP receives royalties from a licensing agreement for amebiasis diagnostics with TechLab, Inc. Those royalties are donated in their entirety to the American Society for Tropical Medicine and Hygiene without benefit to Dr. Petri.