

Development of a Multiplex PCR Diagnostic Test for Diarrheal Parasites Commonly Found in Jordan

Hiba Jamil, Saira Huq and Steven Williams

Department of Biology, Smith College, Northampton, MA



Background

- The World Health Organization reports that diarrhea causes 1.5 million child deaths per year.
- 13% of Jordanian children under age 5 who died in 2004 were killed by diarrhea (WHO).
- Microscopy is the common method for parasite diagnostics in developing countries, but this technique is not sensitive enough to detect all parasites in a stool sample nor sufficient in distinguishing between different parasites.



Giardia lamblia



- E. histolytica* is invasive and causes Amoebiasis, a disease leading to liver abscesses and dysentery.



- E. dispar* is morphologically identical to *E. histolytica* but does not produce harmful effects (Figure 1).

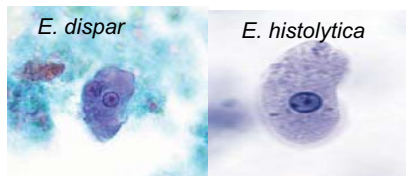


Figure 1: Identical *Entamoeba* trophozoites

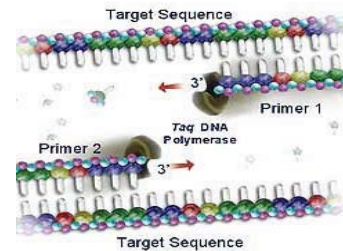
Methods

- Primers were designed to anneal at ribosomal subunits. These DNA regions are variable from species to species and sufficiently conserved within a species to allow for amplification specificity.

- MP Primer Computer Program was used for multiplex primer design.

- Each parasite's specific primers were tested individually on *Giardia* and *Cryptosporidium* DNA.

- Gel electrophoresis was used to analyze the PCR product.



Results

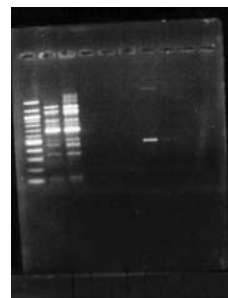
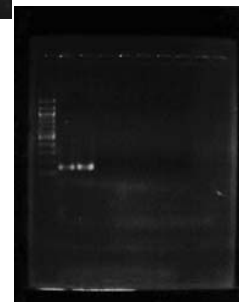


Figure 2: *Giardia* DNA amplified by *Giardia* primers (lane 3 & 4) and *E. histolytica* primers (lane 7). (100 BP ladder lane 1)

Figure 3: *Cryptosporidium* DNA amplified by *Cryptosporidium* primers only (lane 3 & 4). (100 BP ladder lane 1)



Future Experimentation



- PCR with varied combinations of DNA template, primers and adjusted PCR parameters.

•If awarded the Fulbright Fellowship, Hiba Jamil will implement the multiplex PCR test at the Princess Haya Biotechnology Center at the Jordan University of Science and Technology in Irbid, Jordan in September 2011.

- She will be running the assay on samples from immunocompromised patients at the King Hussein Cancer Center in Amman, Jordan.



Acknowledgements: We would like to thank Steven Williams, Nawal Hijawi, Mustafa Saad and Donald Andrew.

Purpose

- We aim to develop a single multiplex PCR test that will distinguish between the genera of *Cryptosporidium* and *Giardia* and between the species of *Entamoeba histolytica* and *E. dispar*.

Parasitic Diseases

- Cryptosporidium* and *Giardia* parasites cause the severe diarrheal diseases Cryptosporidiosis and Giardiasis, respectively.

Cryptosporidium hominis

