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Validation Of A Multiplex Real-time PCR Gastrointestinal Parasites Panel

Author Block: K. Marks-Beaubrun[1], J. Kwan[1], R. Lau[2], F. Ralevski[2], A. Wang[2], R. Cudiamat[2], E. Chen[2], K. Orejana[2], A. K. Boggild[1]; [1]Tropical Disease Unit, Toronto Gen. Hosp., Toronto, ON, Canada, [2]Publ. Hlth.Ontario, Toronto, ON, Canada

Abstract:

Microscopy is the conventional method for identification of gastrointestinal parasitic pathogens in fecal samples, however, it presents numerous challenges including high technical expertise and prolonged turnaround time. Molecular methods provide higher throughput and potentially higher sensitivity and specificity. We sought to validate a commercial multiplex parasitic real time PCR panel detecting 6 protozoal pathogens: *Blastocystis hominis (Bh)*, *Cryptosporidium*, *Cyclospora*, *Dientamoeba fragilis (Df)*, *Entamoeba histolytica (Eh)* and *Giardia lamblia (Gl)* in unpreserved fecal specimens submitted for diagnostic parasitology. We analyzed 192 specimens, including 84 banked, frozen known positive specimens containing all of the targeted pathogens (8 *Bh*, 13 *Crytosporidium*, 13 *Cyclospora*, 10 *Df*, 15 *Eh*, 13 *Gl* and 12 mixed protozoal infections) and 108 fresh specimens randomly selected from our prospective parasitology submissions, including 4 *Bh*, 3 *Df*, 2 mixed infections, and 99 microscopy negatives. DNA extraction and PCR were setup with the Hamilton Starlet automated platform and Seegene's extraction and PCR kits. Microscopy was the reference standard for all organisms with stool ELISA as an additional reference assay for *Eh*. Sensitivity, specificity, positive predictive and negative predictive values of the enteric multiplex were: 96%, 90%, 60%, and 99% for *Bh*; 100% for *Cryptosporidium*; 79%, 100%, 100%, and 98% for *Cyclospora*; 86%, 86%, 86%, and 98% for Df; 81%, 100%, 100%, and 98% for Eh; and, finally, 94%, 85%, 85% and 99% for *Gl*, respectively. The platform had high sensitivity for *Bh*, *Cryptosporidium* and *Gl*, but suboptimal sensitivity for detection of *Cyclospora, Df*, and *Eh*. Low positive predictive value for *Bh* may reflect challenges to accurate microscopic identification of this organism. Negative predictive value was excellent for all targets, supporting that the platform accurately determines true negatives. Limit of detection was as follows: 8 parasites/g stool for *Bh*; 9 parasites/g stool for *Cryptosporidium*; 38 parasites/g stool for *Cyclospora*; 697 parasites/g stool for *Df;* 47 parasites/g stool for *Eh;* and 22 parasites/g stool for *Gl*. This particular enteric multiplex platform provides a useful diagnostic tool for *Bh*, *Cryptosporidium,* and *Gl*. Further optimization of the assay is required for *Cyclospora*, *Df,* and *Eh* prior to clinical use.