Validation Of A Multiplex Real-time PCR Gastrointestinal Parasites Panel

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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 Cryptosporidium, 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.