Low parasite density in human stools is a common hindrance for the parasitological diagnosis of intestinal parasite infections. Commercially available Bailenger-type stool concentration assays are presently not entirely documented, especially their efficacies. The aim of this study was to compare the performance of an commercial concentration kits with those of an optimized, home-made reference assay for Bailenger-type concentrations of clinical stool samples infected with one of 9 helminth and 6 protozoa parasites.

Material and methods

1. stool samples

Ninety-six stools from different patients in which presence of one parasite was previously established using Bailenger-type home-made concentrations as well as other techniques ( flotation, centrifugation, plaque count microscopy) were studied. In 44 stools, parasite consisted of helminth larvae and/or eggs including Pneumocystis jiroveci, Entamoeba moshkovskii var. ameboides, Ascaris lumbricus, Dipylidium caninum, Enterobius vermicularis, Diphyllobothrium latum, Strongyloides stercoralis, and Toxocara canis. Three samples were submitted to microscopic examination (X 71, 7.5, 9, 4, 5, and 6 stool), respectively, of which 10 were found parasite-positive by direct examination i.e. in the absence of concentration. In 52 stools, one prozoan parasite was detected, i.e. Chlostridium novyi, Entamoeba hystolytica, E. histolytica, Blastocystis hominis, Giardia lamblia, and Entamoeba hartmanni. Protozoa were identified in 9, 7, 1, 1, 11, 4, and 2 stool, respectively, of which 33 were found parasite-positive by direct examination i.e. in the absence of concentration.

2. Bailenger-type concentration procedures

Three commercial stool Bailenger-type parasite concentration kits were used according to manufacturer's instructions:

- Easy Pass Bailenger (Ethico, Los Ilia, France): Bailenger-type solution, no solvent. Test sample: 1 ml of faecal solution. Sample processing: flotation system.
- Mini Parasit Bailenger (Ethico, Los Ilia, France): Bailenger-type solution, no solvent. Test sample: 1.5 ml of faecal solution. Sample processing: flotation system.
- Panmap (Kormann-Lax, Los Ilia, Iran): Prozonal prepak using Bailenger-type solution and ethyl alcohol as solvent. Test sample: 1.5 ml of faecal solution. Sample processing: flotation system.

The home-made Bailenger concentrates procedure which was previously validated in the Clinical Parasitology Laboratory, CHU de Rennes, for all above stool samples consisted of using Bailenger (optical at 150x and ethyl at 50x) after solubilization. Test sample 1 ml of faecal solution. Sample processing: submersion in 200 ml of flotation solution.

3. Qualitative microscopic criteria of the presence of parasites in stools ("positive" samples)

Direct stool examination - for protozoa (sample pellet diluted 30x in saline) at least one parasite per 50 microscopic fields (magnification X300)

- for helminths (sample pellet diluted 30x in saline) at least one egg or larva per one 21mm X 21mm cover slip (magnification X 100)

Concentrated stool examination - for protozoa (sample pellet diluted 30x in saline) at least one parasite per 50 microscopic fields (magnification X300)

- for helminths: at least one egg or larva per one 21mm X 21mm cover slip (magnification X100)

Calculating concentration factor of assays:

For helminths: expressing the number of eggs/larvae counted on 1 cover slip and expressed as number of eggs/larvae/ml (one cover slip containing 35 fields)

Concentration factor number of eggs/larvae counted (on 1 cover slip examination) / (number of eggs/larvae/ml (direct stool examination)

For protozoa: concentration factor (number of parasites/ml) / (number of parasites/ml (direct stool examination))

5. Assay performance calculation for helminths

For each concentration procedure, performance was expressed as the ratio: (total number of helminths found in the ex-sis concentration pellet) / (number of helminths in the entire sample) - in which ex-sis pellet number (number of helminths) cover slips found by direct examination / total sample volume (ml)

Results

Table 1: Qualitative parasite detection

<table>
<thead>
<tr>
<th>Stool sample dilution</th>
<th>Parasite</th>
<th>Dilution factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>30x</td>
<td>Protozoa</td>
<td>X300</td>
</tr>
<tr>
<td>30x</td>
<td>Helminths</td>
<td>X100</td>
</tr>
</tbody>
</table>

Table 2: Quantitative evaluation of concentration

<table>
<thead>
<tr>
<th>Assay</th>
<th>helminths</th>
<th>protozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy Pass Bailenger</td>
<td>32/37</td>
<td>4/5</td>
</tr>
<tr>
<td>Mini Parasit Bailenger</td>
<td>34/37</td>
<td>4/5</td>
</tr>
<tr>
<td>Panmap</td>
<td>35/37</td>
<td>5/5</td>
</tr>
</tbody>
</table>

Comments: Present results reveal significant differences in efficacy between commercial concentration kits depending on parasites, which underlines the need for further evaluation and recommendations of commercial assays for each clinical Parasitology laboratories.

1. The Panmap assay: Easy Pass Bailenger and Mini Parasit Bailenger were found much less efficient than the two-phase technique since solubilization pellets were usually larger than the ex-sis phase pellet (0.5 g/drops versus 2.0 drops), implying the occurring examination of at least one hour per concentrated sample of entero-parasites, i.e. at least 11.56 cover slips (magnification: X300), especially for eggs and larvae.

2. The Panmap 3 domain kit was found convenient and provide results close from those of the home made Bailenger reference technique.