Evaluation of Parasite Egg and Cyst Recovery Using Devices Designed for Centrifugal or Stationary Flotation

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ABSTRACT

Two new devices (OT, ST), were recently introduced for the recovery of parasite eggs and cysts for microscopic examination. These devices, two stationary flotation devices, and a standard double-centrifugal sugar-flotation were compared using common flotation solutions and methods recommended by the manufacturers for the recovery of hookworm, ascaridoid, and whipworm eggs from companion animal fecal samples. Additionally, the recovery of Giardia cysts in the OT device using a zinc sulfate versus sodium nitrate solution was evaluated. Double-centrifugal sugar-flotation (1.30 specific gravity) was the most sensitive method for the recovery of the nematode eggs from feces of companion animals. Overall, centrifugation increased the recovery of eggs as compared with standing flotation methods, with the ST performing equivalently to the OT. Although these more recently introduced tests have good sensitivities for the nematodes tested, egg recovery was routinely markedly less than that achieved by standard double-centrifugal sugar-flotation, and false-negatives did occur. Still, the OT and ST generally have increased recoveries over the two standing flotation devices, and are significantly better than these for the recovery of ascaridoid and whipworm eggs from dog and cat samples. Zinc sulfate (1.18 specific gravity) is recommended for the recovery of Giardia cysts when using the OT device.

(Int J Am Anim Hosp Assoc 2018; 54:---–---. DOI 10.5326/JAAHA-MS-6549)

Introduction

Two devices, Device 1 [OT]a and Device 2 [ST]b recently entered the fecal analysis market. Both single-use devices are novel in design, and aim to standardize routine fecal examinations while reducing direct handling of fecal material by technical staff. Although the devices are markedly different in design, both are intended to be used with centrifugal flotation using any of the routine flotation solutions, at the user’s discretion.

Device OT is a three-piece device, designed to allow easy sampling, mixing, in-tube centrifugation, and the generation of a convex meniscus for coverslip placement (Figure 1). A tubular sampler collects, by pressure, about 1 g of feces, and is discarded after transferring and mixing the sample in a tube with flotation solution. Into this tube is placed an insert having small slots on its bottom to catch floating particulates (Figure 2). After centrifugation, the tube is placed upright and the inserted device is screwed down further to form a convex meniscus onto which a coverslip is placed. The tube remains stationary before the coverslip is examined.

Device ST consists of a small squeeze-bottle for collection and processing of fecal samples prior to centrifugation (Figure 3). Feces are sampled with the included spoon, and spoon and specimen are placed in the bottle containing the flotation medium of choice. The sample is mixed by squeezing the sides of the bottle and then transferred to a centrifuge tube (provided by the user) by pouring the material through the bottle’s sieving funnel-tip. The material is examined following centrifugation.

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In a series of trials, the recovery efficiencies of a standard centrifugal sugar-flotation, centrifugal flotation using the ST or OT devices, and two stationary flotation devices, Device 3 [FL] and Device 4 [OP], were compared for the recovery of nematode eggs from companion animal samples using common flotation media. Also evaluated was *Giardia* cyst recovery in the OT using zinc sulfate or sodium nitrate solutions.

**Materials and Methods**

**Fecal Samples**

Convenience fecal samples from dogs and cats with natural or experimental infections with one or more of the following parasites were used: *Giardia*, *Ancylostoma*, *Uncinaria*, *Toxascaris*, *Toxocara*, and *Trichuris*; samples from animals with experimental infections were acquired from Cheri Hill Kennel and Supply (Stanwood, Michigan). All samples were confirmed to contain parasites by qualitative double-centrifugal sugar-flotation. Prior to performing each test, the weight of feces being processed was appropriately determined and recorded.

**Flotation Solutions**

Solutions were prepared of aqueous solutions of 1.30 specific gravity (spg) magnesium sulfate (MgSO₄), 1.30 spg cane sugar, and 1.20 zinc sulfate (ZnSO₄). Commercially prepared solutions consisted of a solution of 1.20 spg sodium nitrate (NaNO₃) and a solution of 1.18 spg zinc sulfate (ZnSO₄). In all cases, specific gravities of the solutions were verified by hydrometer prior to use.

**Flotation Procedures and Eggs per Gram Calculation**

Four devices, OT, ST, FL, and OP, and glass tubes (for centrifugal sugar-flotation) were used with five different flotation solutions (Table 1). Commercial kits were used following manufacturer’s instructions, and are briefly outlined below; the OP device was also used with noted protocol changes. The principal author performed all procedures and examined all coverslips under ×100 or ×400 magnifications, as appropriate. Parasites were counted and eggs per gram (EPG; or cysts per gram) were calculated based on the recorded weight of fecal material mixed for examination.

**Double-centrifugal sugar-flotation [CSI.305]**

In this adaptation of the method described in Bowman a fecal sample was mixed with a small amount of distilled water in an unwaxed 3 oz paper cup and strained through two layers of cheesecloth into a second paper cup. The mixture was then poured into a 16 × 100 mm glass tube, and centrifuged in a swinging centrifuge at
Device OT
A fecal sample was loaded into the OT sampling device/mixer and mixed in the provided tube with flotation solution. The mixer insert was discarded and the filter piece inserted along with additional solution. The tube and filter device were centrifuged at 1318 × g for 5 min in a fixed-angle centrifuge provided to us by the device’s manufacturer. The tube was removed from the centrifuge, and an 18 × 18 mm coverslip was placed on the filter piece. The latter was twisted down until the solution touched the underside of the coverslip. The device and coverslip were allowed to stand upright for 3 additional min prior to examination.

Device ST
A fecal sample and the collection spoon supplied with the device were placed in the ST bottle and mixed with approximately 15 mL of flotation solution (as per manufacturer’s direction, a greater volume of flotation solution than used to fill the tube is initially used for mixing). Contents were mixed thoroughly and then (approximately 13 mL) poured through the sieving spout into a round-bottom 16 × 100 mm glass tube to form a convex meniscus. An 18 × 18 mm coverslip was placed on the tube before centrifugation at 300 × g for 10 min in a swinging bucket centrifuge. After centrifugation, the coverslip was examined.

Device FL
A fecal sample collected in the insert of the FL device was placed in the container and flotation solution added. The material was mixed, and more solution was used to form a convex meniscus. A 22 × 22 mm coverslip was added, and the device was allowed to stand for 15 min before coverslip examination.

Device OP with 1.18 spg zinc sulfate solution [OP1.18Z]
A fecal sample collected in the device’s insert was replaced in the container. The commercially prepared 1.18 spg ZnSO₄ solution was used to mix the material before more media was added to form a convex meniscus. A 22 × 22 mm coverslip was placed on the device and allowed to stand for 5 min before being transferred onto a glass slide for examination.

Device OP with 1.20 sodium nitrate solution [OP1.20N]
In this modification, a fecal sample was processed as above, but a prepared 1.20 spg NaNO₃ solution was used, and the 22 × 22 mm coverslip was allowed to stand for 10 min prior to examination.

Experimental Trials
There are five trials presented. Trial 1 examined the two new devices, OT and ST, compared with the standard sugar flotation using the sugar solution in all tests. The high viscosity of the sugar was thought...
Trial 1
Feline fecal samples (n = 9) containing *Toxocara cati* eggs from naturally infected cats were processed using 1.30 spg sugar in the ST device [ST1.30S] (0.930–1.022 g), OT device [OT1.30S] (0.438–0.663 g), and with the standard double-centrifugal sugar-floation [CS1.30S] (0.470–0.594 g).

Trial 2
Feline fecal samples (n = 38) containing *Ancylostoma braziliense* eggs from experimentally infected cats were processed using either 1.30 spg MgSO₄ in the ST [ST1.30M] (0.722–1.093 g) and OT [OT1.30M] (0.900–1.088 g) devices, or 1.30 spg sugar in the double-centrifugal flotation [CS1.30S] (0.207–1.051 g).

Trial 3
Sodium nitrate solution (1.20 spg) was used in the ST (0.951–1.121 g), OT (0.405–1.097 g), and OP (0.861–1.234 g) devices [ST1.20N,
TABLE 1
Abbreviated Designation for Technique and Flotation Media Used in These Studies

<table>
<thead>
<tr>
<th>Designation</th>
<th>Method—Centrifugal Techniques</th>
<th>Method—Stationary Flotation Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS1.30S</td>
<td>Double-Centrifugal Sugar Flotation using 1.30 spg sugar</td>
<td>FL1.20N</td>
</tr>
<tr>
<td>OT1.30S</td>
<td>Device 1 [OT] using 1.30 spg sugar</td>
<td>OP1.18Z</td>
</tr>
<tr>
<td>ST1.30S</td>
<td>Device 2 [ST] using 1.30 spg MgSO₄</td>
<td>OP1.20N</td>
</tr>
<tr>
<td>OT1.30M</td>
<td>Device 1 [OT] using 1.30 spg MgSO₄</td>
<td>ST1.18Z</td>
</tr>
<tr>
<td>ST1.30M</td>
<td>Device 2 [ST] using 1.30 spg MgSO₄</td>
<td>OT1.20Z</td>
</tr>
<tr>
<td>OT1.20N</td>
<td>Device 1 [OT] using 1.20 spg NaNO₃</td>
<td>OT1.18Z</td>
</tr>
<tr>
<td>ST1.20N</td>
<td>Device 2 [ST] using 1.20 spg NaNO₃</td>
<td>OT1.30S</td>
</tr>
<tr>
<td>OT1.20Z</td>
<td>Device 1 [OT] using 1.20 spg ZnSO₄</td>
<td>CS1.30S</td>
</tr>
<tr>
<td>ST1.18Z</td>
<td>Device 2 [ST] using 1.18 spg ZnSO₄</td>
<td></td>
</tr>
<tr>
<td>OT1.18Z</td>
<td>Device 1 [OT] using 1.18 spg ZnSO₄</td>
<td></td>
</tr>
<tr>
<td>ST1.18Z</td>
<td>Device 2 [ST] using 1.18 spg ZnSO₄</td>
<td></td>
</tr>
</tbody>
</table>

spg, specific gravity.

OT1.20N, and OP1.20N, respectively) to recover eggs of Toxocara spp. (n = 6; 5 feline, 1 canine), Ancylostoma spp. (n = 7; 1 feline, 6 canine), and Trichuris vulpis (n = 19; all canine) from 24 samples (5 cats and 19 dogs with single or multiple natural infections).

**Trial 4**

Four devices, OT, ST, FL, and OP, utilizing two flotation media, were compared with each other and the double-centrifugal sugar-flotation (CS1.30S). The flotation media used in the devices were 1.20 spg sodium nitrate solution and 1.18 spg zinc sulfate solution; these are the two solutions recommended by the manufacturers of the two stationary devices for which it is recommended that FL be used with the 1.20 spg sodium nitrate solution and OP be used with 1.18 spg zinc sulfate solution. Samples included five egg types (one sample had two different species of parasites): Ancylostoma caninum (n = 2; 1 natural, 1 experimental infection), Toxascaris leonina (n = 1; experimental infection), Toxocara canis (n = 2; experimental infections), T. vulpis (n = 1; natural infection), and Uncinaria stenocephala (n = 1; natural infection). The sample containing two different egg types was processed as a single sample; both types of eggs were counted separately and were handled as independent counts in the analyses. Five replicates of each sample were processed with each of seven device/media combinations: CS1.30S (0.423–0.975 g), ST1.20N (0.936–1.071 g) and OT1.20N (0.789–1.126 g), ST1.18Z (0.915–1.076 g) and OT1.18Z (0.860–1.147 g), FL1.20N (0.907–1.074 g), and OP1.18Z (0.932–1.094 g).

**Trial 5**

The OT device was used with either zinc sulfate solution at 1.20 spg [OT1.20Z] (0.582–1.011 g) or a solution of 1.20 spg sodium nitrate [OT1.20N] (0.711–1.002 g) to compare the recovery of Giardia cysts using these two different flotation media from eight fecal samples (three canine and five feline) from naturally infected animals.

**Statistics**

The number of eggs or cysts per gram was determined by dividing the number counted by the weight of the feces examined for each sample; the result was not always an integer. Ranges of counts for herein are rounded to integers; all other numbers are represented with three significant digits. Statistical comparisons were performed using statistical software. Analysis was performed using log-transformed data of the EPGs + 1. One-way analysis of variance with Tukey’s between group comparisons with 95% familial confidence intervals was used to compare the geometric means. In Trial 4, the coefficients of variation around the means for each test were determined by egg type by dividing the standard deviation (SD) of the mean by the mean of each of the 5 replicates, and multiplying by 100. The coefficients of variation were compared without data transformation. For Trial 5, the geometric means of calculated cysts per gram were compared using a two-tailed t test. Arithmetic means are cited in the text, unless indicated otherwise, and are used in textual egg recovery comparisons.

**Results**

The mean EPG recovery and SD for T. cati eggs in Trial 1 were as follows: 307 ± 549 for CS1.30S, 26.7 ± 35.1 for ST1.30S, and 12.2 ± 18.0 for OT1.30S. The CS1.30S consistently recovered more eggs from a sample using the different methods. There is no significant difference (P > .05) in the geometric means between methods sharing the same superscript. EPG, eggs per gram; SD, standard deviation; spg, specific gravity.
**TABLE 3**

**Trial 2: *Ancylostoma braziliense* EPG Recovered from 38 Fecal Samples from Experimentally Infected Cats**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean EPG (Arithmetic) ± SD</th>
<th>Mean EPG (Geometric) ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS1.30S*</td>
<td>2800 ± 2070</td>
<td>1620 ± 4.48</td>
<td>4–6660</td>
</tr>
<tr>
<td>ST1.30M</td>
<td>494 ± 432</td>
<td>297 ± 3.12</td>
<td>0–2090</td>
</tr>
<tr>
<td>OT1.30M</td>
<td>256 ± 366</td>
<td>95.8 ± 4.01</td>
<td>0–1700</td>
</tr>
</tbody>
</table>

Three centrifugal flotation methods were employed: 1.30 spg MgSO₄ solution in Devices 1 [OT] and 2 [ST], and a 1.30 spg double-centrifugal sugar-flotation.

*Results of multiple comparisons within the geometric means for each of the different methods.

EPG, eggs per gram; SD, standard deviation; spg, specific gravity.

**T. cati** eggs than the other methods, and both the ST1.30S and OT1.30S had false-negative results. The ST failed to detect eggs in 2 samples having 6 and 14 EPG recovered by the CS1.30S, and the OT failed to detect eggs in 3 samples with 4, 14, and 21 EPG recovery by the CS1.30S method; both tests shared a false-negative result on one sample. The difference in the mean number of eggs recovered using the different methods was significant (P = .012), and the CS1.30S recovery differed significantly from that of the OT1.30S, but no significant difference was apparent between the mean EPG recoveries of the CS1.30S and ST1.30S or ST1.30S and OT1.30S (Table 2).

In Trial 2, recovery of hookworm eggs by ST1.30M, OT1.30M, and CS1.30S was assessed. The CS1.30S recovered a mean (± SD) of 2800 (± 2070) EPG, about 5 times greater than achieved with the ST1.30M (495 ± 432 EPG) and the OT1.30M (256 ± 366 EPG; Table 3). Both the ST1.30M and OT1.30M had one false-negative result (a sample with 4 EPG evidenced using the CS1.30S). The difference between the mean recoveries of the three techniques was significant (P < .001), and the means differed significantly between each test with CS1.30S > ST1.30M > OT1.30M.

In Trial 3, using the commercially prepared 1.20 spg sodium nitrate solution for the recovery of three different egg types from dog feces, tests utilizing centrifugation (ST1.20N and OT1.20N) had higher recoveries for all three egg types than the stationary flotation technique, OP1.20N (Table 4). The respective mean EPG results for the three assays, ST1.20N, OT1.20N, and OP1.20N were as follows: for *Toxocara* spp., 147 ± 281, 175 ± 133 versus 58.2 ± 96.1; for *Ancylostoma* spp., 295 ± 442, 181 ± 320, 55.4 ± 82.7; and for *T. vulpis*, 38.8 ± 55.0, 44.6 ± 104, 15.5 ± 33.9. For *Toxocara*, the ST1.20N and OP1.20N shared a false-negative result on the same sample. For *Ancylostoma*, the OT1.20N and OP1.20N shared a false-negative result, and the OP1.20N had a second false-negative result (the latter on a sample with a relatively high number of eggs on the other tests, approximately 100 EPG). For *Trichuris*, the OT1.20N and OP1.20N shared a false-negative result on a sample that had a single egg recovered by the ST1.20N. The OP1.20N had two additional false-negative results; one had EPG counts of 22 and 14, and the other 2 and 1 EPG, respectively, with the ST1.20N and OT1.20N methods. The only significant difference in the tests when using the commercial sodium nitrate 1.20 spg solution was with the recovered EPGs for *T. vulpis* (analysis of variance P = .023), for which the OT1.20N was not different from either the ST1.20N or the OP1.20N, but the ST1.20N recovered significantly more eggs than the OP1.20N (Table 4).

Trial 4 was a comparison of the efficacy and variability of seven different device and media combinations for their recovery of nematode eggs from fecal samples containing single or mixed infections of roundworms (*Toxascaris* and *Toxocara*), hookworms (*Ancylostoma* and *Uncinaria*), and the whipworm, *T. vulpis* (Table 5; Figures 4, 5). When recoveries were compared by test and egg type, it was clear that for heavier eggs (T. canis, T. leonina, and T. vulpis) the CS1.30S method routinely recovered more eggs than the other methods. In fact, for these egg types, the recovery ranges for the CS1.30S did not generally overlap those of any of the other methods (the only exception occurred in one of the *T. canis* samples in which the ST1.20N recovery barely overlapped the lower range of the CS1.30S recovery for the same sample (Table 5)). Similarly, for comparisons assessing hookworm egg recovery by the

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**TABLE 4**

**Trial 3: EPG from 24 Fecal Samples from Naturally Infected Dogs (n = 19) and Cats (n = 5)**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean EPG (Arithmetic) ± SD</th>
<th>Mean EPG (Geometric) ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toxocara cati (n = 5)</strong> or <strong>Toxocara canis (n = 1)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1.20N</td>
<td>147 ± 281</td>
<td>16.7 ± 10.2</td>
<td>0–701</td>
</tr>
<tr>
<td>OT1.20N</td>
<td>175 ± 133</td>
<td>28.8 ± 7.87</td>
<td>3–826</td>
</tr>
<tr>
<td>OP1.20N</td>
<td>58.2 ± 96.1</td>
<td>8.47 ± 8.98</td>
<td>0–235</td>
</tr>
<tr>
<td><strong>Ancylostoma caninum (n = 6)</strong> or <strong>Ancylostoma tubaeforme (n = 1)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1.20N</td>
<td>295 ± 442</td>
<td>62.7 ± 7.68</td>
<td>4–1080</td>
</tr>
<tr>
<td>OT1.20N</td>
<td>181 ± 320</td>
<td>39.7 ± 7.77</td>
<td>0–887</td>
</tr>
<tr>
<td>OP1.20N</td>
<td>55.4 ± 82.7</td>
<td>11.8 ± 7.97</td>
<td>0–215</td>
</tr>
<tr>
<td><strong>Trichuris vulpis (n = 19)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1.20N*</td>
<td>38.8 ± 55.0</td>
<td>16.7 ± 2.87</td>
<td>1–208</td>
</tr>
<tr>
<td>OT1.20N†</td>
<td>44.6 ± 103</td>
<td>12.7 ± 3.48</td>
<td>0–452</td>
</tr>
<tr>
<td>OP1.20N†</td>
<td>15.5 ± 33.9</td>
<td>4.07 ± 3.19</td>
<td>0–134</td>
</tr>
</tbody>
</table>

All samples were examined using 1.2 spg sodium nitrate solution spg 1.20 in the Devices 1 [OT], 2 [ST], and 4 [OP].

*Results of multiple comparisons within the geometric means for each of the different methods. There is no significant difference (P > .05) in the geometric means between methods sharing the same superscript.

EPG, eggs per gram; SD, standard deviation; spg, specific gravity.
### Table 5

**Trial 4: EPG of Feces Recovered Using Seven Different Device and Media Combinations with Five Replicate Tests on Each of Six Fecal Samples from Naturally Infected Dogs**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean EPG (Arithmetic) ± SD</th>
<th>Mean EPG (Geometric) ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ancylostoma caninum</strong> 1</td>
<td>423 ± 30.9</td>
<td>423 ± 2.08</td>
<td>389–455</td>
</tr>
<tr>
<td>CS1.30S*</td>
<td>24.9 ± 14.2</td>
<td>24.9 ± 2.76</td>
<td>11–44</td>
</tr>
<tr>
<td>ST1.20N**</td>
<td>185 ± 42.0</td>
<td>183 ± 2.28</td>
<td>121–229</td>
</tr>
<tr>
<td>ST1.18Z</td>
<td>158 ± 56.7</td>
<td>153 ± 2.27</td>
<td>108–255</td>
</tr>
<tr>
<td>OT1.20N</td>
<td>152 ± 38.8</td>
<td>149 ± 2.34</td>
<td>90–192</td>
</tr>
<tr>
<td>FL1.20N</td>
<td>73.3 ± 33.5</td>
<td>69.1 ± 2.61</td>
<td>35–120</td>
</tr>
<tr>
<td>OP1.18Z</td>
<td>8.17 ± 2.03</td>
<td>9.99 ± 2.24</td>
<td>6–11</td>
</tr>
</tbody>
</table>

| **Ancylostoma caninum** 2 | 193 ± 47.6 | 191 ± 2.27 | 142–268 |
| CS1.30S*     | 184 ± 15.8 | 186 ± 2.09 | 162–205 |
| ST1.20N      | 113 ± 12.8 | 114 ± 2.12 | 101–130 |
| ST1.18Z      | 114 ± 49.5 | 99.9 ± 3.07 | 26–143 |
| OT1.20N      | 56.4 ± 30.6 | 50.3 ± 2.96 | 16–93 |
| FL1.20N      | 66.5 ± 17.3 | 66.5 ± 3.33 | 40–82 |
| OP1.18Z      | 9.48 ± 4.80 | 10.4 ± 2.73 | 3–15 |

| **Toxocara canis** 1 | 597 ± 35.0 | 598 ± 2.06 | 558–646 |
| CS1.30S*     | 135 ± 43.4 | 131 ± 2.45 | 75–190 |
| ST1.20N      | 264 ± 96.6 | 245 ± 2.68 | 96–339 |
| ST1.18Z      | 148 ± 47.2 | 144 ± 2.37 | 105–209 |
| OT1.20N      | 270 ± 9.60 | 272 ± 2.04 | 257–282 |
| FL1.20N      | 34.9 ± 13.9 | 34.7 ± 2.51 | 35–18 |
| OP1.18Z      | 20 ± 6.40 | 21.5 ± 2.33 | 15–30 |

| **Toxocara canis** 2 | 96.0 ± 21.51 | 95.8 ± 2.29 | 61–113 |
| CS1.30S*     | 49.6 ± 10.65 | 50.7 ± 2.24 | 37–62 |
| ST1.20N      | 35.3 ± 12.80 | 35.4 ± 2.46 | 19–50 |
| ST1.18Z      | 35.5 ± 14.88 | 34.7 ± 2.57 | 17–53 |
| OT1.20N      | 31.0 ± 11.92 | 30.7 ± 2.58 | 13–46 |
| FL1.20N      | 8.90 ± 3.04 | 10.6 ± 2.35 | 6–14 |
| OP1.18Z      | 6.35 ± 2.22 | 8.10 ± 2.35 | 4–9 |

| **Toxascaris leonine** | 532 ± 61.06 | 530 ± 2.14 | 424–579 |
| CS1.30S*     | 66.9 ± 21.90 | 65.7 ± 2.42 | 41–88 |
| ST1.20N      | 74.8 ± 22.66 | 73.9 ± 2.37 | 51–101 |
| ST1.18Z      | 124 ± 38.00 | 122 ± 2.36 | 85–179 |
| OT1.20N      | 133 ± 60.80 | 118 ± 2.82 | 37–201 |
| FL1.20N      | 13.3 ± 7.93 | 13.3 ± 2.92 | 4–24 |
| OP1.18Z      | 19.0 ± 8.39 | 19.6 ± 2.53 | 11–29 |

(continued)

### Table 5 (Continued)

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean EPG (Arithmetic) ± SD</th>
<th>Mean EPG (Geometric) ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichurus vulpis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS1.30S*</td>
<td>862 ± 116</td>
<td>858 ± 2.15</td>
<td>688–998</td>
</tr>
<tr>
<td>ST1.20N</td>
<td>141 ± 22.8</td>
<td>141 ± 2.17</td>
<td>117–177</td>
</tr>
<tr>
<td>ST1.18Z</td>
<td>54.7 ± 46.0</td>
<td>45.7 ± 3.02</td>
<td>23–134</td>
</tr>
<tr>
<td>OT1.20N</td>
<td>240 ± 68.5</td>
<td>235 ± 2.32</td>
<td>177–333</td>
</tr>
<tr>
<td>OT1.18Z</td>
<td>31.2 ± 4.30</td>
<td>33.0 ± 2.14</td>
<td>26–38</td>
</tr>
<tr>
<td>FL1.20N</td>
<td>18.9 ± 9.98</td>
<td>18.1 ± 2.96</td>
<td>5–28</td>
</tr>
<tr>
<td>OP1.18Z</td>
<td>2.34 ± 1.91</td>
<td>3.82 ± 3.02</td>
<td>0–5</td>
</tr>
</tbody>
</table>

| **Uncinaria stenocephala** |                           |                            |                |
| CS1.30S*    | 55.4 ± 10.9 | 56.6 ± 2.21 | 43–69 |
| ST1.20N     | 51.7 ± 14.5 | 51.9 ± 2.35 | 32–69 |
| ST1.18Z     | 36.9 ± 16.6 | 35.8 ± 2.60 | 17–59 |
| OT1.20N     | 52.6 ± 11.6 | 53.5 ± 2.25 | 38–68 |
| OT1.18Z     | 31.2 ± 15.2 | 29.8 ± 2.73 | 12–50 |
| FL1.20N     | 23.3 ± 8.92 | 23.9 ± 2.47 | 12–34 |
| OP1.18Z     | 5.48 ± 1.17 | 7.28 ± 2.34 | 3–7 |

*1, 5, ***, **Results of multiple comparisons within the geometric means for each of the different methods. There is not a significant difference (P > .05) in the geometric means between methods sharing the same superscript.

Egg, eggs per gram; SD, standard deviation.

Different methods, the CS1.30S recovered the most eggs of each type (Ancylostoma and Uncinaria), but was not always significantly superior to the other centrifugation methods. For T. canis and T. leonina eggs, the two stationary floatations FL (FL1.20N) and OP (OP1.18Z) recovered significantly fewer eggs than any of the other methods. In the case of T. vulpis, the stationary OP device (OP1.18Z) recovered significantly fewer eggs than any of the other methods (only 0.23% the number of eggs recovered by the CS1.30S); overall, for all egg types, the mean EPG recovery of the OP1.18Z was only between 0.2–10% the mean recovery of the CS1.30S for the same egg type. The 1.18 spg zinc sulfate solution recovered significantly fewer T. vulpis eggs than the 1.20 spg sodium nitrate solution when utilized in a centrifugal assay, but the FL1.20N (the stationary method utilizing sodium nitrate) recovered very few T. vulpis eggs compared with any of the methods employing centrifugation. In the case of hookworms, the FL with sodium nitrate spg 1.20 (FL1.20N) tended to recover eggs in the lower range of EPGs recovered by some of the centrifugal methods rather than grouping with recoveries obtained by the other stationary flotation method. In the case of U. stenocephala, the only test with markedly different results was the OP (OP1.18Z).

Overall (Figure 4), the CS1.30S recovered the most eggs, the ST (ST1.20N and ST1.18Z) and OT (OT1.20N and OT1.18Z) performed
FIGURE 4 Geometric mean EPG and its 95% confidence interval for each egg type counted in Trial 4 on tests performed on six different canine fecal samples. Two samples contained Ancylostoma caninum eggs (AC-1 and AC-2), two Toxocara canis eggs (Tc-1 and Tc-2), and three with eggs of Toxascaris leonina (Tl), Trichuris vulpis (Tv), or Uncinaria stenocephala (Us). Five replicate tests were performed on each sample with each of the seven device/media combinations: double-centrifugal sugar-flotation with 1.3 spg sugar [CS1.30S], ST and OT with sodium nitrate at spg 1.20 or zinc sulfate at 1.18 spg [ST1.20N, ST1.18Z, OT1.20N, and OT1.18Z], and FL with sodium nitrate at spg 1.20 [FL1.20N] and OP with zinc sulfate at 1.18 spg [OP1.18Z]. CI, confidence interval; EPG, eggs per gram; spg, specific gravity.

A comparison of the coefficients of variance in Trial 4 (Figure 5) revealed that the amount of variance was similar for all the centrifugation assays and significantly less than that of the two stationary flotation methods (P < .001), which did not differ significantly from each other. The coefficient of variation of the mean for the FL did not differ from that of OT using 1.18 spg zinc sulfate (OT1.18Z).

The final trial, Trial 5, compared 1.20 spg ZnSO₄ or NaNO₃ in the OT device for the recovery of Giardia cysts from eight fecal samples from naturally infected animals (three dogs and five cats). The mean number of Giardia cysts per gram recovered were as follows: for OT1.20Z, 373 (SD = 409, range = 24–1270); and for OT1.20N, 53.1 (SD = 57.1, range = 1–182). The corresponding geometric mean numbers of cysts per gram were 215 (SD = 3.41) and 27.0 (SD = 4.78) cysts per gram, respectively. A two-tailed t test detected a significant difference in recovery between the two flotation solutions (P = .011), with 1.18 spg ZnSO₄ having significantly better recovery.

FIGURE 5 Mean and SD of the coefficients of variation (CV = \[100 \times \{\text{Mean (SD of each of the five replicates/Mean of the five replicates)}\}\]) on the egg counts made in five replicate tests on six canine samples in Trial 4 using seven different device/media combinations: CS1.30S, ST1.20N, ST1.18Z, OT1.20N, and OT1.18Z, FL1.20N, and OP1.18Z. Two fecal samples contained Ancylostoma caninum eggs (AC-1 and AC-2), two Toxocara canis eggs (Tc1 and Tc2), and three with eggs of Toxascaris leonina (Tl), Trichuris vulpis (Tv), or Uncinaria stenocephala (Us). CV, coefficient of variation; SD, standard deviation.

Discussion

Many techniques are described for diagnosing parasitic infections through fecal examination, and care should be taken in choosing a method to fit the objective and parasite in question.¹ For the routine recovery of common nematode eggs from feces, centrifugal flotation (with an appropriate flotation solution for the parasite[s] sought) has repeatedly been shown to be more sensitive than stationary flotation methods.³–⁷ Many, however, still perform stationary flotation for fecal examinations due to its perceived ease and cleanliness. The work here examined two aids for fecal examination, the ST device and the OT device, introduced, in part, with goals of promoting centrifugal flotation as the method of choice through improvements in ease of use and minimization of sample handling. Here it has been shown that both devices provided comparable results with various flotation media, having parasite recoveries markedly greater than those achieved with two common stationary flotation tests.

The first trial compared recoveries of the two new devices to the double-centrifugal sugar-flotation utilizing 1.30 spg sugar for the recovery of T. cati eggs in all assays. The double-centrifugal sugar-flotation had a mean EPG recovery 11.4 times that of the ST and 25.6 times that of the OT, and both devices had false-negative results on samples with apparently low egg counts (4–21 EPG). The 1.30 spg sugar solution was chosen for initial testing of the devices because it has been considered the “gold standard” for egg recovery since its introduction for fecal flotation in 1923.¹,³ However, in light
of the results, it was thought that the high viscosity of this solution may have hampered the performance of these devices.

Therefore, a second trial (Trial 2) was performed using a solution of similar specific gravity with lower viscosity (1.30 spg MgSO₄) in the two devices for comparison with the double-centrifugation 1.30 spg sugar-flotation. Again, the double-centrifugal sugar-flotation recovered more eggs (5.7 and 11 times greater mean EPG than the ST or OT devices, respectively), but the devices did about twice as well at egg recoveries with these hookworm eggs and 1.30 spg MgSO₄ than they had with 1.30 sugar. Both devices had a single false-negative result on a sample with apparently few (four) eggs.

A comparison of the two new devices to a stationary flotation (Trial 3) utilized 1.20 spg sodium nitrate in solution in both devices, OT and ST, along with a stationary device, OP. The samples contained the eggs of *T. canis*, *T. cati*, *A. caninum*, *A. tubaeforme*, or *T. vulpis*. A significant difference between the mean numbers of eggs recovered was only evident when comparing the recovery of *T. vulpis* eggs, and only between the ST and the standing flotation method. All the methods had false-negatives: the ST had one, OT had two, and OP had six (for whipworm, hookworm, and roundworm eggs, respectively, ST: zero, zero, one; OT: one, one, zero; and OP: three, two, one, respectively). Here, the OP misidentified 25% of the samples as “negative.”

In the final trial comparing the recovery of parasite eggs (Trial 4), two commonly used salt solutions at 1.18 spg (ZnSO₄) and 1.20 spg (NaNO₃) were used with the OT and ST in a comparison with these two common standing flotation devices, OP and FL, along with the standard double-centrifugal sugar-flotation method. The results of this trial confirmed the double-centrifugal sugar-flotation to be the most efficient method for nematode egg recovery. As expected, based on our experience, the difference between this and the other procedures was most evident for the recovery of ascarids, and especially so for whipworms. This method also had the least variance in its recovery irrespective of egg type (Figure 5). For hookworm eggs (*Ancylostoma* and *Uncinaria*), most of the tests were comparable in their recoveries; however, the OP performed more poorly than the rest and had the greatest variability (Figures 4, 5). Additionally, for one of the canine fecal samples, the five replicates run by ST had an unexpected and markedly reduced recovery of *A. caninum* eggs that may have been due to an error in processing that went unremarked at the time (e.g., this particular sample was perhaps more difficult to homogenize in the mixing bottle).

Overall, in trials comparing egg recoveries with the different solutions and devices, centrifugal flotation was again shown to be more efficient and sensitive than stationary flotation. For the tests utilizing centrifugation, the double-centrifugal sugar-flotation outperformed the other assays, whereas the two new devices examined were comparable to each other in their egg recoveries and outperformed the stationary flotation methods. For the stationary flotations, the Device FL typically surpassed Device OP. Using sucrose density gradient centrifugation, the eggs of the nematodes have been determined to float in solutions of these specific gravities: *A. caninum* 1.06; *T. leonina* 1.06; *T. canis* 1.09; *T. cati* 1.1; and *T. vulpis* 1.15. Herein it was found that for hookworm eggs, including those of *U. stenocephala*, recoveries were similar with all the methods, the exception being OP utilizing 1.18 ZnSO₄. All three of the ascaridoid eggs had significantly improved recoveries with centrifugation when compared with stationary flotation. For whipworm eggs, a solution with a specific gravity ≥ 1.2 increases egg recovery by all assays and markedly increases the number of eggs recovered in a stationary float. For whipworms eggs, the OP device using 1.18 ZnSO₄ recovered a very low percentage of eggs (0.23% of the eggs recovered by the double-centrifugal sugar-flotation).

Regarding the recovery of *Giardia* cysts in Device OT with 1.20 spg zinc sulfate or 1.20 spg sodium nitrate solution, the zinc sulfate recovered significantly (7 times) more cysts than sodium nitrate. Thus, as previously suggested for the recovery of this protozoan from formed or refrigerated samples, zinc sulfate should be used as the medium of choice.8

In most cases, fecal processing followed the manufacturer’s indications for the testing devices. For Device ST, therefore, a portion of the fecal sample placed in the mixing bottle was not examined (approximately 17%), because more flotation solution for mixing than was used to fill the tube was used. Thus, calculated EPGs in our trials reflect the approximate numbers of eggs that would be recovered from an initial 1 g of feces placed in the mixing bottle and reflect the 17% “loss of eggs” inherent in this procedure. With respect to centrifugation, following the suggestion of the manufacturers of the devices meant that different times and relative centrifugal forces were utilized. Additionally, the OT device used a fixed-angle rotor requiring coverslip placement on the vertical tube after centrifugation, and a 3 min wait for egg collection on its surface, whereas the other two methods used a swinging bucket rotor having coverslips in place during centrifugation. In the case of the double-centrifugal sugar-flotation with 1.30 spg sugar, there were two centrifugations at 800 ×g, one in water for 1 min to remove suspended particulates for improved visibility within the final sample, and a second for 10 min to float the eggs onto a coverslip at the top of the tube. For Device OT, all centrifugations were at 1318 ×g for 5 min. For Device ST, all centrifugations to float the eggs were at 300 ×g for 10 min. Time and relative centrifugal force are critical components of centrifugal flotation, and changes to the times or speeds could have produced markedly different results. Thus, if all centrifugations to float eggs had been for 10 min at the same relative centrifugal force of 800 ×g, the results might have been significantly different.
Conclusion

Double-centrifugal sugar-flotation using a wash step and 1.30 spg sugar was the best fecal examination procedure for the recovery of the nematode eggs studied and consistently had the highest recovery rate with the least variance. Zinc sulfate was verified as a better flotation media for Giardia cysts than sodium nitrate at 1.20 spg. The newly introduced ST and OT devices were observed to have good sensitivity for the helminths tested, generally recovered more helminth eggs than stationary flotation, and recovered significantly more ascarid and whipworm eggs. Because these two newer centrifugation assays performed similarly in the experiments presented here, in practices the choice of one over the other may ultimately be based upon cost, ease of use, and subtle reasons for staff preferences of one device over the other.

The authors would like to acknowledge BGS Medical Products for providing the SqueezeTest devices and IRIS Sample Processing for providing the StatSpin Ovatube devices and the fixed-angle centrifuge for its use. The authors received no financial support for this work from outside parties.

FOOTNOTES

a StatSpin Ovatube; Iris Sample Processing, Inc., Westwood, Massachusetts
b SqueezeTest; BGS Medical Products Inc., Venice, Florida
c Fecalyzer; Evsco Pharmaceuticals, Division of Vétoquinol USA, Fort Worth, Texas
d Ovassay Plus; Zoetis, Madison, New Jersey
e Magnesium sulfate heptahydrate (epsom salts); Topco Assoc LLC, Elk Grove Village, Illinois
f Sugar, pure cane, granulated; Wegmans, Rochester, New York
g Zinc sulfate heptahydrate; Fisher Scientific, Pittsburgh, Pennsylvania
h Fecasol; EVSCO Pharmaceuticals, Division of Vétoquinol USA, Inc., Fort Worth, Texas
i Ovassay Plus solution; Sybioscience Corporation, St. Louis, Missouri
j Dixie 3 oz bath cups; Dixie Consumer Products, Atlanta, Georgia
k Fisherbrand cheesecloth wipers; Fisher Scientific, Pittsburgh, Pennsylvania
l 16 × 100 round-bottom borosilicate glass tube; Kimble-Chase Life Science, Rochester, New York
m IEC Centra CL2 centrifuge; Thermo Fisher Scientific, Waltham, Massachusetts
n VanGuard V6500 centrifuge; Hamilton Bell Co., Inc., Montvale, New Jersey
o Minitab Statistical Software, Release 16.2.4; State College, Pennsylvania

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