The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals

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The FLOTAC is a sensitive, accurate, and precise technique for the diagnosis of protozoan and helminth infections in humans and animals. However, it requires centrifugation, and hence might be out of reach in resource-constrained settings. As an extension of the original FLOTAC protocol, this protocol describes the Mini-FLOTAC technique, a logical evolution of FLOTAC conceived to perform multivalent, qualitative, and quantitative diagnosis of helminth and protozoan infections in human and animal feces, and urine. This has been found to be of most use in the processing of large numbers of samples with rapid laboratory workup, and for veterinary applications directly on-farm. In addition to the Mini-FLOTAC apparatus, we describe the use of the Fill-FLOTAC, a closed system used to facilitate the performance of the first four consecutive steps of the Mini-FLOTAC technique: fecal sample collection and weighing, homogenization, filtration, and filling of the Mini-FLOTAC chambers. Processing of an individual sample using this protocol requires ~12 min.

INTRODUCTION

Diagnostics: needed but neglected

An analysis of the research and development (R&D) pipeline of therapeutic products revealed that only 37 of the 850 (4.4%) new drugs and vaccines registered in the period 2000–2011 had an indication for neglected diseases[1]. Within the limited funding for R&D against neglected diseases, results from the initial G-FINDER report in 2007 emphasized that the situation is particularly severe with regard to diagnostics (e.g., <1% of the malaria R&D funding was spent on diagnostics)[2]. Yet, accurate and precise diagnostic tests are the first step on the path toward individual patient treatment and the foundation for population-based disease control, prevention, surveillance, and elimination[3–5]. Prior studies on drug efficacy and the detection of low-intensity infection with parasites in humans and animals pointed to the need for accurate, precise, low-cost, easy-to-perform, and quantitative tests to be used in the public health and the veterinary sectors[6–10].

Still today, copromicroscopic techniques are widely used for the diagnosis of helminths and protozoa in both humans and animals. However, copromicroscopic techniques have a number of shortcomings, such as low sensitivity (high number of false-negative test results), low accuracy (the number is either underestimated or overestimated), low precision (poor repeatability of the test results), and, for some methods, complex and varying protocols that introduce discrepancies in diagnosis across laboratories[4,8,11–13]. **Table 1** summarizes different characteristics (e.g., sensitivity, accuracy, precision, cost, processing time, and equipment needs) and the main limitations for the most commonly applied copromicroscopic techniques, including the direct smear method[14], the simple tube flotation technique[14], the Wisconsin technique[14], the McMaster technique[14], the FecPak system (https://www.techiongroup.com/), the Kato-Katz method[15,16], the formalin-ether concentration method[17,18], and FLOTAC[4], the protocol for which this paper is an extension.

From FLOTAC to Mini-FLOTAC

In 2004, the FLOTAC technique, which is based on a combination of flotation and translation facilitated by a novel apparatus, was featured in the peer-reviewed literature[19]. Subsequently, numerous studies in different laboratories showed that the FLOTAC technique outperformed classic copromicroscopic techniques[4] in terms of sensitivity, accuracy, and precision of detecting and quantifying eggs, larvae, oocysts, and cysts of parasites in human and animal fecal samples. Moreover, the FLOTAC technique allows for simultaneous detection of a wide range of helminths and protozoa. Despite these excellent diagnostic features, FLOTAC has been found to be time- and labor-intensive, requiring laboratory equipment that can impede its wide application in resource-constrained settings, where it is most needed[4].

Mini-FLOTAC[20] is a logical evolution of the FLOTAC technique. It is user-friendly, produces highly reproducible results, and is particularly useful for monitoring and surveillance, for which large numbers of fecal samples must be rapidly, yet reliably, examined. It is recommended that Mini-FLOTAC be used in combination with Fill-FLOTAC, a kit that protects laboratory personnel against potential biohazards during the preparation (i.e., collection, weighing, homogenization, filtration, and filling of the apparatus) of fecal samples for subsequent microscopic examination[20].

Mini-FLOTAC

The Mini-FLOTAC apparatus is a disk-shaped device made of polycarbonate amorphous thermoplastic. This material has been chosen because of its excellent light transmission, high heat resistance, robustness, high-dimensional stability, and good electrical insulation properties.

The Mini-FLOTAC apparatus is composed of two physical components (i.e., the base and the reading disk) and two accessories (i.e., the key and the microscope adaptor; see **Fig. 1**). There are two 1-ml flotation

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TABLE 1 | Characteristics (diagnostic performance and technical performance) and main limitations of different copromicroscopic techniques used for the diagnosis of helminth and protozoan infections in humans and animals.

Technique	Diagnostic performance			Technical performance			Main limitation
	Sensitivity	Accuracy	Precision	Cost	Processing time	Equipment needs	
Direct smear	Very low	Very low	Very low	Inexpensive	Fast	Basic laboratory equipment	Gives positive results only if there are high levels of parasites
Simple tube flotation	Very low	Very low	Very low	Inexpensive	Long	Basic laboratory equipment	Only allows for qualitative, not quantitative, diagnosis
Wisconsin	Low	Low	Very low	Inexpensive	Long	Fully equipped laboratories	Lack of precision, owing to the absence of a grid on the coverslip
McMaster	Medium	Low	Low	Expensive	Medium	Basic laboratory equipment	Diagnosis of intestinal protozoa limited to coccidia
FecPak	Medium	Low	Low	Very expensive	Long	All the equipment is provided by the manufacturer	Utility is limited to gastrointestinal strongyles
Kato-Katz	Medium	Medium	Low	Inexpensive	Long	Basic laboratory equipment	Requires fresh feces
Formalin–ether concentration	High (for intestinal protozoa only)	High (for intestinal protozoa only)	Very low	Expensive	Very long	Fully equipped laboratories	Use of formalin and ether, which are hazardous to humans and the environment
FLOTAC	Very high	Very high	Very high	Inexpensive	Long	Fully equipped laboratories	Requires centrifugation steps with two different rotors and so an equipped laboratory
Mini-FLOTAC	High	High	High	Expensive	Medium	Basic laboratory equipment	Detection of some parasites (e.g., trematoda) requires centrifugation and so, for very accurate applications, it is suggested that the FLOTAC technique be used

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chambers in the apparatus that are designed for optimal microscopic examination of fecal sample suspensions within the chambers (total volume: 2 ml) using the two ruled grids on the surface of the reading disk, which divide each chamber into 12 sections (**Fig. 1**, ii). A step-by-step assembly of the Mini-FLOTAC is shown in **Figure 2**.

The Mini-FLOTAC permits a maximum magnification of 400×. This level of magnification is required for the detection and diagnosis of intestinal protozoa, which are small in size, and for the identification of animal lungworm larvae.

Fill-FLOTAC

Fill-FLOTAC is a kit made of polycarbonate amorphous thermoplastic, composed of a graduated container, a lid (with two screw caps), a collector/homogenizer pole, and a filter (**Fig. 3**). There are two versions of Fill-FLOTAC: Fill-FLOTAC 2, which permits analysis of up to 2 g of feces (usually used for examination of feces collected from cats, dogs, and humans), and Fill-FLOTAC 5, which enables analysis of up to 5 g of feces (usually used for examination of feces from livestock; see **Fig. 3**).

The containers for the two Fill-FLOTAC versions are graduated, semitransparent, and screw into the lid, allowing hermetic closure. There is a cone on the bottom of the graduated container, which permits homogenization of samples within the closed system of the container. On the top of the lid, there are two holes with screw caps: one central (with a large screw cap) for the collector/homogenizer pole and one lateral (with a small screw cap) for passage of filtered samples. The upper end (handle) of the collector/homogenizer pole is slightly thicker, whereas the lower part is conical and fits over the cone on the bottom of the graduated container. The conical end of the collector/homogenizer pole has a volume of either 2 g (Fill-FLOTAC 2) or 5 g (Fill-FLOTAC 5). As the name suggests, this part of the Fill-FLOTAC allows the collection and homogenization of the fecal sample in the Fill-FLOTAC container before laboratory processing. The filter is in the lower part of the lid. The thin plastic layer is perforated with 250-μm holes to ensure an optimal filtration of the fecal suspension. There are also two accessories: a tip to fill the Mini-FLOTAC and a device to disassemble the Fill-FLOTAC filter (**Fig. 3**).

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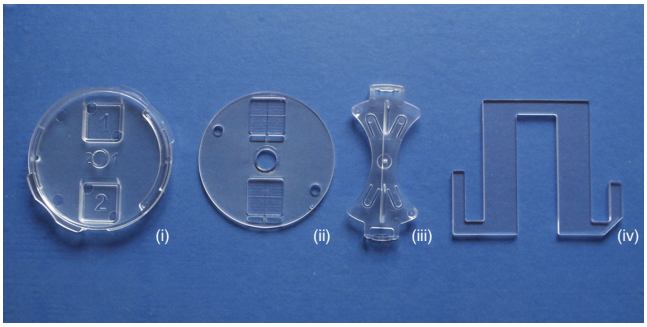

Figure 1 | Mini-FLOTAC components. (i) Base; (ii) reading disk with two ruled grids; (iii) key; and (iv) microscope adaptor.

The Fill-FLOTAC enables the first four steps of the Mini-FLOTAC technique—i.e., (i) sample collection and weighing; (ii) homogenization; (iii) filtration; and (iv) filling of the Mini-FLOTAC chambers—to be easily and safely performed. The Fill-FLOTAC permits the collection and analysis of fresh fecal samples, but also offers the opportunity to add fixative, so that samples can be transferred and stored, pending diagnostic workup.

Characteristics of the Mini-FLOTAC technique

Mini-FLOTAC[20] allows the simultaneous diagnosis of helminth eggs/larvae, and protozoa oocysts/cysts, offering an advantage over other copromicroscopic techniques, such as the Kato-Katz method, which is unsuitable for larval diagnosis, or the McMaster technique, which does not allow identification of intestinal protozoa and larvae (see also **Table 1**). Moreover, the Mini-FLOTAC also permits the diagnosis of yeasts (*Macrorhabdus ornithogaster*) in bird feces, as described by Borrelli *et al.*[21]

The most important difference between Mini-FLOTAC and FLOTAC is in their analytical sensitivity; whereas the sensitivity of FLOTAC is 1 egg/larvae/oocyst/cyst per gram of feces (EPG/LPG/OPG/CPG), the respective sensitivity of Mini-FLOTAC is 5 EPG/LPG/OPG/CPG. This is explained by a 5-fold-lower sample volume in the Mini-FLOTAC chambers as compared with that of FLOTAC (2 ml of fecal suspension held in the Mini-FLOTAC device versus 10 ml in FLOTAC).

Barda *et al.*[22,23] reported the main advantages of the Mini-FLOTAC technique. The Mini-FLOTAC technique can be performed on either fresh or fixed fecal samples, offering an opportunity to process samples days or weeks after transfer to the laboratory. In fact, use of the Fill-FLOTAC closed system apparatus enables a fecal sample to be preserved, and then allows the user to progress to the diagnosis without requiring sample transfer to another container, thus minimizing exposure of humans to potentially infectious fecal material and fixatives. The use of fixatives is not possible in more widely used techniques, such as the Kato-Katz technique for the diagnosis of helminth eggs in human stool samples. Moreover, the Mini-FLOTAC technique allows processing of pooled fecal samples (as described in the 'Experimental design')[24]. As the Mini-FLOTAC technique is easy to use, it is of particular value for laboratories with limited resources or for application directly on-farm. These logistical advantages facilitate fieldwork in remote areas, where laboratories are far away from collection sites[25], and they also permit quality control after fixed samples have reached laboratories where trained microscopists assess the samples. Importantly, both the Mini-FLOTAC and Fill-FLOTAC devices are reusable (up to 50 times) after thorough washing, which lowers costs per fecal sample analyzed. The Mini-FLOTAC can also be used for the diagnosis of parasites in human and animal urine, but the protocol for this, at the moment, requires centrifugation to obtain the pellet, as described in Maurelli *et al.*[26].

One of the main limitations of the Mini-FLOTAC technique, as with any copromicroscopic technique based on flotation (e.g., simple flotation, Wisconsin, and McMaster), is that the choice of fixative used for fecal preservation (e.g., 5% (vol/vol) formalin and sodium acetate–acetic acid–formalin), the duration of fecal preservation before Mini-FLOTAC analysis, and the selection of the flotation solution (FS) might influence the performance of the Mini-FLOTAC technique[22], specifically affecting the percentage of parasitic elements (PEs) recovered. In fact, as was reported for the FLOTAC technique by Kochanowski *et al.*[27] and by Ruzicova *et al.*[28], the percentage of recovery can sometimes be low. Two studies have been performed on the percentage of recovery of eggs by Mini-FLOTAC; Noel *et al.*[29] used the Mini-FLOTAC technique for the diagnosis of equine strongyles and recovered 42.6% of the eggs, whereas Bosco *et al.*[30] performed Mini-FLOTAC for the diagnosis of ovine and equine gastrointestinal strongyles and reported an egg recovery rate of 100%. Various factors might explain this difference, such as the procedure for egg isolation and the flotation solution used. Additional research is necessary to identify key factors explaining incomplete recovery of eggs and to verify further the correlation between the theoretical and real sensitivity using the Mini-FLOTAC and other flotation-based diagnostic techniques.

Moreover, care is indicated with some ingredients because of environmental (e.g., zinc sulfate) and human toxicity (e.g., formalin). Another limitation of the Mini-FLOTAC technique is the lower sensitivity as compared with the FLOTAC technique, and hence, for very accurate applications, it is suggested that the FLOTAC technique should be used.

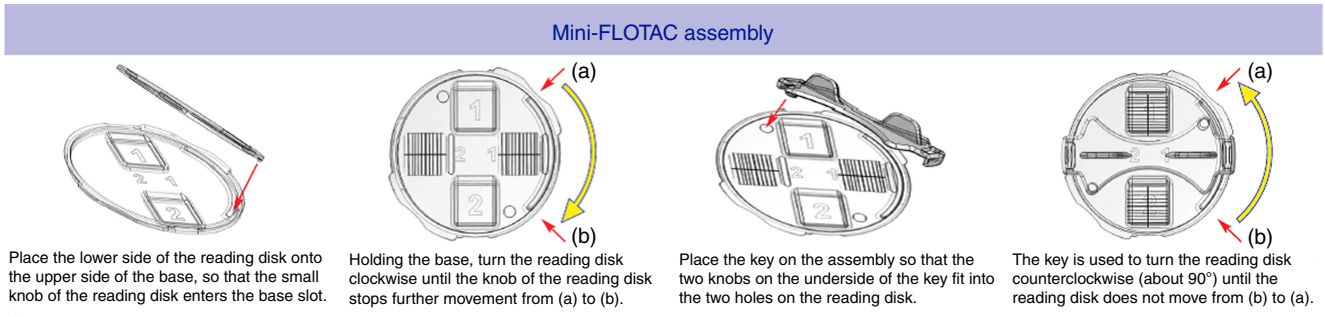

Figure 2 | Step-by-step assembly of the Mini-FLOTAC.

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Figure 3 | Fill-FLOTAC 2 and Fill-FLOTAC 5. (i) Fill-FLOTAC 2 container; (ii) Fill-FLOTAC 2 lid (with collector/homogenizer and filter); (iii) small screw cap; (iv) large screw cap; (v) tip to fill Mini-FLOTAC; (vi) Fill-FLOTAC 5 container; (vii) Fill-FLOTAC 5 lid (with collector/homogenizer and filter); (viii) device used to disassemble the Fill-FLOTAC.

The diagnosis of an individual sample using the Mini-FLOTAC approach takes ~12 min (refs. 22,31). There are some more rapid copromicroscopic techniques in the medical and veterinary fields (e.g., direct smear), but these are less sensitive, accurate, and precise than Mini-FLOTAC[32].

Experimental design

The accuracy and precision of any copromicroscopic technique depends on how fecal samples are processed (e.g., amount of feces examined, use of individual or pooled fecal samples) and preserved[33]. PEs are not equally distributed within a fecal sample, rather they are clustered[34,35], and hence it is recommended to sample a large proportion of the fecal sample and to thoroughly homogenize the sample to ensure an accurate and precise diagnosis. When applying the Fill-FLOTAC, the Mini-FLOTAC technique is one of the few methods that allows users to sample and homogenize a large amount of feces, up to 5 g, and to analyze 200 mg of feces, which is in contrast to other commonly applied techniques for the diagnosis of human (Kato-Katz: 41.7 mg of feces) and animal (McMaster: up to 66.7 mg of feces) samples. It can be demonstrated that the accuracy and precision of the diagnosis of PE is correlated with the amount of feces examined[33,36], with the results being more accurate and precise when more feces are examined.

Moreover, regarding sample pooling, Kenyon *et al.*[24] and Rinaldi *et al.*[37] validated the use of pooled fecal samples for the assessment of nematode infection intensity and anthelmintic drug efficacy (by fecal egg count reduction; FECR) in sheep. The pooling of ovine fecal samples proved to be a valid procedure for assessing nematode FEC and FECR in sheep by both the McMaster and Mini-FLOTAC techniques, and hence it can be used for a rapid and economic detection of PEs. Recently, pooling of biological samples has also been tested in human samples by using the McMaster and Kato-Katz techniques for feces and by filtration technique and point-of-care circulating cathodic antigen test for urine, including cost-effectiveness analysis[38–41], but more research remains to be conducted.

Sample preservation. To store feces for long periods (several weeks), different methods of preservation for human, pet animal, and livestock feces are recommended. For humans and pets, the feces can be stored in 5% formalin, with a minimal fixation ratio of 1:1 (one part feces plus one part 5% formalin)[31,42]. This quantity of formalin is low, to ensure safety of the operator, and it is sufficient to fix the feces for up to 3 weeks before microscopic examination[42]. It is important to homogenize fixed feces thoroughly as soon as they are put into the Fill-FLOTAC. Experience obtained thus far suggests that the use of fresh feces produces the most accurate results, but 5% formalin produced more accurate results than other fecal preservatives (e.g., 10% formalin and sodium acetate–acetic acid–formalin)[4]. For livestock, the feces can be stored by vacuum packaging at +4 °C for up to 3 weeks[36,37,43].

Barda *et al.*[42] evaluated the temporal effect of 5% formalin on preservation of human feces positive for soil-transmitted helminths (STHs; *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*), using the Mini-FLOTAC technique. The feces were fixed at a ratio of 1:1 with 5% formalin and subsequently examined at time periods up to 31 d after collection. For *T. trichiura*, the prevalence over the 31-d observation period remained at 100%, and for *A. lumbricoides* the prevalence remained fairly constant (77.4%). However, for hookworm, the prevalence gradually declined, starting at around day 15 post collection. Hence, for an accurate quantitative diagnosis of hookworm, with a 1:1 dilution with 5% formalin, it was suggested that fecal samples should be examined within a maximum of 15 d of preservation. For a longer period of preservation, a different dilution ratio, different formalin concentration, or another fixative would have to be evaluated.

MATERIALS

REAGENTS

FS

- Sodium chloride (NaCl; AppliChem, cat. no. A1149)
- Zinc sulfate heptahydrate ($ZnSO_4$ $7H_2O$; AppliChem, cat. no. A1000) **! CAUTION** It is an irritant to humans and is dangerous to the environment.

Fixative

- Formaldehyde (CH_2O) solution, 40% (for 5% formalin; Lab-Scan, cat. no. A3548M) **! CAUTION** It is toxic to humans and dangerous to the environment.

EQUIPMENT

- Fill-FLOTAC (http://www.parassitologia.unina.it/flotac/fill-flotac/how-to-get/?lang=en)
- Mini-FLOTAC (http://www.parassitologia.unina.it/flotac/mini-flotac/how-to-get/?lang=en)
- Wooden spatula (Doc, cat. no. TTT-ABB 01)
- Timer (Control Company, cat. no. 5004)
- Microscope adaptor for Mini-FLOTAC (http://www.parassitologia.unina.it/flotac/mini-flotac/how-to-get/?lang=en)
- Tip for Fill-FLOTAC (http://www.parassitologia.unina.it/flotac/fill-flotac/how-to-get/?lang=en)
- Device to disassemble the filter of the Fill-FLOTAC (http://www.parassitologia.unina.it/flotac/fill-flotac/how-to-get/?lang=en)
- Conventional optical microscope (Leica, model no. DM 1000, cat. no. 10052-382)

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• A hand tally counter (Carlo Erba, cat. no. 284000211)
• Cylinder (Biosigma, cat. no. 068771)
• Hydrometer (LabCenter, cat. no. 9004060)
• Magnetic stirrer (Falc, cat. no. 601.0122.60)
• Chemical safety cabinet (Momoline, cat. no. Ecoair 2006)

REAGENT SETUP

FS Thus far, three FSs have been widely used with the Mini-FLOTAC technique: FS2 (sodium chloride, specific gravity (s.g.) = 1.20), FS3 (zinc sulfate, s.g. = 1.20), and FS7 (zinc sulfate, s.g. = 1.35). You can find the details about chemical composition, s.g., and recipe in our previous FLOTAC protocol[4]. ▲ **CRITICAL** It should be noted that not all the FSs available in specialized parasitology laboratories can be used with the Mini-FLOTAC technique. Until new data become available, it is advisable not to use any FS other than those listed here.

Fixative You can find the recipe for 5% formalin in our previous FLOTAC protocol[4].

PROCEDURE

Preparation of the sample ● TIMING 1–5 min

1| Prepare the sample, following one of the five options below, based on different types of feces: use option A for fresh human feces, option B for fixed human feces, option C for fresh feces of cats and dogs, option D for fixed feces of cats and dogs, or option E for fresh feces of livestock.

(A) Fresh human feces

(i) Add 38 ml of FS (dilution ratio 1:20) to the Fill-FLOTAC 2 container.

(ii) Carefully homogenize the fecal sample by mixing it with a wooden spatula, and fill the conical collector (2 g of feces) of the Fill-FLOTAC 2 and level the surface.

▲ **CRITICAL STEP** The PEs are not distributed homogeneously in the sample, so it is very important (especially if infection intensities are low) to carefully homogenize the fecal sample for ~10–20 s (depending on the consistency of the sample) before filling the conical collector to avoid false-negative results.

▲ **CRITICAL STEP** To obtain accurately weighed samples, they must be pressed into the conical collector and then the sample must be made level with the edge of the cone.

(B) Fixed human feces

(i) Repeat Step 1A(ii).

(ii) To preserve the sample, add 2 ml of 5% formalin into the Fill-FLOTAC container.

(iii) Homogenize the fecal suspension from Step 1B(i) in the fixative from Step 1B(ii) by pumping the conical collector up and down in the container (10 times), while turning to the right and left.

▲ **CRITICAL STEP** To ensure that samples are fixed properly, accurately homogenize the feces in the fixative. Make sure that all the feces are suspended.

■ **PAUSE POINT** Wait at least 30 min before the analysis. The samples can be stored at room temperature in 5% formalin for up to 15 d for human feces[42] and up to 1 month for animal feces[43].

(iv) To allow analysis of the sample, add up to 40 ml (1:20 dilution ratio) of FS to the Fill-FLOTAC container.

(C) Fresh feces of cats and dogs

(i) Repeat Step 1A(i–ii), using 18 ml of FS (dilution ratio 1:10).

(D) Fixed feces of cats and dogs

(i) Repeat Step 1B(i–iv), adding up to 20 ml of FS (1:10 dilution ratio).

(E) Fresh feces of livestock

(i) Repeat Step 1A(i–ii), using 45 ml of FS (dilution ratio 1:10) in a Fill-FLOTAC 5 container and fill the conical collector with 5 g of feces.

2| Homogenize the fecal suspension by pumping the conical collector up and down (10 times) in the container, while turning to the right and left.

▲ **CRITICAL STEP** It is important to accurately homogenize the sample to ensure that all the sample is mixed with FS. Make sure that all the fecal material is suspended. Mix the sample carefully to avoid the formation of too many bubbles.

3| Put the tip on the lateral hole of the Fill-FLOTAC. Invert the Fill-FLOTAC 5 times to mix the sample and squeeze the Fill-FLOTAC container to filter the sample (because under the lid of the Fill-FLOTAC there is a filter) and pour it from the tip. Use the tip to fill the flotation chambers of the Mini-FLOTAC through the filling holes, until a meniscus is formed.

▲ **CRITICAL STEP** It is important that the suspension be mixed five times, by inverting the Fill-FLOTAC, just before filling each of the chambers to ensure that the PEs in the fecal suspension are homogeneously distributed in the two chambers.

▲ **CRITICAL STEP** To avoid formation of air bubbles, the chambers should be filled with the Mini-FLOTAC apparatus held at an angle of ~45°.

? TROUBLESHOOTING

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4| After 10 min, use the key to turn the reading disk clockwise (~90°) until the reading disk stops, to completely separate the floating PEs from the parts of the Mini-FLOTAC apparatus that contain the fecal debris. Remove the key.
▲ **CRITICAL STEP** To allow the PEs to fully disperse into the flotation solution, the Mini-FLOTAC device should rest for ~10 min.

Examination of the Mini-FLOTAC apparatus under a microscope ● TIMING 1–5 min

5| Put the microscope adaptor (**Fig. 1**, iv) on the stage of the microscope and place the Mini-FLOTAC on the microscope adaptor (**Fig. 4**) with the ruled grid of chamber no. 1 on the right. Focusing on the grid (**Fig. 4**), start from one corner and count the different PEs in all 12 sections of the first chamber, using a hand tally counter to record the PE numbers; then repeat the count for the second chamber. It is important to perform these counts in one sitting.
▲ **CRITICAL STEP** The density of PEs can affect the timing and the accuracy of microscopic readings. Our experience thus far indicates that count results are accurate when the number of PEs is <250 per ruled grid, because it is a number that is easy to count. When the number of PEs per ruled grid is >250, it is advisable to use a higher-dilution ratio (at Step 1A(i) for fresh feces or 1B(iv) for fixed feces) and/or to examine only a part (half chamber, reading alternate sections) of each ruled grid.
? TROUBLESHOOTING

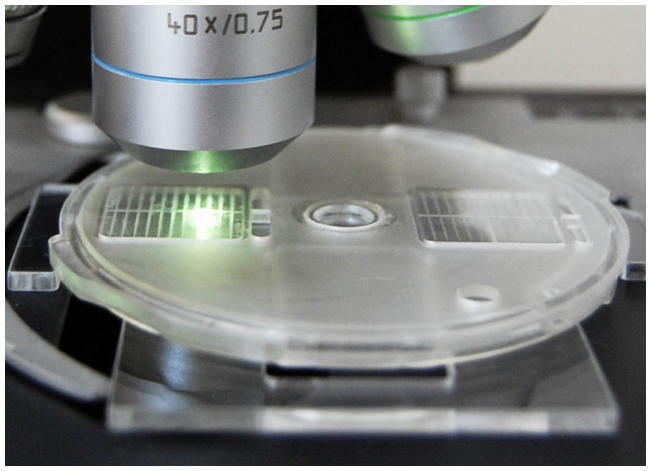

Figure 4 | The Mini-FLOTAC apparatus being analyzed under a microscope. The microscope is focused on one of the two flotation chambers, each of which is divided into 12 sections by the ruled grid on the reading disk to facilitate counting of PEs in the sample.

Analysis of results ● TIMING 5–40 s

6| Calculate the multiplication factor used to obtain the EPG/LPG/OPG/CPG by dividing the dilution ratio by the volume, e.g., if you use a dilution ratio of 1:10 and read both whole chambers of the Mini-FLOTAC, with a total volume of 2 ml, the multiplication factor is 10/2 = 5. Therefore, for fresh and fixed feces of cats, dogs, and livestock, the multiplication factor is 5 (dilution ratio is 1:10 and 2 chambers of 1 ml are examined), whereas the multiplication factor is 10 for fresh and fixed human feces (dilution ratio is 1:20 and 2 chambers of 1 ml are examined). Calculate EPG/LPG/OPG/CPG in your sample by multiplying the number of PEs counted in Step 5 by the appropriate multiplication factor.

Cleaning ● TIMING 3–4 min

7| To disassemble the Mini-FLOTAC device, turn the reading disk clockwise (~90°) and open the Mini-FLOTAC device, bringing out the raised portion from the arched slot. If need be, use the key to remove the disk. To disassemble the Fill-FLOTAC, remove all the components, using the appropriate accessory device to remove the filter.

8| Wash all the components thoroughly using neutral dish soap.
▲ **CRITICAL STEP** It is important to thoroughly wash all the components of the Mini-FLOTAC and Fill-FLOTAC devices before reuse to avoid contamination and, hence, false-positive results (the apparatus may be reused up to 50 times).

? TROUBLESHOOTING
Troubleshooting advice can be found in **Table 2**.

TABLE 2 | Troubleshooting table.

Step	Problem	Possible reason	Solution
3	Formation of air bubbles	Incorrect filling of the chambers	Fill the chambers of the Mini-FLOTAC while inclining the apparatus at 45°, until a meniscus is formed
		Mini-FLOTAC has been reused too many times (>50 times)	Replace the Mini-FLOTAC
5	Difficulty visualizing PEs	Presence of too much debris	Increase the dilution ratio, adding a higher quantity of FS. This can be very important, especially in feces obtained from sheep and goats, which are often very dark

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● TIMING

Steps 1–4, for fresh feces (Steps 1A, 1C, and 1E), preparation of the sample: 1–2 min
Steps 1–4, for feces fixed in 5% formalin (Steps 1B and 1D), preparation of the sample: 4–5 min
Step 5, examination of the Mini-FLOTAC apparatus under a microscope: 1–5 min, depending on the number of PEs and the experience of the technician
Step 6, analysis of results: 5–40 s, depending on how many species of parasites are found in the sample
Steps 7 and 8, cleaning: 3–4 min
As the Mini-FLOTAC technique allows the simultaneous detection of both helminths and protozoa, the number of parasitological diagnoses possible in a given time varies. For example, 20 Mini-FLOTAC devices can be analyzed, and hence up to 300 parasitological diagnoses (nearly 15 different PEs per device contemporaneously in a total of 1–5 min) can be performed daily by one investigator for sheep.

ANTICIPATED RESULTS

Results obtained thus far suggest that the Mini-FLOTAC technique has a higher sensitivity than other diagnostic techniques for detecting PEs in human and animal feces and urine[22–24,26,29,30,32,37,44–50]. The Mini-FLOTAC technique has been successfully used for the diagnosis of STHs and other helminths (e.g., *Schistosoma mansoni* and *Hymenolepis nana*)

TABLE 3 | Experience obtained thus far with the Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in different animal host species (cattle, buffalo, sheep, goat, cat, dog, and horse) and humans, with differing fecal preservation methods, PEs, and FSs.

				FS (specific gravity)			
Host	Preservation method	Parasite	PE	NaCl (1.20)	$ZnSO_4$ (1.20)	$ZnSO_4$ (1.35)	Glucose–salt solution (1.24–1.26)
Cattle, buffalo, sheep, and goat	Fresh or vacuum-packaging preserved	*Eimeria* spp.	Oocysts	+++	++	+	NT
Cattle, buffalo, sheep, and goat	Fresh or vacuum-packaging preserved	Gastrointestinal strongyles	Eggs	+++	++	+	++
Cattle, buffalo, sheep, and goat	Fresh or vacuum-packaging preserved	*Calicophoron daubneyi*	Eggs	–	–	+++	NT
Horse	Fresh	*Parascaris equorum*	Eggs	+	+	+++	+++
Horse	Fresh	Strongyles	Eggs	+++	++	+	+++
Dogs and cats	Fresh or F5% preserved	*Toxocara* spp.	Eggs	++	++	+++	NT
Dogs and cats	Fresh or F5% preserved	Hookworm	Eggs	+++	++	+	NT
Dogs and cats	Fresh or F5% preserved	*Trichuris* spp.	Eggs	+++	+++	+++	NT
Dogs and cats	Fresh or F5% preserved	Lungworms	Larvae	+	+++	++	NT
Dogs and cats	Fresh or F5% preserved	*Giardia* spp.	Cysts	+	+++	++	NT
Dogs and cats	Fresh or F5% preserved	*Isospora* spp.	Oocysts	+++	++	+	NT
Birds	Fresh	*Macrorhabdus ornithogaster*	Spores	++	+++	NT	NT
Birds	Fresh	*Isospora* spp.	Oocysts	+++	NT	+	NT
Humans	Fresh or F5% preserved	*Ascaris lumbricoides*	Eggs	++	NT	+++	NT
Humans	Fresh or F5% preserved	Hookworm	Eggs	+++	NT	+	NT
Humans	Fresh or F5% preserved	*Schistosoma mansoni*	Eggs	+	NT	+++	NT
Humans	Fresh or F5% preserved	*Hymenolepis nana*	Eggs	+++	NT	+	NT

+++, most efficient; ++, efficient; +, less efficient; –, did not work for this parasite; F5%, 5% formalin; NT, not tested.

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in humans[22,23,42,44–46,51–54], and is reported to be more cost-effective than using, for example, the Kato-Katz technique for the diagnosis of STHs in humans[51–53]. Further improvements are required, however, to enhance accuracy when using the Mini-FLOTAC technique for the diagnosis of intestinal protozoa in humans[22,23,42,44–46]. With regard to animals, the Mini-FLOTAC technique has been successfully used for the diagnosis of *Toxocara canis*, hookworm, *Trichuris vulpis*[32,47], lungworms, *Giardia* spp., and *Isospora* spp. (unpublished data, G.C., P. Pepe, D. Ianniello, R. Vascone (Department of Veterinary Medicine and Animal Productions, University of Naples), & L.R.) in feces of dogs. In cat feces, the Mini-FLOTAC technique was more sensitive than the Kova slide for the diagnosis of *Toxoplasma gondii*[48]. In goats, promising results were obtained with the Mini-FLOTAC technique for *Eimeria* spp.[49] In sheep, accurate detection of gastrointestinal nematodes has been reported[24,37,50]. In cattle, the technique was successfully used for the diagnosis of the rumen fluke *Calicophoron daubneyi*[55]. In horses, the Mini-FLOTAC technique was successfully used for the diagnosis of *Parascaris* spp. and strongyle eggs[29,31,56]. Finally, in birds, the Mini-FLOTAC technique was used for the detection of the yeast *M. ornithogaster* and the protozoa *Isospora* spp.[21] The technique also proved useful for the diagnosis of *Capillaria plica* (*Pearsonema plica*) in dog urine[26]. **Table 3** summarizes the experience gained thus far with the Mini-FLOTAC technique for diagnosis of PEs in feces of different hosts. For parasite species not reported in this paper, validation of the Mini-FLOTAC technique is required, and those who are interested in using and further validating the Mini-FLOTAC technique are invited to contact the corresponding author.

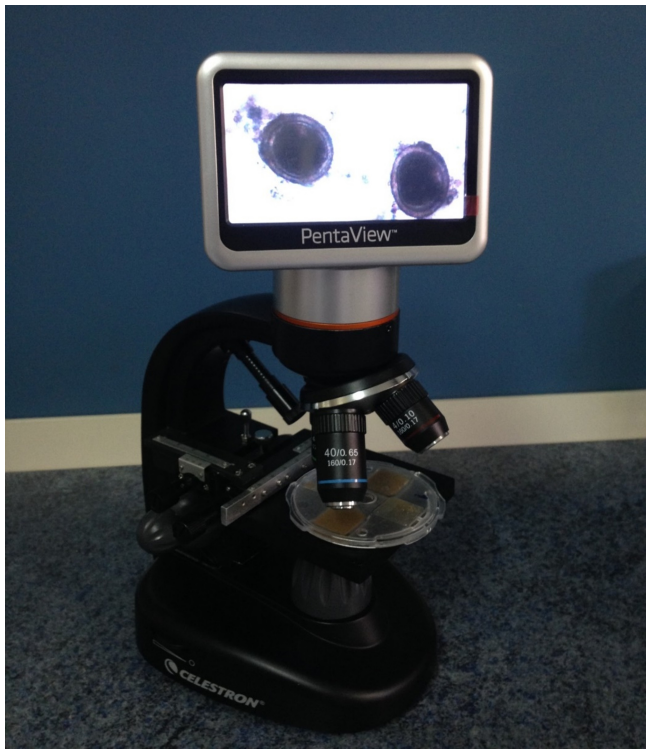

Figure 5 | Mini-FLOTAC under a portable microscope for analysis directly in the field.

Comparative studies performed with Mini-FLOTAC and other FEC techniques revealed that the Mini-FLOTAC technique produces (i) markedly fewer negative results than those obtained by other, currently more widely used techniques, thus indicating high sensitivity, and (ii) variances in PE counts per gram of feces that are lower and ranges that are more narrow than those observed for other techniques, thus indicating high precision and accuracy of Mini-FLOTAC[26,47,49,50].

Taken together, these results indicate that the Mini-FLOTAC technique is a promising method for more precise and more accurate detection and quantification of PEs in human and animal feces than widely used copromicroscopic techniques (e.g., McMaster and Kato-Katz), especially in laboratories in which the centrifugation step cannot be performed[57] or for veterinary use directly on-farm. Moreover, the Mini-FLOTAC technique enhances the safety of the operator in comparison with other copromicroscopic techniques, because the Fill-FLOTAC device acts as a closed system so that the technician is in contact with sample only to homogenize the sample before filling the collector of the Fill-FLOTAC.

The Mini-FLOTAC can be used also with portable microscopes (**Fig. 5**), and further developments of the Mini-FLOTAC technique in the veterinary field are in progress, using online decision support tools to assist veterinarians and farmers in the detection of anthelmintic drug resistance and to optimize control strategies so that evidence-based parasite control strategies can be effectively implemented in the future.

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