Standard Operating Procedures (SOPs) for the diagnosis of *Fasciola* spp. and Paramphistomidae eggs in cattle using the Mini-FLOTAC technique

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ETHICS APPROVAL NO: **PG/2022/0008127**

1. GOAL

The Mini-FLOTAC technique is aimed to perform multivalent quali-quantitative diagnosis of helminth and protozoan infections in animals and humans. These SOPs provide instructions on the laboratory activities, i.e. the preparation and copromicroscopic analysis of cattle faecal samples for the diagnosis of *Fasciola* spp. and Paramphistomidae eggs using the Mini-FLOTAC technique, in the framework of the PREPARE4VBD project.

2. EQUIPMENTS, MATERIALS AND REAGENTS

- Gloves
- Fill-FLOTAC 5g
- Mini-FLOTAC
- Wooden spatula
- Tip for Fill-FLOTAC
- Timer
- Microscope adaptor for Mini-FLOTAC
- Conventional optical microscope
- A hand tally counter
- Device to disassemble the filter of the Fill-FLOTAC
- Flotation solution (Zinc sulphate heptahydrate; ZnSO4 7H2O; specific gravity 1,350)
- Cylinder
- Hydrometer
- Magnetic stirrer

3. METHODOLOGY AND PROCEDURES

3.1 Preparation of flotation solution (FS)

- Combine 685 ml of water and 685 grams of zinc sulphate
- Dissolve the zinc sulphate in the water by stirring on a magnetic stirrer
- Check the s.g. (1,350) with a hydrometer

3.2 Mini-FLOTAC Technique

1. Add 45 ml of FS-Zinc sulphate 1,350 in the Fill-FLOTAC beaker (dilution ratio= 1:10).
2. Fill the conical collector of the Fill-FLOTAC with 5 g of faeces and level the surface.

CRITICAL STEP: The eggs are not distributed homogeneously in the sample, so it is very important (especially if infection intensities are low) to carefully homogenize the faecal 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.

3. Homogenize thoroughly the suspension.

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 faecal material is suspended. Mix the sample carefully to avoid the formation of too many bubbles.

4. 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 to fill the flotation chambers of the Mini-FLOTAC apparatus with the faecal suspension until a little meniscus if 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 eggs in the faecal 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°.

5. After 10 minutes, use the key to turn the reading disc clockwise (about 90°) until the reading disk stops, to completely separate the floating eggshells from the parts of the Mini-FLOTAC apparatus that contain the faecal debris. Remove the key and examine the Mini-FLOTAC under the microscope (10X objective), using the microscope adaptor. Place the Mini-FLOTAC on the microscope adaptor with the ruled grid of chamber no. 1 on the right. Focusing on the grid, start from one corner and count the eggshells of *Fasciola* spp. and Paramphistomidae in all 12 columns of the first chamber, using a hand tally counter to record the eggshells number; then repeat the count for the second chamber. It is important to perform these counts in one sitting.

Calculate the multiplication factor used to obtain the eggshells per gram (EPG) by dividing the dilution ratio by the reading 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. Calculate EPG in your sample by multiplying the number of eggshells counted in Step 5 by the appropriate multiplication factor.

CRITICAL STEP: The density of eggshells can affect the timing and the accuracy of readings. Our experience thus far indicates that count results are accurate when the number of eggs is <250 per ruled grid, because it is a number that is easy to count. When the number of eggs per ruled grid is >250, it is advisable to use a higher-dilution ratio (at Step 1 and/or to examine only a part (half chamber, reading alternate sections) of each ruled grid.

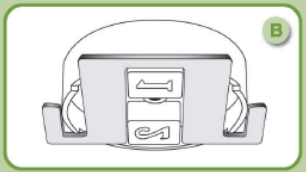
4. TROUBLESHOOTING

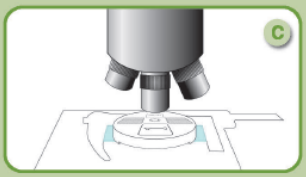
Step	Problem	Possible reason	Solution
3	Formation of air bubbles	Incorrect filling of the chambers Mini-FLOTAC has been reused too many times (>50 times)	Fill the chambers of the Mini-FLOTAC while inclining the apparatus at 45°, until a meniscus is formed Replace the Mini-FLOTAC
5	Difficulty visualizing eggshells	Presence of too much debris	Increase the dilution ratio, adding a higher quantity of FS.

Fill-FLOTAC manual

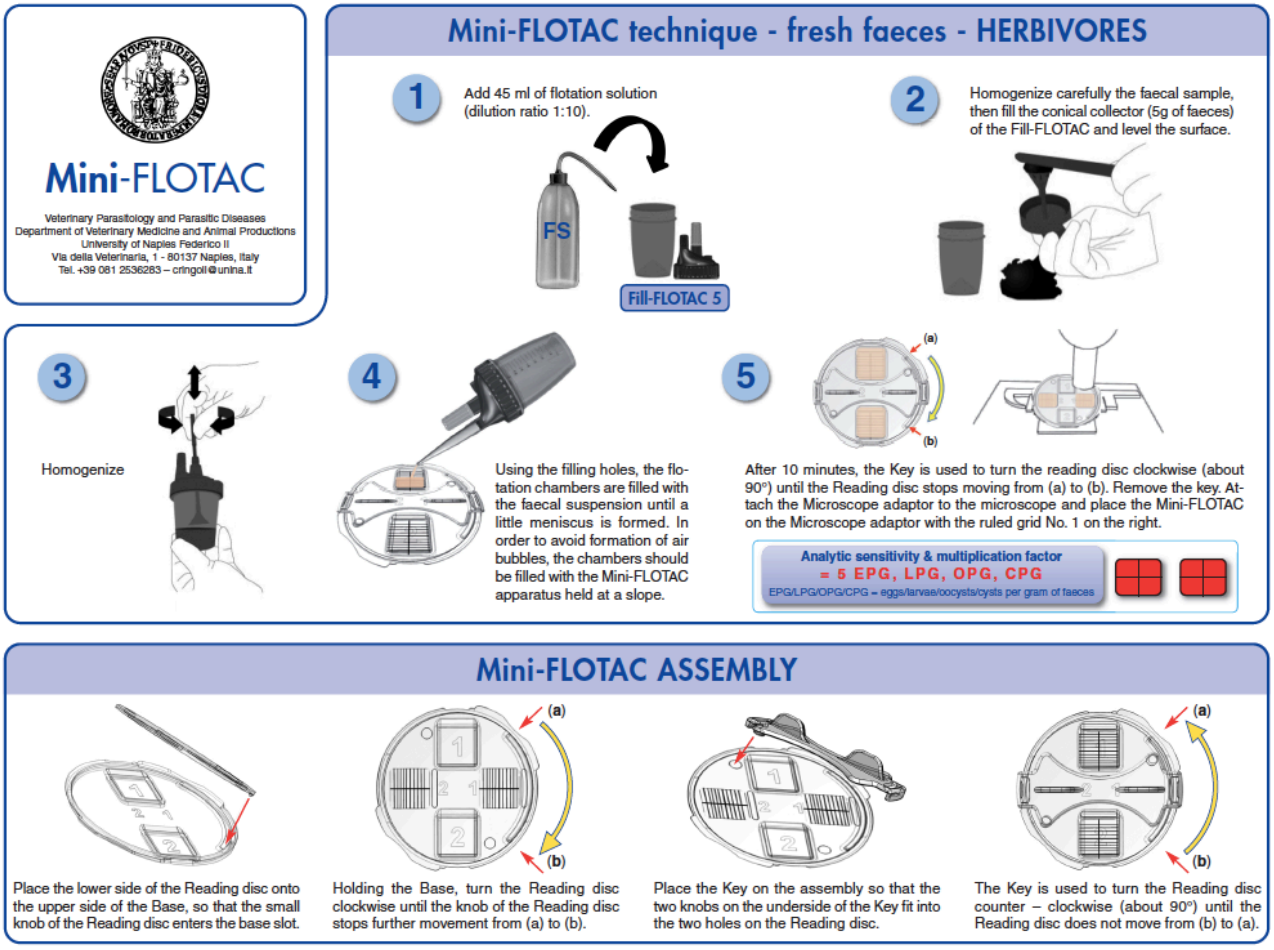
The **Microscope adaptor (A)** maintains the Mini-FLOTAC apparatus securely under the microscope, and it is transparent in colour in order to allow the unhindered passage of the microscope light **(B)**.

If the **Microscope adaptor** is inconsistent with the microscope translation table, the Mini-FLOTAC can be placed over a microscope slide on the microscope translation table **(C)**.





Mini-FLOTAC technique manual



References

Cringoli G, Maurelli MP, Levecke B, Bosco A, Vercruysse J, Utzinger J, Rinaldi L. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat Protoc. 12(9):1723-1732, 2017.

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Appendix 1. Faecal sample analysis sheet - Mini-FLOTAC technique

Farm ID: _____

City: _____ Province: _____

GPS coordinates: Longitude (N/S): _____
Latitude (E/W): _____ Altitude: _____

Name of Analyst: _____ Date of Analyzes:_____

Cattle ID	*Fasciola* spp.		Paramphistomidae	
	Number of eggshells	EPG*	Number of eggshells	EPG*
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

* Eggshells per gram of faeces