

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

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1. GOAL

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 Flukefinder technique, in the framework of the PREPARE4VBD project.

2. EQUIPMENTS, MATERIALS AND REAGENTS

2.1 Flukefinder Kit® that includes:

- Flukefinder apparatus (two units provided with a sieve each one: the Top unit (labelled) and the Bottom unit)
- graduated plastic cup
- plastic squirt bottle
- test tube (15 ml)
- concentric dish (Petri plate)
- disposable pipette
- wooden spatula

2.2 Other equipments and materials that are not included in the Flukefinder Kit:

- gloves
- balance
- conventional optical microscope
- a hand tally counter

3. METHODOLOGY AND PROCEDURES

3.1 Flukefinder Technique

1. Fit the two units together with the label upright and on top.
2. Holding the two joined pieces at a slight angle with the vent hole up, wet the screens by running water through the top.
3. Weight two grams of faeces and mix them with 30 ml of water in a plastic cup and pour into the top section.

CRITICAL STEP: Be sure the sample is mixed well with water.

4. Tap the Flukefinder against the side of the sink to expedite the passing of water through the screens.

CRITICAL STEP: Do not use spatulas on screens. Flush sample through screens with moderate force from tap water (3 or 4 times).

5. Briefly hold the column under the cold running tap until about half full.

6. Repeat steps 4 and 5 three times, then separate the top unit from the bottom and backwash debris (waste) from the top section into the sink until clean.
7. Invert the bottom unit over a cup and backwash the eggs and debris from the screen into the cup with a strong stream from a squirt bottle of water.
8. Swirl suspension and pour into a 15 ml test tube and allow to settle (about 4 minutes for 12-15 ml of water or about 3 minutes for 9-10 ml of water).
9. Slowly, in one movement, pour supernatant (liquid above the sediment) from the tube without disturbing the sediment and refill tube with 9-10 ml of water.
CRITICAL STEP: Pour off the supernatant slowly and in one movement to avoid mixing.
10. Let stand for 2 minutes only and pour supernatant from the tube.
11. Repeat steps 9 and 10 two or three times (until no more debris remains suspended after 2 minute of sedimentation).
12. Pour off the supernatant, swirl to suspend the sediment and pour quickly into the shallowest concentric scribed dish. Add a squirt of water to the tube, swirl and again quickly pour into the dish. Look for eggs using a dissecting microscope at 25x magnification or a standard microscope using the 4x objective. A drop of methylene blue dye will greatly enhance viewing.
CRITICAL STEP: Check the surface of the water when viewing, some eggs may float.

3.2 Cleaning Flukefinder kit

You can wash all the plastic materials. Thoroughly rinse the Flukefinder after use to insure accuracy of the next assay. Do not place Flukefinder in hot water or steam sterilizer as it will damage the device simply rinse in cool water.

References

Richards Dixon, www.flukefinder.com

Prof. Maria Paola Maurelli

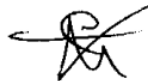


04/05/2022

Dr. Antonio Bosco



Dr. Lavinia Ciuca

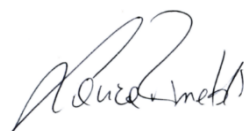


Reviewers

Signature

Date

Prof. Laura Rinaldi



05/05/2022

Approver

Signature

Date

Appendix 1. Faecal sample analysis sheet - Flukefinder Technique

Farm ID: _____		
City: _____ Province: _____		
GPS coordinates: Longitude (N/S): _____ Latitude (E/W): _____ Altitude: _____		
Name of Analyst: _____ Date of Analyzes: _____		
Cattle ID	<i>Fasciola</i> spp.	Paramphistomidae
	Number of eggs	Number of eggs
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
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20		