**Techniques for parasite assays and identification in faecal samples**

To diagnose gastro-intestinal parasites of ruminants, the parasites or their eggs/larvae must be recovered from the digestive tract of the animal or from faecal material. The following are the main tasks involved in this process:

 Collection of faecal samples
 Separation of eggs/larvae from faecal material, and their concentration
 Microscopical examination of prepared specimens

**Collection of faecal samples**

[Faecal samples for parasitological examination should be collected from the rectum of the animal](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e07.jpg). [If rectal samples cannot be obtained, fresh faecal samples may be collected from the pasture.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e08.jpg)

* Several samples should be collected. Samples should be dispatched as soon as possible to a laboratory in suitable containers such as: plastic containers.
[Each sample should be clearly labelled with animal identification, date and place of collection.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e09.jpg)
* Samples should be packed and dispatched in a cool box to avoid the eggs developing and hatching. If prolonged transport time to a laboratory is expected, the following may help to prevent the eggs developing and hatching.
* Adding 3% formal in to the faeces (5-20 ml, depending on the volume of faeces). This is to preserve parasite eggs. When samples are received in the laboratory they should immediately be stored in the refrigerator (4 °C) until they are processed. Samples can be kept in the refrigerator for up to 3 weeks without significant changes in the egg counts and the morphology of eggs.

SAMPLES SHOULD NEVER BE KEPT IN THE FREEZER.

**Equipments**

 Beakers or plastic containers
 A tea strainer or double layer cheesecloth
 Measuring cylinder or other container graded by volume
 stirring rod
 Test tube
 Test tube rack or a stand
 Microscope
 Microslides, coverslips
 Balance or teaspoon
 Flotation fluid. Ex.

1. Saturated salt solution (Sodium chloride: 400 grams, Water: 1000 ml)
2. Salt/sugar solution (Sodium chloride: 400 grams, Water: 1000 ml, Sugar: 500 grams)
3. Sodium nitrate (Sodium nitrate: 400 grams, Water: 1000 ml)

Methylene blue

 Microscope

 Funnel (size according to need)
 Funnel stand
 Rubber or plastic tubing
 Rubber bands

 Pasteur pipette
 Small petri dish(es)

**Simple test tube flotation**

**Principle**

The simple test tube flotation method is a qualitative test for the detection of nematode and cestode eggs and coccidia oocysts in the faeces. It is based on the separating of eggs from faecal material and concentrating them by means of a flotation fluid with an appropriate specific gravity.

**Application**

This is a good technique to use in initial surveys to establish which groups of parasites are present.

**Procedure:-**

[(a) Put approximately 3 g of faeces into Container 1.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0a.jpg)

[(b) Pour 50 ml flotation fluid into Container 1.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0b.jpg)

[(c) Mix faeces and flotation fluid thoroughly with a stirring device .](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0c.jpg)

[(d) Pour the resulting faecal suspension through a tea strainer into Container 2.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0d.jpg)

[(e) Pour the faecal suspension into a test tube from Container 2.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0e.jpg)

[(f) Place the test tube in a test tube rack or stand.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0f.jpg)

[(g) Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully place a coverslip on top of the test tube.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0g.jpg)

[(h) Let the test tube stand for 20 minutes.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0h.jpg)

(i) Carefully lift off the coverslip from the tube, together with the drop of fluid adhering to it, and immediately place the coverslip on a microscope slide.

**Sedimentation technique (for trematode eggs)**

**Principle**

The sedimentation technique is a qualitative method for detecting trematode eggs (Paramphistomum) in the faeces. Most trematode eggs are relatively large and heavy compared to nematode eggs. This technique concentrates them in a sediment.

**Application**

This is a procedure to assess the presence of trematode infections. It is generally run only when such infections are suspected (from previous postmortem findings on other animals in the herd/flock area), and is not run routinely. The procedure can be used to detect liver fluke (Fasciola) and Paramphistomum eggs.

**Procedure:-**

[(a) Weigh or measure approximately 3 g of faeces into Container 1.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0p.jpg)

[(b) Pour 40-50 ml of tap water into Container 1.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0q.jpg)

[(c) Mix thoroughly with a stirring device.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0r.jpg)

[(d) Filter the faecal suspension through a tea strainer into Container 2.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0s.jpg)

[(e) Pour the filtered material into a test tube.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0t.jpg)

(f) Allow to sediment for 5 minutes.

[(g) Remove the supernatant very carefully.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0u.jpg)

[(h) Resuspend the sediment in 5 ml of water.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0v.jpg)

[(i) Allow to sediment for 5 minutes.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0w.jpg)

[(j) Discard the supernatant very carefully.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0x.jpg)

[(k) Stain the sediment by adding one drop of methylene blue.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0y.jpg)

[(l) Transfer the sediment to a microslide. Cover with a coverslip.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e0z.jpg)

**Microscopical examination of prepared samples**

The prepared samples on microslides from the simple test tube flotation method and the sedimentation method are examined under a microscope at the magnifications listed in Table 1.

**Table 1 MAGNIFICATION LEVELS FOR EXAMINING PREPARED SAMPLES**

|  |  |
| --- | --- |
| **Magnification**  | **Parasites**  |
| 10 x 10  | Nematode and cestode eggs  |
| 10 x 40  | Coccidia oocysts  |
| 10 x 4  | Trematode eggs  |

|  |
| --- |
|  |

**Isolation and identification of lungworm larvae and infective larvae harvested from faecal cultures (the Baermann technique)**

**Principle**

The Baermann technique is used to isolate lungworm larvae from faecal samples and infective larvae from faecal cultures. It is based on the active migration of larvae from faeces suspended in water and their subsequent collection and identification.

**Application**

This is a procedure for harvesting infective larvae for identification purposes.

**Procedure**

[(a) Support the funnel by a stand.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1h.jpg)

[(b) Weigh or measure about 5-10 g of faecal culture/faeces and place it on a piece of double-layer cheesecloth.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1i.jpg)

[(c) Form the cheesecloth around the faeces as a "pouch".](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1j.jpg)

[(d) Close the pouch with a rubber band.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1k.jpg)

[(e) Fix a supporting stick under the rubber band .](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1l.jpg)

[(f) Place the pouch containing faecal culture material or faeces in the funnel.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1n.jpg)

[(g) Fill the funnel with lukewarm water, covering the faecal material.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1o.jpg)

[(h) Leave the apparatus in place for 24 hours, during which time larvae actively move out of faeces and ultimately collect by gravitation in the stem of the funnel.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1p.jpg)

[(i) Draw a few ml of fluid from the stem of the funnel into a small petri dish.](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1q.jpg)

[(j) Examine under dissecting microscope for live lungworm larvae (L1).](http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e1r.jpg)