SAMPLING

د.نور عبد الحميد عبدالله

Common laboratory procedures for diagnosing parasitism:

Parasitic infections are usually diagnosed from samples of

feces,

urine,

Blood

and tissue.

Collection of fecal samples:

Fecal samples for parasitological examination should be fresh collected from the rectum of the animal. collected from the animal during the act of defecation or from the rectum using a fecal loop during the physical examination. If rectal samples cannot be obtained, fresh fecal samples may be collected from the pasture.

Several samples should be collected. Samples should be dispatched as soon as possible to a laboratory in suitable containers such as:

screw cap bottles,

plastic containers with lids ,

disposable plastic sleeves/gloves used for collecting the samples ,

plastic bags.

Each sample should be

clearly labeled with animal identification(age, sex, no. , name of owner)

date(day season)

place of collection

and any other information to the case .

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,
to prevent the eggs developing and hatching or oocysts may have sporulated and these parasite may have died. the following may help :

(a) Filling the container to capacity or tightening the glove as close to the feces as possible. This is to exclude air from the container.

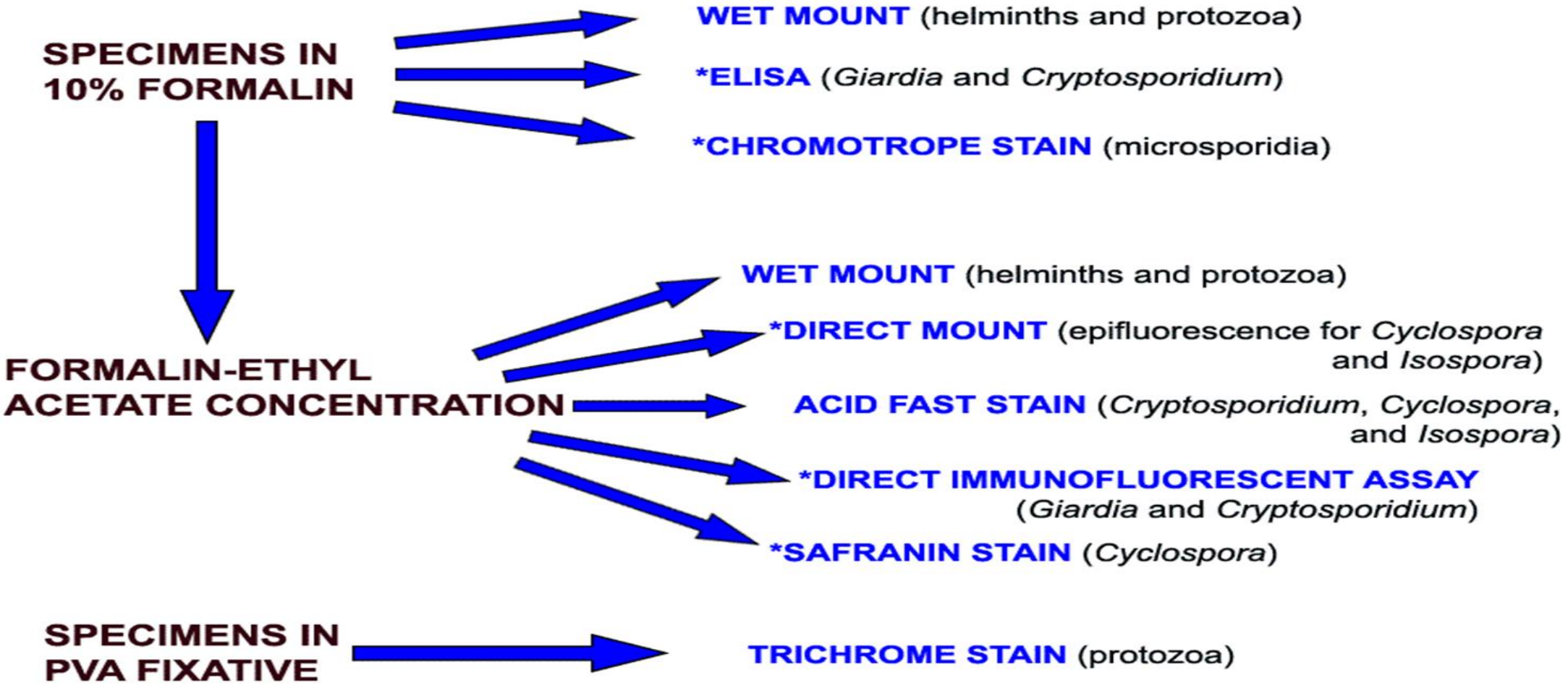
(b) If fresh feces can not promptly submitted , the sample should be refrigerated for no more 24 hours to stop the parasites development and reduce unpleasant odors.

(c) The amount of feces needed to perform a fecal examination approximately the size of an adult mans thumb.

(d) When samples are received in the laboratory they should immediately be stored in the refrigerator (4 °C) until they are processed

(e) Adding preservative solution as 10% formalin to the feces (5-20 ml, depending on the volume of feces). This is to preserve parasite eggs. (N.B Formalin-fixed feces cannot be used for fecal cultures.) . Other preservative solutions (eg, sodium acetate formalin, polyvinyl alcohol, merthiolate –iodine –formaldehyde.

Testing of Fecal Specimens Preserved in Formalin and PVA: (*indicates special test)



A-Gross examination of feces:

Consistency: the condition of the feces ,that is soft , watery (diarrheic) or very hard (constipation) , should be noted. This description will vary with animal species. For example, cattle feces are normally softer than those of horses or sheep. F=formed , S=soft , L=loose, W=watery

Color : unusual fecal colors should always be reported . for example , light- gray feces may indicated excessive fat in feces , assign of poor intestinal absorption.

Blood : blood may indicated severe parasitism as well as other intestinal disease.

Mucus : mucus on the surface of fresh feces may be associated with intestinal parasitism or some other metabolic disease.

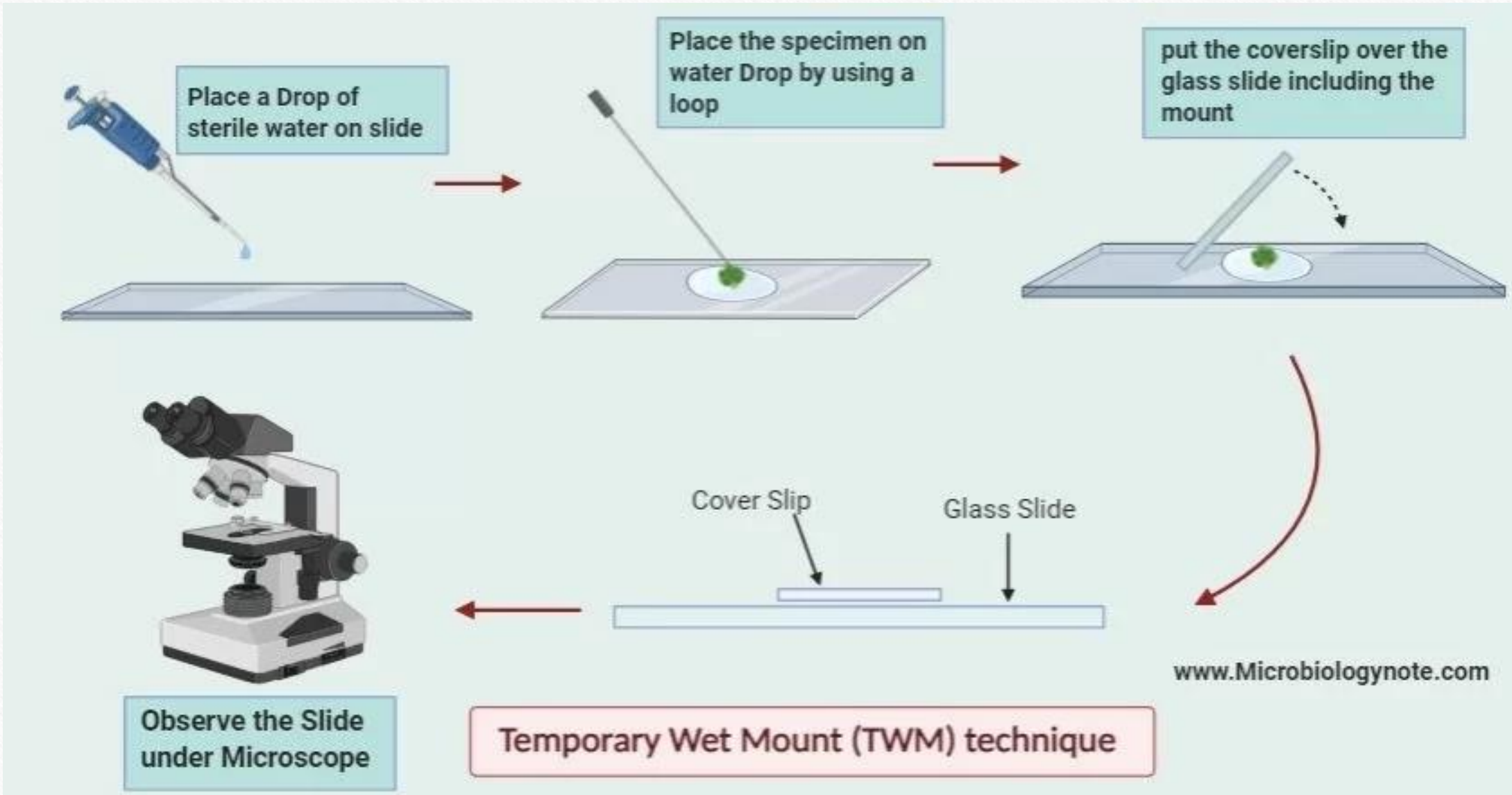
Age of the feces :in aged samples as noted earlier, parasite eggs may have embryonated or larvated ,oocysts may have sporulated or pseudoparasites may have present. Some protozoan parasites are recognized by their distinctive movements. In old feces these parasite may have died.

B-Direct smear:

Wet mount: The simplest method of microscopic fecal examination for parasites in the direct smear, the aim of its to observe Helminth eggs and oocyst of protozoa.

Procedure:

1. place several drops of normal (0.85% NaCl)saline or fecal flotation solution on slide with an equal amount of feces. Use normal saline (for motile organisms) and Lugol's iodine stain (to see internal morphologic structures, eg, those of Giardia),
2. mix the solution and feces together with a wooden applicator until the solution is homogenous.
3. smear the solution over the slide into a thin film.
4. remove any large pieces of feces.
5. place a coverslip over the smear.
6. examine the slide under the microscope and record any protozoan cysts, eggs ,larvae of parasite.


Place a Drop of sterile water on slide
Place the specimen on water Drop by using a loop
put the coverslip over the glass slide including the mount
Cover Slip
Glass Slide
Observe the Slide under Microscope
www.Microbiologynote.com
Temporary Wet Mount (TWM) technique

C- Stained smears: This type of smear is essential for accurate diagnostic detail and is also suitable for long-term storage for record purposes. The stains generally used are gemsa , malachite green , haematoxylin and trichrome .

Preparation of fixed stained smears by using giemza stain :

1 -make a light smears of stool on glass slides and air dry.

2-Fix in absolute methanol (methyl alcohol) (95%) for 3 minute and air dry.

3-Place smears in coplin gar (a glass pot) filled with giemza stain & let for 10 minutes .

4-Wash by buffered distal water .

5- air dry and examine under x10 , x 40 , x100.

D-concentration methods:

The aim is concentration eggs count in examined part of stool when the parasitic infection is light , it include :

a-flotation: The simple test for the detection of nematode and cestode eggs and some protozoan cysts oocysts in the feces by using flotation solutions as sheather sugar solution, sodium nitrate, zinc sulfate , magnesium sulfate , saturated sodium chloride flotation solution.(Zinc sulfate flotation repeated on 3 consecutive days, is the method of choice to reveal Giardia cysts, which are intermittently shed in feces.

Sheather's sugar can be used to detect small oocysts of Cryptosporidium or Toxoplasma)

400. (Figure 2)

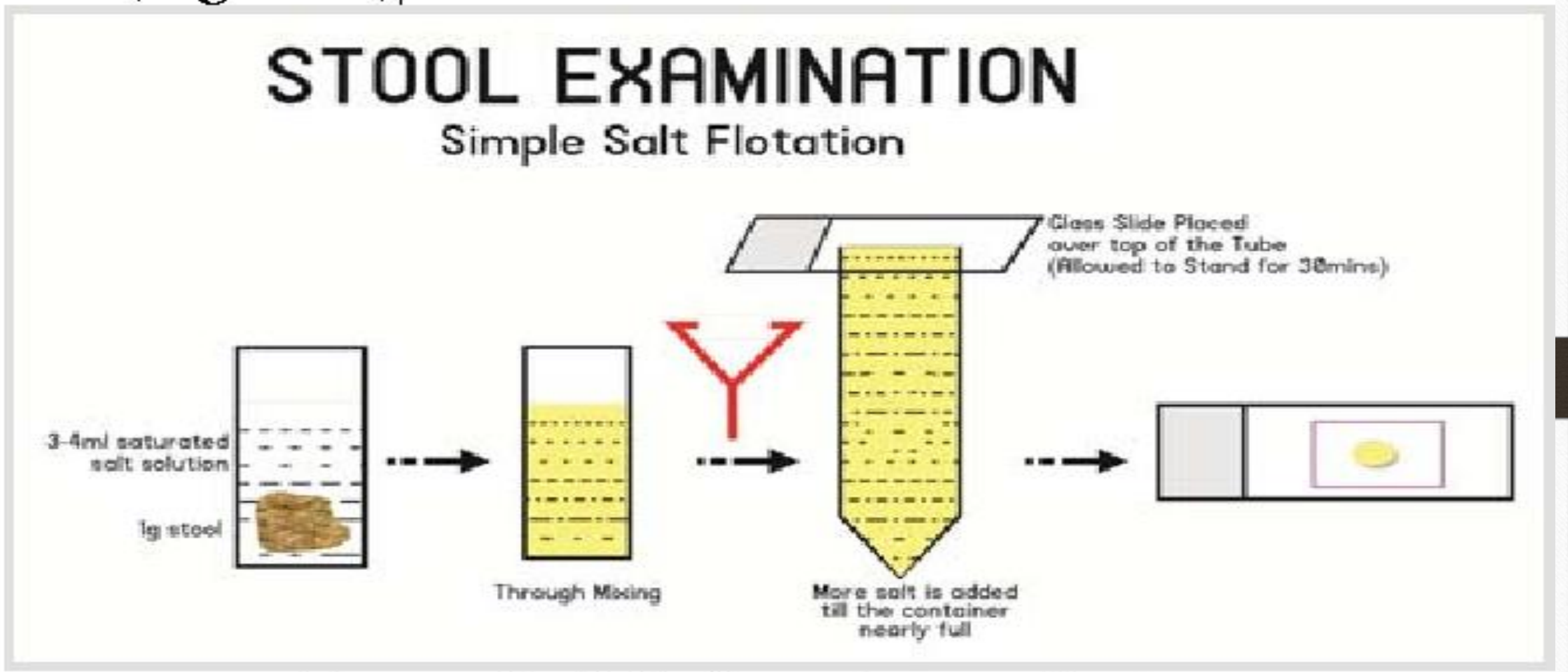


Figure 2: Salt flotation technique

Procedure:

-Place about 2 gm of fecal sample in 10 ml distilled water in 15 ml shell vial, un waxed paper cup. Using a tongue depressor, make an emulsion by thoroughly mixing the solution with feces.
-Filter this material through two layer of gauze into 10 ml centrifuge tube.
-Centrifuge for 5 minute at 1000 (rpm).
-Remove tubes from the centrifuge, pour off the supernatant fluid, divided sediment into 2 tubes one added Sheather's solution and other added saturated sodium chloride, using an applicator stick, thoroughly mix the contents.
-Centrifugalize again at the same rate and time.
-Remove fluid from the of centrifuge tube place it on a glass microscopic slide, apply a coverslip , and examine under 40x and 100x.

b-Sedimentation technique (for trematode eggs)

The sedimentation technique is a qualitative method for detecting trematode eggs in the feces. Most trematode eggs are relatively large and heavy compared to nematode eggs. By using tab water ,formalin –ether acetate ,formalin ethyle acetate.

Procedure:

-Place about 2 gm of fecal sample in 10 ml tab water in 15 ml in a cup or beaker.

-Filter this material through two layer of gauze into 10 ml centrifuge tube.

-Centrifuge for 5 minute at 1000 (rpm).

-Pour off the liquid in the top of the tube without disturbing the sediment at the bottom.

- using a pipette transfer a small amount of the top layer of sediment to a microscopic slide. if the drop too thick, dilute it with a drop of water. Apply a coverslip to the drop.

-examine the slide under microscopic.

floatation technique). (Figure 3)

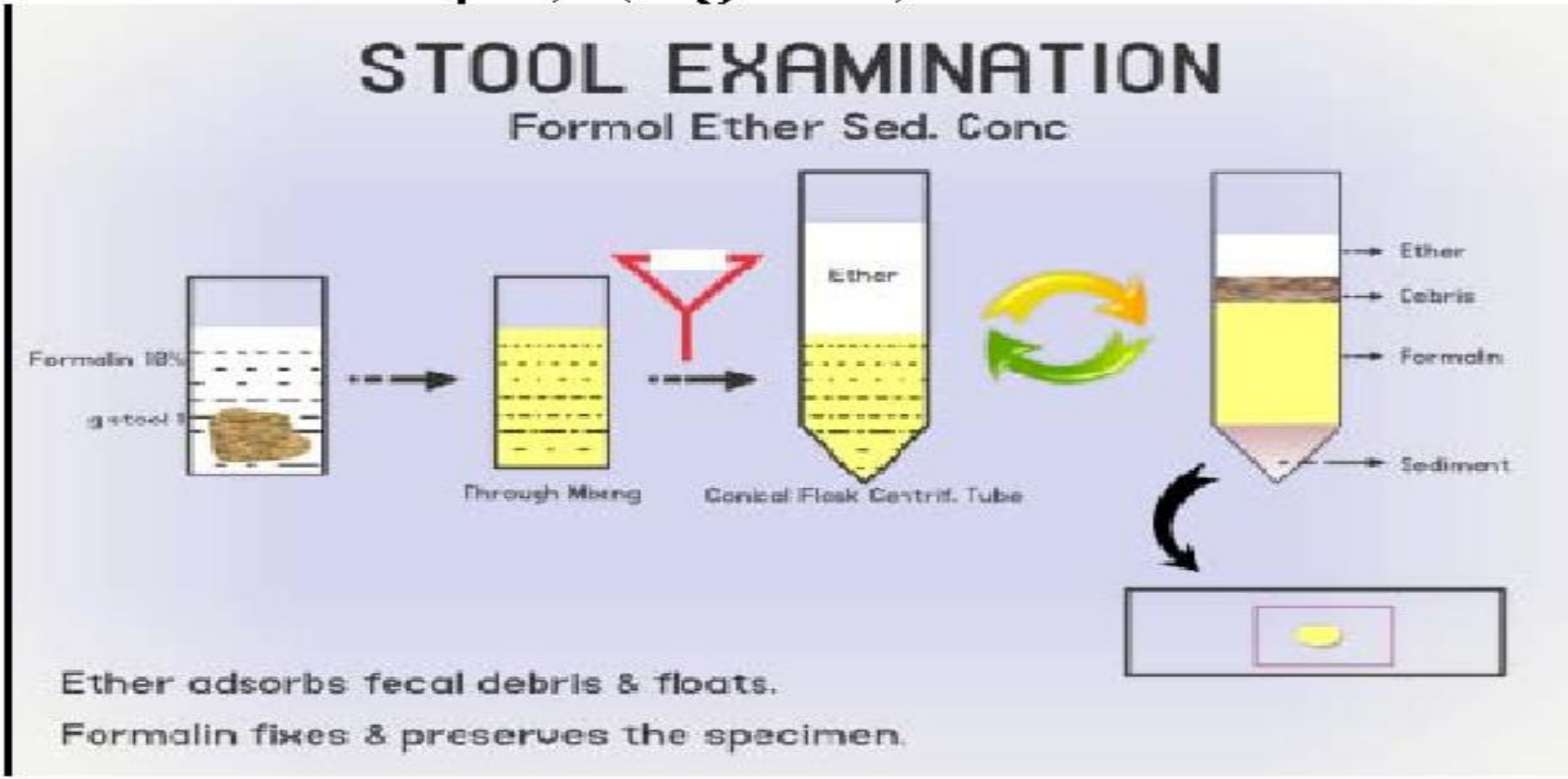


Figure 3 Formal Ether Sedimentation Method

Preparation of faecal cultures

Fecal culture is used in diagnostic parasitology to differentiate parasites whose eggs and cysts cannot be distinguished by examination of a fresh fecal sample. for example the eggs of large strongyles in horses are very similar to those of small strongyles.

Fecal culture of Roundworm eggs:

1.place 20-30 gram of fresh fecal sample in a Jar. Break up the feces with a tongue depressor and moisten slightly with tap water . the mixture should not be so wet as to appear soupy .

2. place the jar on a shelf , away from direct sunlight , and allow it to incubate at room temperature for 7 days . there should be enough moisture so that droplets of condenses water can be seen on the side of the glass jar.

3. some species of nematode larvae can migrate up the walls of the jar. these may be recovered by removing condensation drops from the glass with an artists paintbrush and transferring them to a drop of water on microscope slide.

4. apply a coverslip to the slide and pass it over the open flame of a Bunsen burner once or twice to kill the larvae. Place the slide on the microscopic slide stage and identify the larvae.

The Isolation and identification of lungworm larvae and infective larvae harvested from fecal cultures (the Baermann technique)

Baermann technique is used to isolate lungworm larvae from fecal samples ,soil or animals tissue and infective larvae from fecal cultures. It is based on the active migration of larvae from feces suspended in water and their subsequent collection and identification.

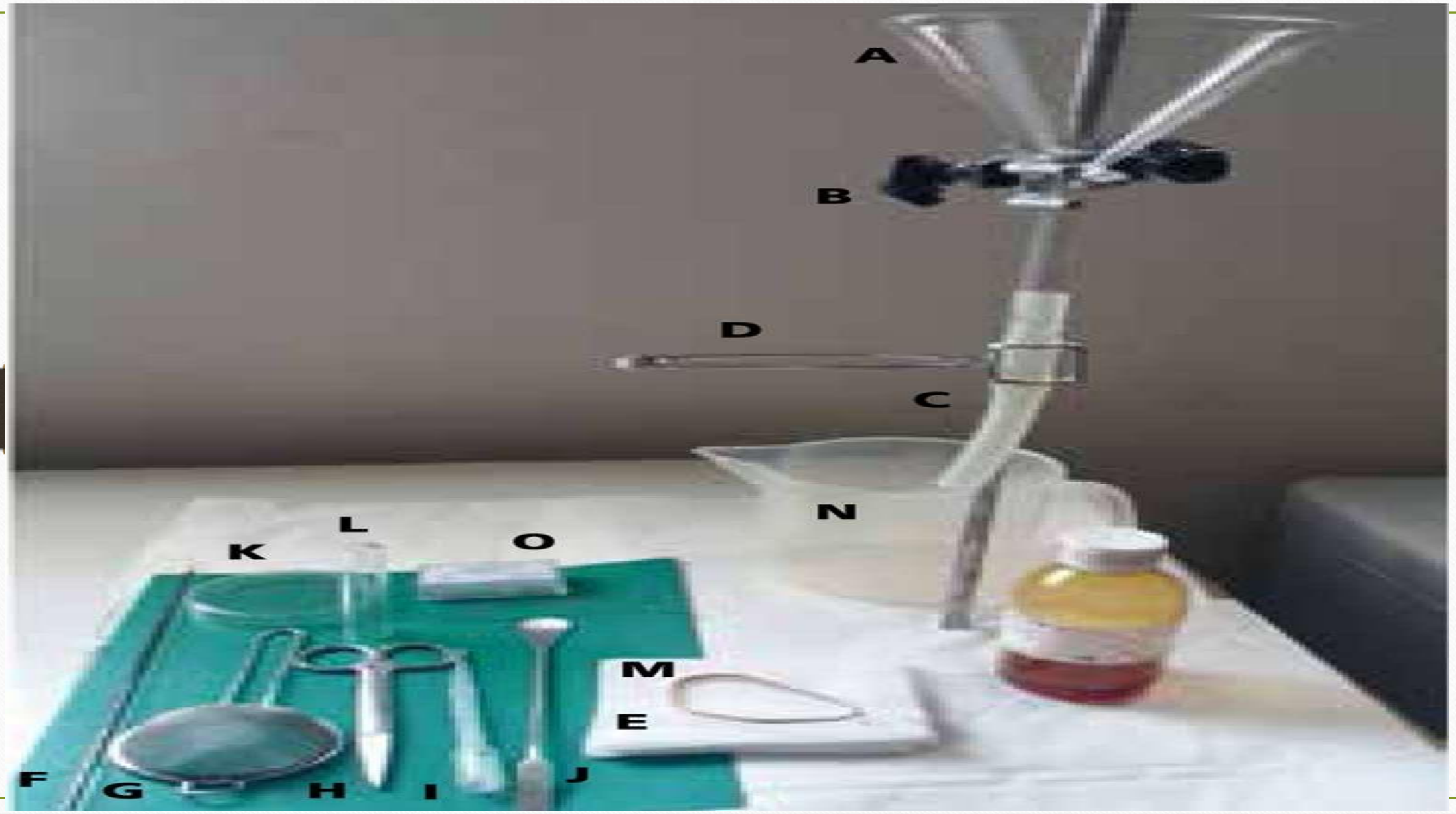
Procedure :

-Spread a piece of cheesecloth or a gauze square out on the support screen in the baermann apparatus. Place 5-15 g of fecal, soil, or tissue sample on the cheesecloth. Be sure that the sample is covered by the warm water or physiologic saline.

-Allow the baermann apparatus to remain undisturbed overnight .

-hold a glass microscopic under the cut-off pipette, and open the pinch clamp long enough to allow a large fluid to fall on slide.

Apply a coverslip to the slide and examine it.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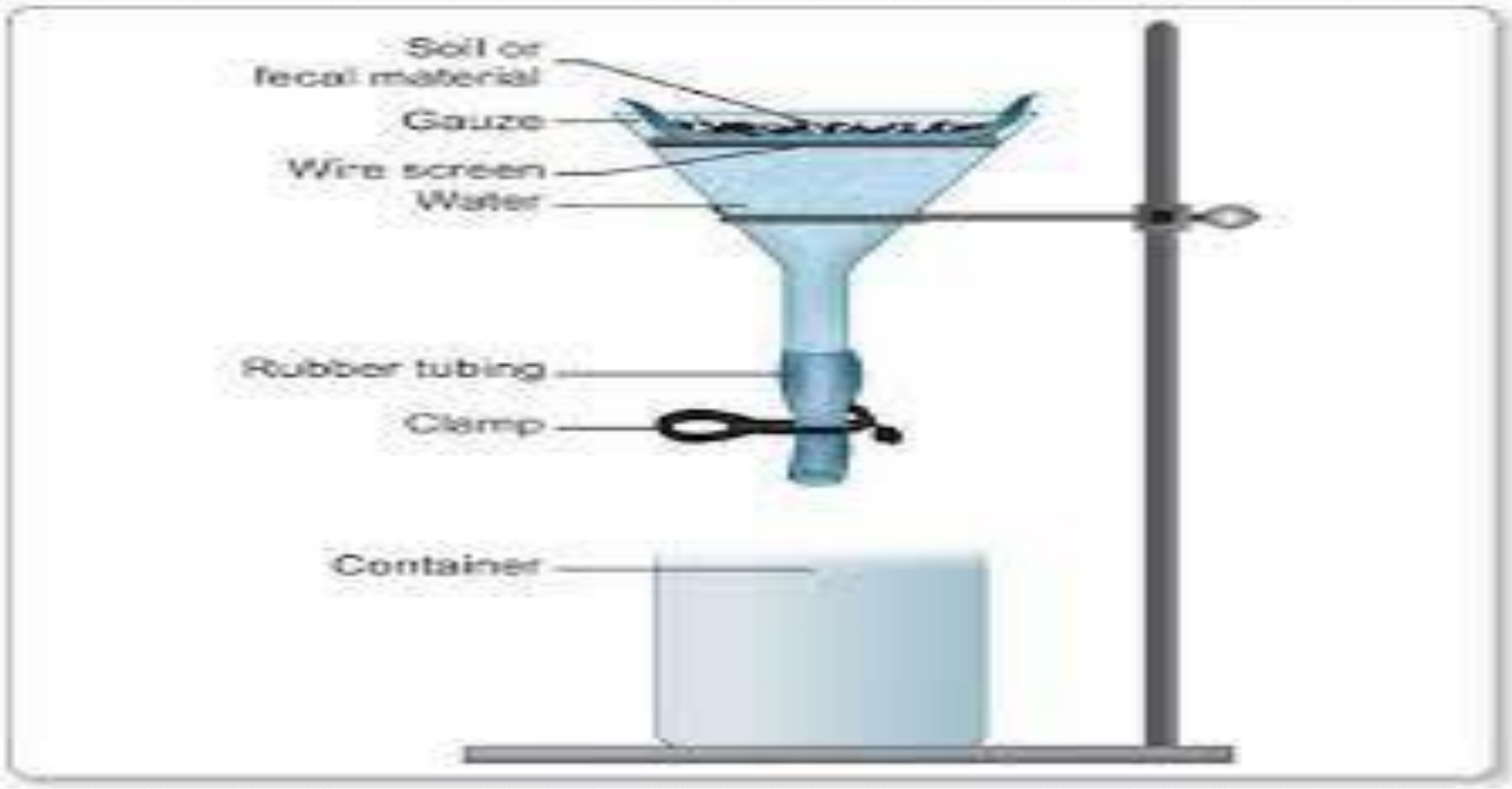
Soil or
fecal material
Gauze
Wire screen
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Rubber tubing
Clamp
Container