



TroCCAP

Tropical Council for Companion Animal Parasites

Standard Operating Procedures (SOPs) for the diagnosis of
canine and feline endo- and ecto-parasites in the tropics

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Disclaimer

The guidelines presented in this booklet were developed by members of the Tropical Council for Companion Animal Parasites Ltd.

These best-practice guidelines are based on evidence-based, peer-reviewed, published scientific literature. The authors of these guidelines have made considerable efforts to ensure the information upon which they are based is accurate and up-to-date.

Individual circumstances must be considered where appropriate when following the recommendations in these guidelines.

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General Considerations and Recommendations

Diagnosis

- Dogs living in the tropics and subtropics should be tested for gastrointestinal parasites at least once every 3 months to monitor the efficacy of parasite control regimes and owner compliance.
- Cats should be tested for endoparasites regularly (two times a year) to monitor the efficacy of control regimes and owner compliance.
- Clinical signs might occur prior to shedding of parasite stages in the faeces, in which case, history and clinical signs should guide treatment decisions.
- Standard or modified faecal flotation is recommended for the diagnosis of most, but not all internal parasites of cats and dogs. In some cases, other methods such as faecal sedimentation or more sensitive diagnostic methods may be appropriate for specific parasites and these are indicated in the guidelines for canines and felines (<https://www.troccap.com/canine-guidelines> and <https://www.troccap.com/feline-guidelines>).
- Blood smears from animals suspected of haemoparasitic infections should be performed using either fresh capillary blood collected via ear-tip or outer lip margin (see video <https://www.youtube.com/shorts/8KCCQ1gqX9Hk>) or fresh venous blood. For buffy coat smears, fresh venous blood or blood collected into an EDTA tube can be used. Blood smears should be made immediately after blood collection in order to best preserve the parasite morphology, and also because some blood microbes can leave their host blood cell (e.g. haemoplasmas).
- Vector-borne pathogens can be detected using various specific laboratory methods, some being available as in-clinic commercial tests.
- In some cases, complementary methods (e.g. blood counts, urinalysis, x-ray, and echocardiography) should be conducted to better guide treatment and management of the patient. In some instances, imaging tools may also be helpful to confirm the diagnosis; e.g. echocardiography may reveal the presence of heartworms in the right heart or arteries and computed tomography scan may indicate the presence of *Onchocerca lupi* in the retrobulbar space.
- Infestations by relatively large ectoparasites (e.g. ticks, fleas, and lice) usually can be seen macroscopically.
- Mite infestations should be diagnosed by microscopic examination of skin scrapings (for *Demodex* spp., *Sarcoptes scabiei*, and *Notoedres cati*), hair plucks or adhesive tape (for *Lynxacarus radovskyi* and *Cheyletiella* spp.) or ear examination using an otoscope (specifically for *Otodectes cynotis*).
- No technique is 100% sensitive, so in some case, parasitic infections can remain undetected.

Optimal microscope technique for examining slide

- Start with the condenser up near the stage of the microscope. While scanning, lower it as needed to increase contrast.
- Under the 4X objective, use the coarse focus knob to gain a clear view.

- Methodically examine under 10X and 40X objectives (20X or 60X if available) ensuring entire slide is scanned. Ensure the fine focus is constantly adjusted while scanning to achieve a focused view.
- Ensure the iris diaphragm is used to adjust illumination intensity and contrast when switching lens objectives.
- Some specimens (e.g. blood smears) should be examined under oil immersion for a complete evaluation (objective lens 100X). Only when scanning the specimen at lower powers is complete should the 100X objective be used. This requires a drop of oil to be placed on the slide/cover slip and then moving in the 100X lens. It is very important to avoid contaminating the other (40X, 60X) dry lenses with oil. If this happens, the contaminated objective lens should be gently wiped clean with lens tissue and in some cases a small amount of solvent is recommended. You should check first with the microscope's manufacturer about the best formulation.

Faecal-based methods

- Faecal-based methods are "a snapshot in time". That is, faecal analysis methods represent only a certain point in time, the time of that faecal collection. Hence, pathogens shed intermittently or in low numbers may be missed. Also, dogs and cats can have immature stages at the time of the analysis and begin shedding eggs within days of a negative faecal result.
- Intermittent shedding of oocysts/cysts/eggs/larvae or their absence in the faeces, even in symptomatic cases, complicates diagnosis.
- Testing 3 to 5 samples, collected on alternate (preferred) or consecutive days, may increase the probability of finding diagnostic stages in the faeces.
- Fresh material provides the best results. If faeces cannot be examined at the time of their collection, they can be kept refrigerated at 3-5°C (not frozen!) for several days.
- Faeces should be examined macroscopically for blood, mucus, proglottids and nematodes prior to analysis. Consistency and colour (may be an indication of upper or lower gastrointestinal bleeding) should also be considered in interpretation of results.

Faecal flotation methods

- The sensitivity of flotation methods is determined by the specific gravity (S.G.) of the flotation solution used and the oocyst/cyst/egg being detected. A sufficient difference between the two is needed for oocysts/cysts/eggs to float. See Table 1.
- Flotation solutions with a S.G. between 1.18 and 1.28 are recommended for the diagnosis of most internal parasites of cats and dogs. See Table 2.
- Some flotation solutions can distort oocysts/cysts/eggs/larvae.
- More viscous solutions require centrifugation.
- The selection of flotation solution depends on the flotation method used and the parasites being targeted.
- Using weighed quantities of faeces and measured quantities of flotation solution can enable quantitative assessments.
- Recovery of parasite stages can be altered if faeces are frozen prior to analysis or preserved with formalin (2% formaldehyde)

Table 1. Ability to float some parasite eggs based on average S.G.

<u>Float relatively easily with all flotation solutions</u>
<i>Cystoisospora</i> spp. (coccidia)
<i>Ancylostoma</i> spp. and <i>Uncinaria</i> sp. (hookworms)
<i>Toxocara canis</i> (roundworm-ascarid)
<i>Toxocara cati</i> (roundworm-ascarid)
<i>Toxascaris leonina</i> (roundworm-ascarid)
<u>Require a higher S.G. solution and centrifugation</u>
<i>Trichuris vulpis</i> (whipworm)
Some trematode eggs, e.g. <i>Opisthorchis</i> spp., <i>Clonorchis</i> spp., <i>Haplorchis</i> spp., <i>Platynosomum</i> sp. (liver and small intestinal flukes of cats) N.B. False negatives common; sedimentation can be more sensitive
<i>Taenia</i> spp., <i>Echinococcus</i> spp., N.B. False negatives common
<i>Linguatula</i> spp. (tongue worm)
<u>Difficult to float; either collapse in solutions or too heavy</u>
<i>Physaloptera</i> spp. (stomach worm); collapses
<i>Spirocerca lupi</i> (esophageal worm); collapses
<i>Dipylidium caninum</i> (common tapeworm); heavy N.B. False negatives common
Trematode eggs e.g., <i>Diphylobothrium</i> spp., <i>Spirometra</i> spp., <i>Paragonimus</i> spp., <i>Echinostoma</i> spp., <i>Alaria</i> spp.; heavy and fluid can enter the egg if the operculum opens

Table 2. Common flotation solutions and their S.G.

Solution	S.G.	Formulation
Sheather's sugar	1.27-1.3	454 g granulated sugar dissolved in 355 mL hot water and 6 mL formaldehyde (37-40% formaldehyde) Note: 30 mL of 10% formalin can be used instead; decrease the 355 mL water to 325 mL
Saturated salt	≈ 1.2	350 to 400 g NaCl in 1 L of water
Magnesium sulphate	≈ 1.2	350 to 400 g MgSO ₄ in 1 L of water; 700 g of MgSO ₄ if heptahydrate is used
Zinc sulphate	≈ 1.18 – 1.2 (1.25)	330 to 390 g ZnSO ₄ in 900 mL of water
Sodium nitrate	≈ 1.18 – 1.2	315 to 400 g in 700 mL of water
All formulations are approximate. A hydrometer should be used to confirm the S.G. Solutions should be at room temperature to test the S.G.		
For an example on preparing and measuring the S.G. of a flotation solution, refer to: https://youtu.be/Skx7JDUL6IE		

Baermann technique

- This method is for the collection and identification of nematode larvae from faeces.
- The quantity of faeces used impacts the sensitivity of this method with less faeces resulting in lower sensitivity when the number of larvae present is low.
- Refrigeration of faeces prior to a Baermann might decrease larval recovery.
- The temperature of the water and room in which the Baermann is placed can impact larvae recovery. Temperatures should be similar to that which the larvae would encounter in the environment.
- Water used in the Baermann apparatus should be warm (e.g., 42 °C or to a temperature still tolerable to skin).
- Cheese cloth is preferred over gauze and the minimal number of layers should be used to prevent larvae from being trapped.

Sedimentation technique

- While sedimentation is most frequently used for large heavy eggs, such as those of flukes and *Dipylidium* egg packets, all diagnostic stages will sink in water.
- The challenge with sedimentation is the ability to see smaller objects. Hence this is not the preferred method for many diagnostic stages in faeces. Consider using centrifugal flotation with a solution with a S.G. >1.25 or filtering the homogenized faecal sample through a 0.1 mm filter prior to sedimentation.
- The quantity of faeces used and sediment examined influences the sensitivity of sedimentation.
- This method is also used for the collection and identification of larvae from faeces.

Haemoparasite detection techniques

- Haemoparasites include microfilaria of nematodes and different stages of protozoa, many of which are vector-borne. There are also some important vector-borne haemotropic bacteria such as *Ehrlichia* and haemoplasmas.
- In general, methods that use some form of blood concentration (e.g., Modified Knott's Test for microfilariae, buffy coat smear for intraleukocytic pathogens) and staining are more sensitive than direct smears.
- In addition to examining blood for organisms, commercial kits and reference laboratories can identify the presence of antibodies, antigens or DNA of different parasites. Antibody results must be interpreted with an understanding of the life cycle, since these can indicate exposure to and not presence of the parasite. Antibodies to pathogens may be retained for up to a year following cure. See the dog/cat guidelines for information on using these for diagnosis.

Ectoparasite diagnostic techniques

- In determining the location for examination and sample collection (hair pluck, adhesive tape), the predilection site of the parasite should be considered.
- In dogs and cats with undercoats or long coats, parting the hair is important to find parasites on ear tips and skin.

Faecal Analysis Standard Operating Procedures (SOP)

Faecal collection and storage

Good faecal analysis starts with the faecal collection method. Faeces should be collected immediately after defecation (from the ground or a litter box), stored between 3 and 5°C prior to analysis (unless performing a Baermann test) and analysed within 5 days of collection. Collection immediately after defecation helps ensure that the source dog/cat is clear and decreases the chance of contamination with soil nematodes. Rectal collection is possible, but the larger quantity typically obtained from voided faeces can increase the number of tests feasible and ensure an adequate quantity for flotation, sedimentation and/or a Baermann.

Faeces should be placed in a clean container, labelled with the animal identification and the date of collection.

Clients should be asked to bring collected faeces as soon as possible to the clinic for analysis. After collection and during transport to the clinic, the faeces should be kept in a cool environment. Once at the clinic, refrigeration until analysis will slow down parasite development making identification easier.

While many parasites are not infective in fresh faecal material, in geographic locations with *Echinococcus* spp., extra precautions should be taken during collection and analysis.

Revolutions per minute (RPM) vs. g-force

For many centrifuges, a table is available to determine the g-force (or relative centrifugal force) achieved based on the rpm (revolutions per minute). However, if this is not available it can be calculated as follows:

$$g\text{-force} = 1.12 \times \text{Radius of rotor in mm} \times (\text{rpm}/1000)^2$$

Online calculators also can be used (e.g., http://insilico.ehu.es/mini_tools/rcf_rpm.php).

S.G. (specific gravity)

S.G. is the weight per unit volume compared with water and can be determined by the weight of a solution or via a hydrometer. Hydrometers should be used at room temperature.

If using weight, a solution that is 1 L that weighs 1.2 kg has a S.G. of 1.2.

If using a hydrometer, ensure the range is appropriate for the solution. Typical hydrometer ranges are >1.0 to 1.22 and 1.2 to 1.4.

For an example on preparing and measuring the S.G. of a flotation solution, refer to: <https://youtu.be/Skx7JDUL6IE>

SOP 1: Simple faecal flotation

Simple faecal flotation is suitable for the recovery and identification of most nematode eggs, some tapeworm eggs, tongue worm (*Linguatula serrata*) and protozoan cysts and oocysts in canine and feline faeces. The method is quick, inexpensive and does not require the use of a centrifuge. However, it does not include a concentration step and viscous flotation solutions with high S.G. cannot be used, decreasing the sensitivity when there are few eggs/cysts in faeces and for some parasites with slightly higher S.G. eggs (e.g., *Trichuris*).

Reagents/Materials

- Flotation solution (e.g. saturated salt or sodium nitrate)
- Slide and coverslip
- Wide mouth cup (e.g., reusable/washable urine collection cup or disposable plastic/paper cup)
- 10 to 15 mL disposable test tube or 10 to 15 mL narrow mouth reusable glass jar
- Tea strainer, gauze or cheese cloth
- Stir stick (e.g., tongue depressor)

Preparation of flotation solutions of S.G. 1.20

- Sodium nitrate:
Dissolve 315 to 400 g sodium nitrate in approximately 700 mL warmed distilled water (dH₂O). Add more dH₂O until the entire solution weighs 1200 g (this equates to a S.G. of 1.20). Mix solution and then check S.G. with hydrometer.
- Saturated salt
Dissolve table salt (NaCl, ~300 to 400 g) in 1000 mL warmed dH₂O while stirring continuously. Add salt until no more dissolves (i.e. salt remains precipitated out of solution once cooled). Check S.G. with hydrometer.

Procedure

1. Using the stir stick, place ~2 g faeces into a wide-mouthed cup (plastic disposable cup/washable reusable cup (e.g., urine cup)/sterile urine jar)
2. Add ~4 mL flotation solution to the cup and mix with faeces thoroughly using the stir stick
3. Add a further ~4 mL flotation solution to the cup and mix again
4. Pour/filter this faecal suspension through a tea strainer/gauze/cheese cloth into a new cup
5. Empty the contents of the cup into a 10 to 15 mL test-tube supported in a rack or stand or into a narrow mouth 10 to 15 mL glass jar
6. Keep adding contents or top up with flotation solution until a positive meniscus forms over the lip of the test tube/jar. The last mL of flotation solution can be poured with a dropper to ensure the meniscus is carefully formed
7. Carefully place a coverslip (approximately 22 x 22 mm) on top of the test tube
8. Stand for 10 to 15 min
9. Carefully lift off the coverslip from the tube, with the drop of fluid adhered to the bottom of it, and place it on a microscope slide

10. Examine under a light microscope at low power (10X objective; 100X magnification) for helminth stages and at high power (40X objective; 400X magnification) for protozoal stages.

For an alternative step-by-step guide with useful images of this procedure, refer to: [http://www.rvc.ac.uk/review/parasitology/Flotation/Simple flotation/Purpose.htm](http://www.rvc.ac.uk/review/parasitology/Flotation/Simple%20flotation/Purpose.htm)

Safety precautions

- Wear lab coat and disposable gloves
- Wash hands thoroughly when finished

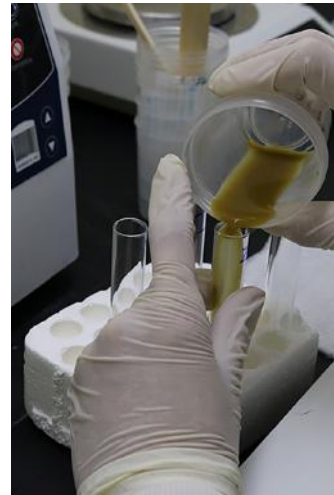
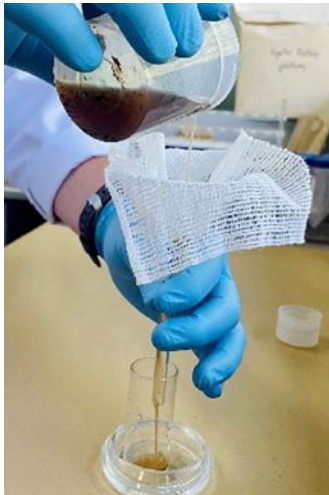
Clean up procedures

- Pour sodium nitrate into appropriate chemical waste container.
- Dispose of all slides, cover slips and disposable test tubes in a sharps container
- Clean all equipment (tea strainer, reusable cups) thoroughly with a 10% bleach solution
- Wipe down work area with 70% ethanol



Place approximately 2 g of faeces into a wide mouth cup. Add approximately 4 mL of flotation solution. Mix thoroughly. Add approximately 4 mL more flotation solution and mix.

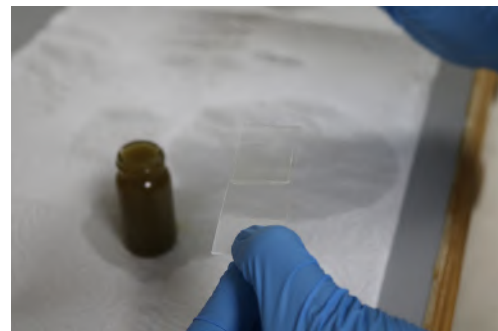




Strain the solution through gauze, cheese cloth or a tea strainer into a clean cup.



Pour the filtered solution into a test tube or a 10 to 15 mL narrow mouth glass jar. Add additional flotation solution until a positive meniscus has formed. Place coverslip on jar.



After 10 to 15 minutes, remove the coverslip and place on a slide.

SOP 2: Centrifugal faecal flotation

The zinc sulphate [S.G. 1.18] centrifugal flotation procedure is suitable for the isolation and identification of protozoan cysts and oocysts in faeces, in particular cysts of *Giardia duodenalis*. Sheather's sugar [S.G. 1.27] centrifugal flotation is more sensitive for the isolation of heavier nematode eggs such as those of *Trichuris vulpis* and *Spirocerca lupi* and the trematode *Platynosomum*. Centrifugal flotation is inexpensive; however, it does require use of a centrifuge.

Reagents/Materials

- Flotation solution (e.g., zinc sulphate solution or Sheather's sugar solution)
- Lugol's iodine
- Slide and coverslip
- Wide mouth cup or jar (e.g., reusable/washable urine collection cup or disposable plastic/paper cup)
- 10 to 15 mL disposable or reusable test tube
- Tea strainer, gauze or cheese cloth
- Stir stick (e.g., tongue depressor)
- Centrifuge for 10-15 mL tubes; a full-swing bucket preferred

Preparation of flotation solutions

- Zinc sulphate solution (S.G. 1.18)
Dissolve 331 g zinc sulphate in 900 mL warmed distilled water (dH₂O). Add more dH₂O until the entire solution weighs 1180 g (this equates to a S.G. of 1.18). Mix solution and then check S.G. with hydrometer. Note: if zinc sulphate heptahydrate is used, then additional quantities will be needed (e.g., approx. 750 g)
- Sheather's sugar (S.G. 1.27)
To 355 mL hot water, add (while stirring) 454 g sugar. Add 6 mL 10 % formalin (10 mL 40% formaldehyde in 90 mL distilled water) per 454 g sugar to avoid fungal contamination. Adjust to ensure S.G. is 1.27 using a hydrometer

Procedure

1. Using the stir stick, place ~2 g faeces into a wide-mouthed cup/jar
2. Add ~4 mL flotation solution to the cup/jar and mix with faeces thoroughly using the stir stick
3. Add a further 4 mL flotation solution to the cup/jar and mix again
4. Pour/filter this faecal suspension through a tea strainer, gauze or cheesecloth into a new cup/jar
5. Empty the contents of the cup/jar into a 10 to 15 mL test-tube supported in a rack or stand
6. Centrifuge at 500 g for 5 min
7. Carefully add more flotation solution until a positive meniscus forms at the top of the test tube and place a coverslip (approximately 22 x 22 mm) on top
8. Stand for a further 5 to 10 min.

9. Carefully lift the coverslip from the tube with the drop of fluid adhered to the bottom of it and place it on a microscope slide. Adding a drop of Lugol's iodine to the slide before placing the coverslip on it can make the *Giardia* cysts easier to see
10. Examine under a light microscope at low power (10X objective; 100X magnification) for helminth stages and at high power (40X objective; 400X magnification) for protozoal stages

Procedure with full swing centrifuge

1. Follow steps 1 through 5 above
2. Carefully add more flotation solution until a positive meniscus forms at the top of the test tube and place a coverslip (approximately 22 x 22 mm) on top
3. Centrifuge at 500 *g* for 10 min
4. Follow steps 9 and 10 above

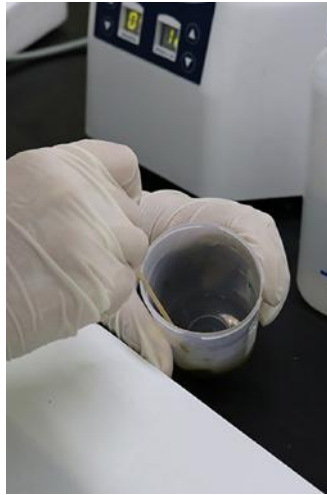
Safety precautions

Wear lab coat and disposable gloves
Wash hands thoroughly when finished

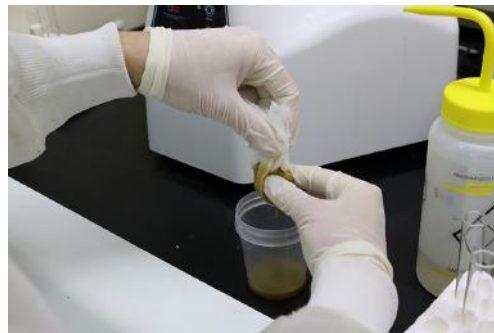
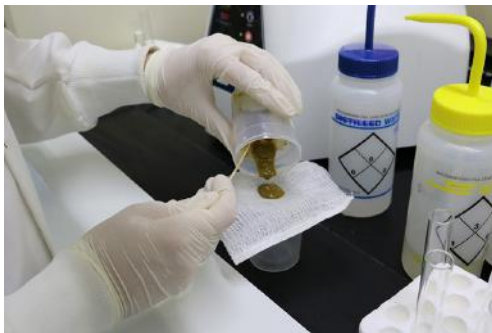
Clean up procedures

Pour zinc sulphate into appropriate chemical waste container
Dispose of all slides and cover slips in a sharps container
Clean all equipment (tea strainer, glass test tubes) thoroughly with a 10% bleach solution
Wipe down work area with 70% ethanol.





Weigh approximately 2 g of faeces into a clean cup. Mix faeces with flotation solution.



Pour solution through cheesecloth/gauze/tea strainer; press gently to collect the solution. Pour solution into test tube.



Place tube into the centrifuge and spin for 5 min at 500 g.

With a full swing bucket centrifuge, the tubes can be spun with the coverslip on. For all other centrifuges, after centrifugation fill with flotation solution until there is a positive meniscus and then place on the coverslip; allow to sit for 5-10 min.

Remove the coverslip and place on a slide. Examine at 100X magnification.

SOP 3: Baermann technique

The Baermann technique is suitable for the isolation and identification of nematode larvae (e.g. *Strongyloides stercoralis* and lungworms) in fresh faeces.

Reagents/Materials

- Distilled water (dH₂O)
- Plastic or glass funnel, rubber tube and clamp OR 50 mL centrifuge tube
- Tea strainer and gauze or cheese cloth
- Toothpick, rubber band or string

Equipment set up

Secure a funnel to a stand; connect a rubber tube with a clamp to the funnel stem.

Procedure

1. Place 3 to 5 g of faeces in the centre of a large square of cheese cloth/gauze and tie with a rubber band, string or toothpick to form a pouch
2. Place the pouch within a tea strainer and suspend in the funnel. If using a 50 mL centrifuge tube, suspend the pouch directly in the tube without a tea strainer
3. Add warm dH₂O to the funnel until the water covers the top of the faecal pouch or add water to the 50 mL tube until the faeces is covered
4. Leave standing for 12 to 24 h or overnight for lungworm larvae or 6 hours for *Strongyloides stercoralis*
5. If utilising a funnel, open the stopper on the rubber tubing and collect 2 mL of the filtered sediment into a test tube. If using a 50 mL centrifuge tube, go to step 7
6. Leave the test-tube standing for 30 min or centrifuge at 500 to 1000 g for 2 min
7. Carefully remove the supernatant with a pipette, leaving ~0.5 mL of the sediment undisturbed
8. Take 1 to 2 drops of the sediment and place on a microscope slide with a cover slip. Repeat as needed
9. Examine under a light microscope at low power (10X objective; 100X magnification) for detecting larvae and at high power (40X objective; 400X magnification) to confirm the presence of a genital primordium and oesophagus and tail shape

Safety precautions

Wear lab coat and disposable gloves
Wash hands thoroughly when finished

Clean up procedures

Dispose of all slides and cover slips in a sharps container
Clean all equipment (tea strainer, funnel, glass test tubes) thoroughly with a 10% bleach solution
Wipe down work area with 70% ethanol

Baermann apparatus method (funnel with tubing and clamp)



Weigh 3 to 5 g of faeces and place on cheesecloth/gauze. Place into a tea strainer and then in the funnel. Fill with water. After sitting overnight, open the clamp and collect 2mL of the sediment.

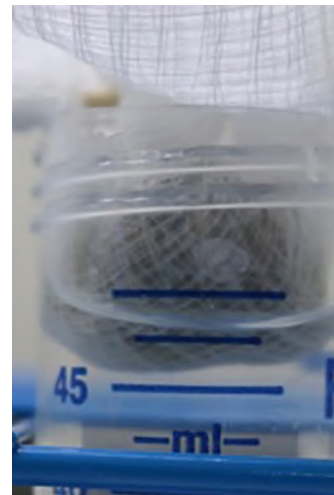
Centrifuge tube (50 mL) method.



Step 1.



Step 2.



Step 3.

Step 1: Place faeces in center of gauze/cheesecloth.

Step 2: Suspend in 50 mL tube with warm water; add water until faecal material is covered. More water is needed.

Step 3: After sitting for 6-24 h (depending on parasite), remove sediment for analysis.

SOP 4: Simple sedimentation technique

The faecal sedimentation technique is suitable for the isolation and identification of heavier eggs, especially those of flukes (e.g. *Alaria* spp., *Paragonimus* spp., etc.) and some tapeworms (e.g. *Spirometra* spp., *Diphylobothrium latum*). The method is quick, inexpensive and does not require the use of a centrifuge.

Reagents/Materials

- Distilled water (dH₂O)
- 5% aqueous methylene blue solution
- Tea strainer or sieve (approximately 0.1 mm aperture)
- Plastic cup/jar
- 50 mL conical tube

Procedure

1. Soak 5 g faeces in 50 mL dH₂O and mix thoroughly
2. Pass through tea strainer/gauze/sieve into a plastic jar to filter. Aperture must be at least 150 µm.
3. Pour all contents into a conical test tube (50 mL)
4. Allow to sediment for 5 min or alternatively centrifuge the tube at 650 g for 10 min
5. Pour off supernatant
6. Add water, mix and allow to sediment 5 min
7. Pour off supernatant carefully
8. Can add 1 to 2 drops of 5% aqueous methylene blue solution in test tube to aid in identification (yellow or colourless fluke eggs against a blue background)
9. Transfer 1 to 2 drops of the sediment to a microscope slide, place a cover slip and examine using a light microscope at low power (4X objective (40X magnification) and 10X objective (100X magnification))

Safety precautions

Wear lab coat and disposable gloves
Wash hands thoroughly when finished

Clean up procedures

Dispose of all slides and cover slips in a sharps container
Clean all equipment (tea strainer, glass test tubes) thoroughly with a 10% bleach solution
Wipe down work area with 70% ethanol.

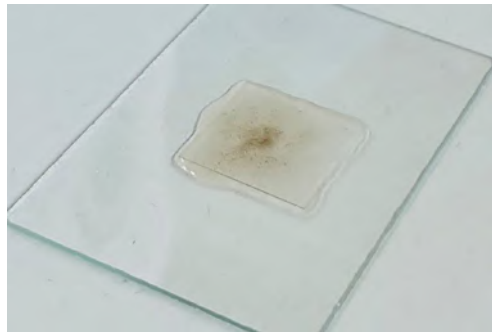
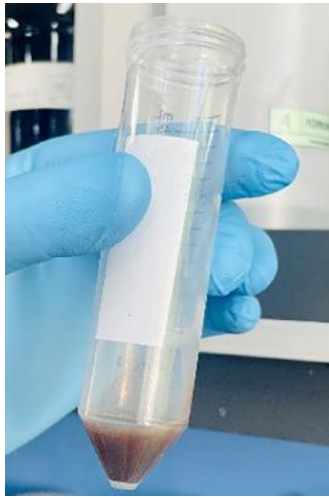


Soak 5 g faeces in 50 mL dH₂O and mix thoroughly.



Pass the faecal/water mixture through tea strainer/gauze/sieve into a plastic jar to filter. Pour all contents into a conical test tube (50 mL). Allow to sediment for 5 min.

Not pictured: Add water to tube, mix, allow to sediment for 5 min and the pour off supernatant carefully.



Transfer 1 to 2 drops of the sediment to a microscope slide, place a cover slip and examine using a light microscope at low power.

SOP 5: Acid fast stain for *Cryptosporidium* oocysts

As the oocysts of *Cryptosporidium* spp. are very small and difficult to detect by inexperienced examiners, this method provides specific staining and allows an easier detection.

Reagents

- Absolute methanol
- Kinyoun's carbol fuchsin
- 10% sulfuric acid solution (H₂SO₄)
- 3% Malachite green

Procedure

1. Make a thin faecal smear and allow to air dry
2. Fix with absolute methanol for 10 min and allow smear to dry
3. Stain with cold Kinyoun's carbol fuchsin strong stain (filtered) for 5 min
4. Wash thoroughly in tap water until no further stain comes out (very important step that can take 3 to 5 min)
5. Decolourise in 10% H₂SO₄ (for very thin smears a rapid dip in Coplin jar of acid followed by an immediate rinse in tap water is sufficient)
6. Counterstain with 3% Malachite green for 2 to 5 min
7. Wash in tap water and blot dry
8. Examine under a light microscope at high power (40X objective; 400X magnification) for oocysts

Results

Oocysts are seen as acid fast (bright pink) oval to round bodies (4 to 6 µm in diameter), surrounded by a colourless halo. Bacteria and yeasts stain green.

Safety precautions

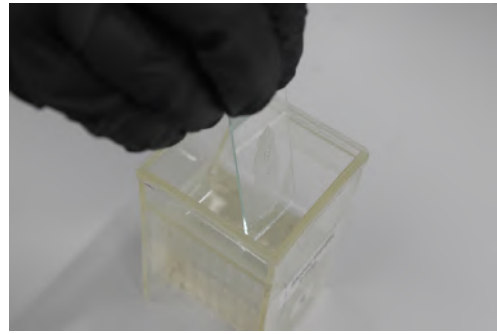
Wear lab coat and disposable gloves
Wash hands thoroughly when finished

Clean up procedures

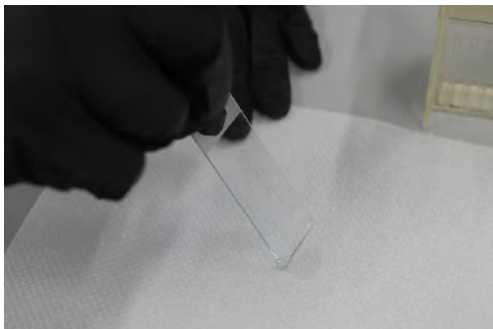
Dispose of all disposable equipment in clinical waste bin or sharps container as appropriate



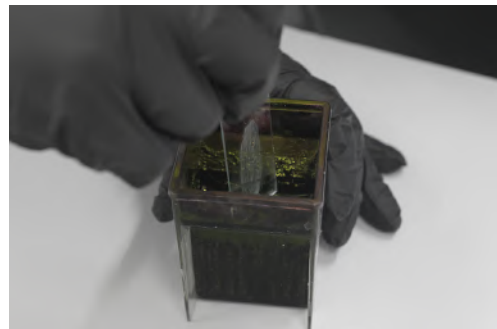
Step 1.



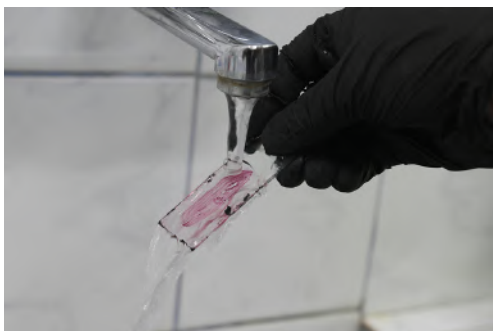
Step 2.



Step 2.



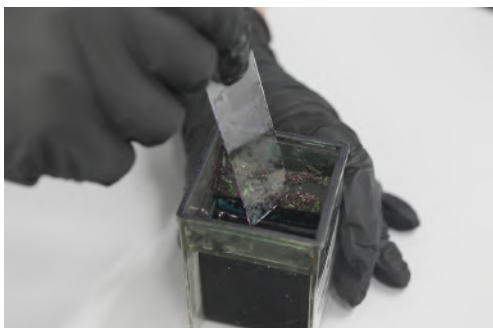
Step 3.



Step 4.



Step 5.



Step 6.



Step 7.

Blood Analysis SOPs

SOP 6: Modified Knott's test

This method is used for the detection of microfilariae in the blood. The method is more sensitive than a direct smear with fresh blood as it concentrates the microfilariae from a large volume of blood. In addition to serological tests, this method also allows detection and identification of microfilaria of species other than *D. immitis* (i.e. *D. repens*, *Acanthocheilonema* spp., *Brugia* spp.). Blood samples should be collected in the evening for increased sensitivity in the detection of *Dirofilaria* spp. microfilaria.

Reagents/Materials

- 2% formalin (2 mL of 40% formaldehyde in 98 mL of distilled water)
- 1% methylene blue
- Conical centrifuge tube
- Slide and coverslip
- Pipette

Procedure

1. Mix 1 mL uncoagulated blood (in heparin or EDTA) with 9 mL of 2% formalin in a conical centrifuge tube
2. Invert the tube gently 4 times to mix the solution
3. Centrifuge at 500 *g* for 5 min
4. Discard supernatant
5. Stain sediment for 1 to 2 min with 1 to 2 drops of 1% methylene blue
6. Add a drop of the sample on a glass slide and cover with a coverslip. Repeat this step so that 2 or more slides are prepared; this increases sensitivity
7. Note: Steps 1-6 can be repeated to increase sensitivity
8. Examine the slides under a light microscope at low power (10X objective; 100X magnification) for microfilariae. Higher magnifications may be required for specific morphological identification of microfilariae

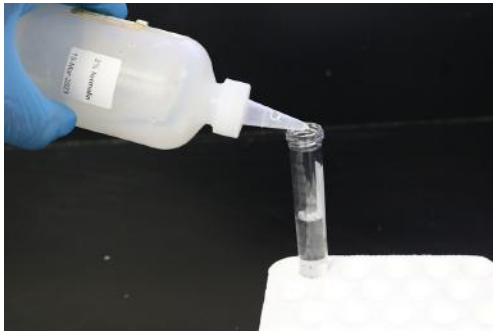
Note: The addition of 9 mL of 2% formalin and steps 2 through 4 may be repeated if cleaner sediment is desired.

Safety precautions

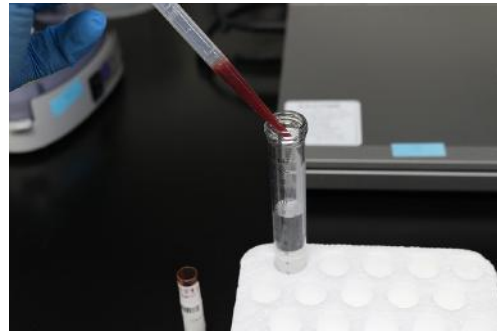
Wear lab coat and disposable gloves

Clean up procedures

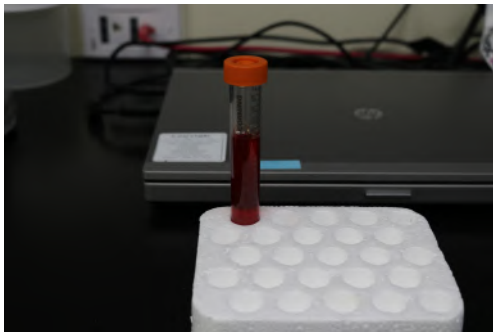
Dispose of all slides and cover slips in a sharps container



Step 1.



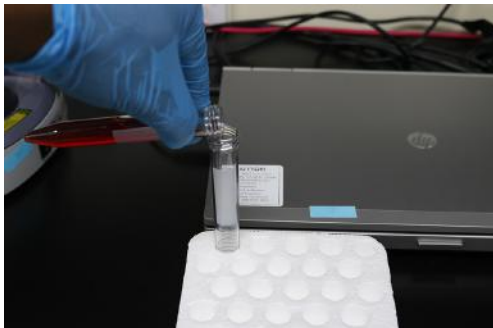
Step 1.



Step 2.



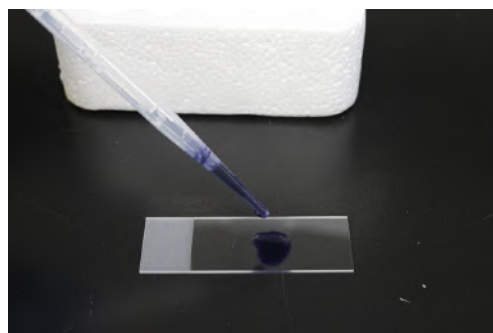
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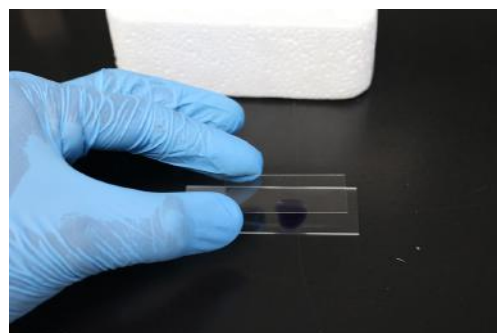
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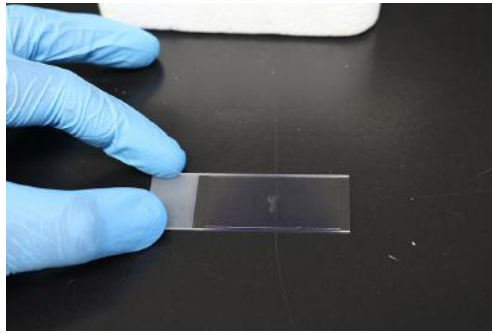
Step 5.



Step 6.



Step 6.



Step 6.

Photos courtesy of Ian Branford and Shadrach Hobson-Tyson, Ross University School of Veterinary Medicine

SOP 7: Microhematocrit method for detection of microfilariae

Microhematocrit is a widely used hematological method. In blood samples which are positive for microfilariae of *Dirofilaria* spp. and *Acanthocheilonema* spp., these larvae tend to concentrate in the buffy coat fraction of the microhematocrit tube. They can be observed under the microscope while still alive and motile.

Reagents/Materials

- Microhematocrit tubes

Procedure

1. Whole blood collected on anticoagulant (EDTA, heparin, etc.) is used to fill-in the microhematocrit tubes (75 mm long, 1 mm in diameter)
2. Centrifuge in a microhematocrit centrifuge at 13,000-15,000 *g* for 4 to 5 minutes
3. The microhematocrit tube is gently removed from the centrifuge and placed horizontally under a microscope, focusing on the buffy coat fraction, located between the plasma and the red blood cell layer
4. Examine under a light microscope first using a 20X objective (200X magnification). and observe the motile microfilariae

Results

Microfilariae cannot be differentiated to species level due to the movement and lack of staining for the visualisation of differentiating features. Microfilariae have the tendency to move from the buffy coat area to the plasma while heated by the microscope light.

Safety precautions

Wear lab coat and disposable gloves

Clean up procedures

Dispose of all slides and cover slips in a sharps container

SOP 8: Blood smear (including ear tip capillary blood smear)

Thin blood smears are used for intra- and extra-cellular parasites in peripheral blood such as haemoprotozoans (*Babesia*, *Theileria*, *Rangelia*, *Cytauxzoon*, *Hepatozoon*, *Trypanosoma*). Thick smears are more concentrated and more sensitive than thin smears but should be followed up with a thin smear for parasite morphological identification. Thin and thick smears also can be used for microfilariae but have low sensitivity compared to the Knott's modified test.

In most cases two or more smears should be made to increase sensitivity of the method. In addition to this SOP, see:

Fresh capillary blood smear. <https://youtu.be/EkYXXHSILYk>; and <https://www.youtube.com/shorts/yfUN89hsZqs>

EDTA blood smear. <https://youtube.com/shorts/j-KNiVnIAi0?feature=share>

Reagents/Materials

- Giemsa stain: 1:20 solution (e.g., 2 mL of stock Giemsa and 40 mL of distilled or buffered water). The 1:20 solution should be no more than 2 days old
- Staining jars
- Ensure slides are clean; wipe with alcohol before use handling the slide by the edge

Procedure thin blood smear

1. Place a small drop of fresh blood (before clotting occurs) or EDTA anticoagulated blood close to one end of the slide
2. Use another slide (the spreader) to spread the blood
3. Hold the spreader at an angle of approximately 45 degrees
4. Touch the far side of the blood with the spreader. The far side is the side furthest away from the edge of the slide
5. The blood should run along the edge of the spreader
6. Gently push the spreader along the length of the slide
7. Note, this is pulling the blood behind the spreader. It is not pushing the blood in front of the spreader
8. Push the spreader to close to the end of the slide. This should result in a "feathered edge", an area where the blood cells are separated
 - a. If there is insufficient blood, the smear is short
 - b. If there is too much blood, a feathered edge is not created
9. Air dry
10. After air drying the blood film, fix in absolute methanol for 5 min and air dry

Procedure thick blood smear

1. Place a small drop of blood in the centre of the slide
2. Use a stick or corner of another slide and spread the blood drop in a circular pattern
3. The resulting smear should be approximately 1.5 cm in diameter
4. If the slide is placed over newsprint, it should be difficult to read through the blood

5. Let the smear dry in a horizontal position for at least 30 minutes. The smear can dry for several hours
6. Do not fix thick smears with methanol or heat
7. Stain with Giemsa
8. If staining is to be delayed, dip the smear briefly in water to haemolyse the erythrocytes

Procedure for Capillary Blood Smear from the ear

1. Hold the ear and clip the fur from a small area at one edge of the pinna
2. Wipe with a dry gauze to remove cut hair, dust and squames. Do NOT use any liquid (e.g. disinfectant) as this will stop the bleb of blood from forming.
3. Gently prick the ear with a fine (e.g. 25G or 26G) needle. (This should be done so gently that no blood is produced.)
4. Squeeze the ear around the needle-prick site to push the capillary blood onto the surface of the skin. There should be a small bleb of blood
5. Apply a microscope slide to the bleb and then make a smear as previously described for a thin blood smear

Staining and viewing (all types of smears)

1. If a commercial stain kit such as Diff-Quik is not available, the following method can be used.
2. Place the slide in the 1:20 Giemsa solution for 20 to 30 min
3. Wash gently using tap water or by dipping into a jar of tap water. Do not over-wash; this will result in removing the colour.
4. Air dry in a vertical position
5. Examine the slide under a light microscope first using a 10X objective (100X magnification). Magnification can be increased when searching for intracellular protozoa and for identification of any microfilariae.

Results

Parasite cytoplasm will stain light blue and nuclei will stain dark magenta.

See the Dog and Cat endoparasite guidelines for images of the various haemoprotozoa.

If microfilariae are seen in a blood smear, a Knott's test should be performed to aid in identification.

Be wary of artefacts related to drying or staining.

Safety precautions

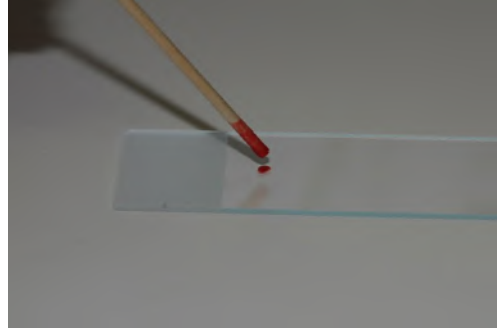
Wear lab coat and disposable gloves

Clean up procedures

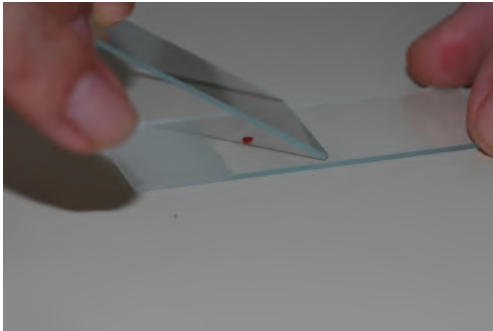
Dispose of all slides and cover slips in a sharps container



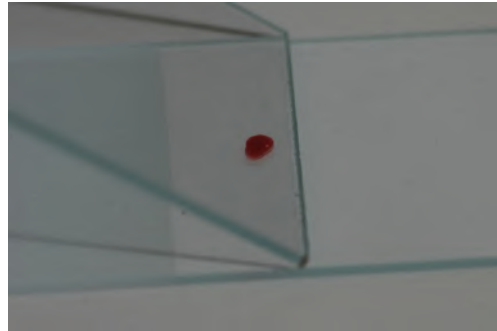
Step 1.



Step 1.



Step 2.



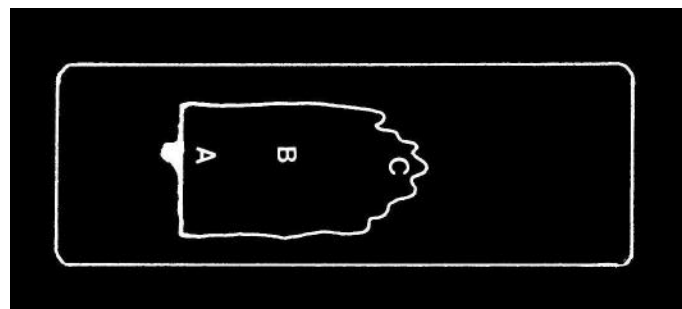
Step 3.



Steps 4-7.



Step 8.



Step 8. Feathered edge is in area C.

SOP 9: Buffy coat smear

Buffy coat is the fraction of the blood which contains the majority of the white blood cells and platelets. It contains 10 to 20 times concentrated leukocytes. It is expressed following the centrifugation of the blood collected on anticoagulants. The buffy coat appears as a buff-coloured thin layer (hence the name), representing about 1% of the total blood volume, located in the centrifugation tube between the plasma (above) and the red blood cells (below). In parasitology, the buffy coat-stained smear represents a more sensitive way than the standard whole blood smear to microscopically detect certain parasites and other pathogens located in the white blood cells, such as *Leishmania*, *Hepatozoon*, *Anaplasma*, or *Ehrlichia*. The buffy coat might also include a higher concentration of microfilariae and/or trypomastigote forms of *Trypanosoma* spp. than the whole blood. The buffy coat fraction can be also used for DNA extraction followed by PCR for the molecular detection of the same parasites.

Reagents/Materials

- EDTA blood tube
- Pipette
- Haematocrit or glass capillary tube
- Giemsa stain
- Glass slides

Procedure

1. Whole blood collected on anticoagulant (EDTA, heparin, etc.) is centrifuged at 200 g for 10 minutes at room temperature
2. The tube is gently removed from the centrifuge and placed in a tube holder
3. With a fine pipette, a small drop of the buffy coat layer is aspirated and placed on a slide
4. Use another slide (the spreader) to spread the blood
5. Hold the spreader at an angle of approximately 45 degrees
6. Touch the far side of the drop with the spreader. The far side is the side furthest away from the edge of the slide
7. The buffy coat drop should run along the edge of the spreader
8. Push the spreader along the length of the slide
9. Note, this is pulling the drop behind the spreader. It is not pushing the drop in front of the spreader
10. Push the spreader to close to the end of the slide. This should result in a “feathered edge”, an area where the blood cells are separated
 - a. If the drop is too small, the smear is short
 - b. If drop is too big, a feathered edge is not created
11. Air dry
12. After air drying the smear, fix in absolute methanol for 5 min and air dry
13. Place the slide in the 1:20 Giemsa solution for 20 to 30 min
14. Wash gently using tap water or by dipping into a jar of tap water. Do not over-wash; this will result in removing the colour
15. Air dry in a vertical position

16. Examine the slide under a light microscope first using a 10X objective (100X magnification). Magnification can be increased when searching for intracellular protozoa and for identification of any microfilariae

Results

See the Dog and Cat guidelines for images of the various parasites

Safety precautions

Wear lab coat and disposable gloves

Clean up procedures

Dispose of all slides and cover slips in a sharps container

Skin Analysis SOPs

SOP 10: Adhesive tape/acetate strip method

The adhesive tape method can be used around the perianal region to collect cestode eggs and at various predilection sites to collect fur mites.

Reagents/Materials

- Slide and coverslip
- Forceps
- Clear adhesive tape

Procedure

1. Use clear adhesive tape or an acetate strip
2. The tape or strip should be approximately 2.5 cm long
3. Place on the hair or skin surface
4. Pull in the direction of the hair
5. Place (sticky side down) on a glass slide
6. Examine using a 4X or 10X objective (40 or 100X magnification) for mites and lice and 10X or 40X objectives (100 or 400X magnification) for cestode eggs

Results

Lice and mite eggs can be seen in addition to a variety of fur mites and lice.

Safety precautions

Wear lab coat and disposable gloves
Wash hands thoroughly when finished

Clean up procedures

Dispose of all disposable equipment in clinical waste bin or sharps as appropriate

SOP 11: Trichogram / Hair pluck method

A trichogram is the examination of hair that has been plucked. It is used primarily for fur mites but also can be used for lice. In situations where skin scraping is difficult (sensitive area), hair plucks can be used for skin mite retrieval, although sensitivity is lower compared to skin scrapes. The plucked hair can be placed on a slide and examined using a compound microscope or in a petri dish and examined under a dissecting (stereo) microscope. Hair obtained when shaving areas for skin scrapes also can be viewed using the hair pluck method.

Reagents/Materials

- Slide and coverslip
- Forceps
- Mineral oil/glycerine/paraffin oil

Procedure

1. Use forceps to pluck hair. Pluck in the direction of hair growth
2. If possible, squeeze skin prior to and while plucking
3. A minimum of 20 hairs should be plucked with 40 or more hairs improving sensitivity
4. For viewing using a compound microscope, place hair on a slide with a drop of mineral oil/glycerine/paraffin oil and add a coverslip
5. For viewing using a stereo microscope, place hair on a petri dish with a drop of mineral oil/glycerine/paraffin oil
6. Examine at low power 4X to 100X magnification

Safety precautions

Wear lab coat and disposable gloves
Wash hands thoroughly when finished

Clean up procedures

Dispose of all disposable equipment in clinical waste bin or sharps as appropriate

SOP 12: Skin scrape for mites and Adhesive tape

Deep skin scrapes can be used for collection of *Demodex* spp. by scraping in the region of alopecia or for *Sarcoptes* and *Notoedres* by scraping approximately 1-2 cm from the suspected papules. Superficial skin scrapes can be used for fur mites.

Skin scrapes should be performed in the predilection areas and/or in the vicinity of lesions. Typically, several scrapes are collected for examination.

If a microscope is not available in the examination room or a skin scrape is being performed during a home consultation visit, adhesive tape can be used to preserve the skin scrape. Samples should be viewed for the presence of mites and eggs within 3 days.

Reagents/Materials

- Slide and coverslip
- Blunt scalpel blade
- Mineral oil/glycerine/paraffin oil
- Clear adhesive tape

Procedure

1. If needed, gently shave the area to be scraped
2. Collect the hairs to examine (See SOP 12: Trichogram)
3. Place a drop of mineral oil/glycerine/paraffin oil on a blunt scalpel blade
4. If looking for *Demodex*, gently pinch the skin between thumb and forefinger before scraping
5. Gently scrape the skin longitudinally and laterally with the blunt scalpel blade until slight capillary bleeding
6. Place the collected material on a slide for immediate viewing
7. If viewing cannot be done within a short period of time, place the material collected on the blade to the sticky side of a piece of adhesive tape. Place the tape, sticky side down, on a slide.
8. Optional: apply a strip of tape (approximately 2.5 cm long) firmly to the lesion that has been scraped and pull it off rapidly. Place the tape, sticky side down, on a slide.
9. Examine at low power (4X objective (40X magnification) and 10X objective (100X magnification))

Safety precautions

Wear lab coat and disposable gloves
Wash hands thoroughly when finished

Clean up procedures

Dispose of all disposable equipment in clinical waste bin or sharps as appropriate

SOP 13: Skin biopsy

The method is used for the detection and identification of microfilariae of onchocercid nematodes of the genera *Onchocerca* (i.e., *Onchocerca lupi*) and *Cercopithifilaria* (i.e., *Cercopithifilaria bairdii*, *Cercopithifilaria* sp. II and *Cercopithifilaria grassii*) in the skin through the observation of the sediment.

Reagents/Materials

- Biopsy punches (4 mm in diameter) OR disposable scalpels
- Saline solution (NaCl 0.9%)
- Rubber gasket to secure the membrane
- Glass slides
- Coverslip (10X10 mm)
- Light microscopy
- Methylene blue (1%)

Procedure

1. Collect skin samples using disposable scalpels (about 0.5 × 0.5 × 0.6 cm) OR biopsy punches (sample of 4 mm in diameter)
2. Soak sample in saline solution for 10 min at 37°C or 3 hrs at room temperature (about 20°C)
3. Remove skin sample
4. Centrifuge the samples at 650 g for 10 min
5. Place two drops of sediment on a glass slide
6. Add a drop of methylene blue (1%)
7. Observe under light microscopy at low power (10X objective; 100X magnification) and at high power (40X objective; 400X magnification) for confirmation of species
8. Identify microfilariae according to their morphology

Safety precautions

Wear lab coat and disposable gloves

Clean up procedures

Dispose of all slides and cover slips in a sharps container

Other Procedures

SOP 14: Examination for ear mites

Ear mites (*Otodectes*) can be seen macroscopically and microscopically. Often ear mites can be seen with an otoscope examination, although this method is not as sensitive as examination of debris collected with a swab. Dogs and cats with ear mite infestations can have secondary bacterial infections. These can be painful and might require placing a muzzle on a dog before examination or administration of an analgesic.

Reagents/Materials

- Otoscope
- Swab
- Mineral oil / paraffin oil
- Slide and coverslip

Procedure (Otoscope)

1. Lift up the ear pinna.
2. Gently place the speculum into the opening of the ear canal
3. While looking through the otoscope, slowly move the speculum down the vertical ear canal
4. Observe the wax and debris for movement. *Otodectes* can appear as white dots moving on the dark wax
5. If the ear is particularly full of debris, using a wider speculum can be beneficial
6. Debris and wax can fill the tip of the speculum. This debris and wax can be examined as described below for a swab

Procedure (Swab)

1. Using a cotton tipped swab lightly coated with mineral oil/paraffin oil, remove the dark waxy debris from both ears
2. Observe the swab to see if there is movement. Movement is likely ear mites.
3. Place 2 to 3 drops of mineral oil/paraffin oil on a glass slide
4. Mix debris collected from the ear on the swab with the oil
5. Remove large pieces of debris
6. Place a coverslip on the slide
7. Examine at low power (4X and 10X objective (40X and 100X magnification))

Results

With both procedures the mites can be seen by their movement, Microscopic examination confirms identification and increases sensitivity of the method.

Safety precautions

Wear lab coat and disposable gloves. Wash hands thoroughly when finished

Clean up procedures

Dispose of all disposable equipment in clinical waste bin

SOP 15: Pustular Demodicosis

Skin scraping often is negative when a dog has pustular demodicosis. This method can increase recovery and confirmation of infestation in these circumstances.

Reagents/Materials

- Slide and coverslip

Procedure

1. Squeeze one or two pustules
2. Collect the material by pressing a slide firmly against the skin
3. Place a coverslip on the slide
4. Examine the preparation at low power (10X objective, 100X magnification) and at higher power (40X objective, 400X magnifications)

Safety precautions

Wear lab coat and disposable gloves
Wash hands thoroughly when finished

Clean up procedures

Dispose of all disposable equipment in clinical waste bin or sharps container as appropriate

SOP 16: Urine sedimentation

Urine sedimentation can be used to identify eggs of *Diocotophyme renale* and *Pearsonema plica* (syn. *Capillaria plica*).

Reagents/Materials

- Slide and coverslip
- 10-15 mL centrifuge tube
- Lugol's iodine
- Acetic acid

Procedure

1. Collect a urine sample in a disposable plastic cup
2. Fill a 10 or 15 mL tube with the sample and centrifuge at 3000 rpm for 10 min. Pour off supernatant
3. Take 1-2 drops of the sediment into a glass slide and add a coverslip
4. Sample can be mixed with a drop of Lugol's iodine to add contrast
5. If the sample is covered with red blood cells, it can be mixed with 2 to 3 drops of acetic acid
6. Examine the preparation at low power (10X objective, 100X magnification) and at higher power (40X objective, 400X magnifications)

Safety precautions

Wear lab coat and disposable gloves
Wash hands thoroughly when finished

Clean up procedures

Dispose of all disposable equipment in clinical waste bin or sharps container as appropriate

Identification References

Eggs and oocysts in faeces

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