Parasitological laboratory

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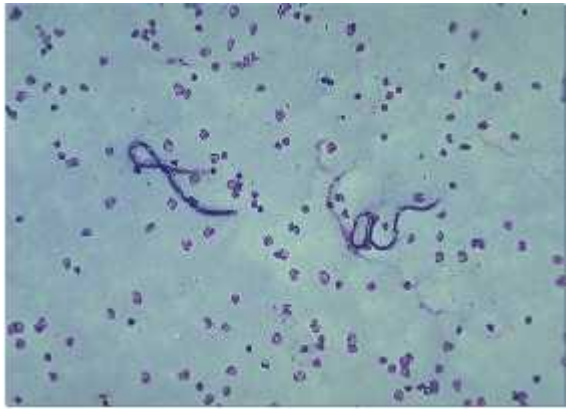
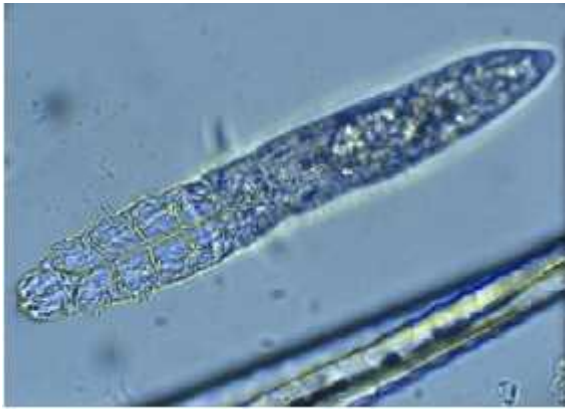




Diagnosis

- Diagnostic techniques: radiography, anatomical pathology, necropsy, microscopic examination of tissue sections, clinical pathology, microbiology, hematology, blood chemistry, immunoserology, parasitology and urinalysis

Collection and Processing of Samples for Parasitology

1 Internal parasites

- Blood examination
- Fecal Examination
- Necropsy/Biopsy

2. External parasites

- Skin scraping

Reasons for Diagnosis

- **Therapy and prevention depend on accurate ID of a parasite**

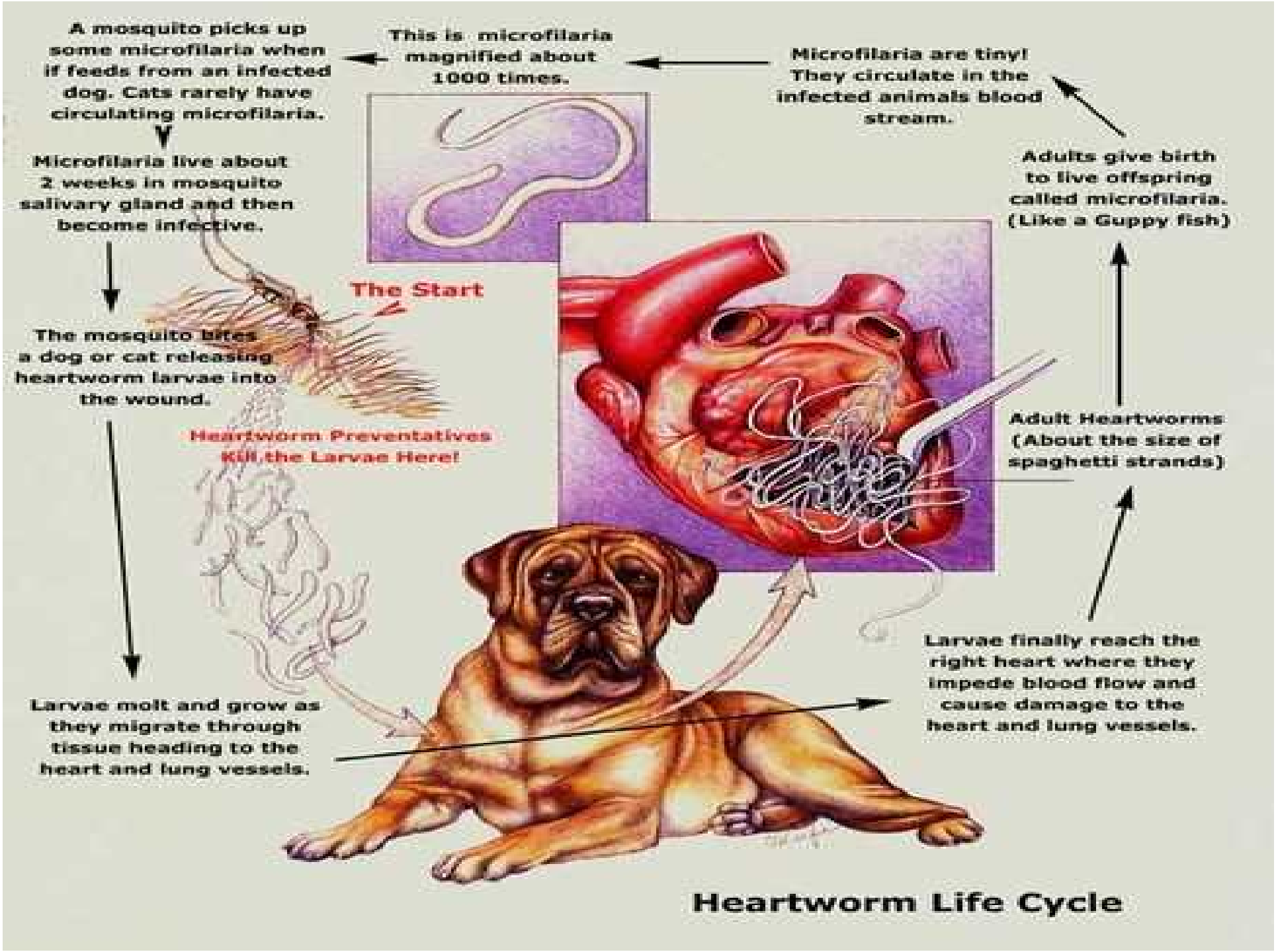
- **Blood examination**

-Heartworm infestation of dogs and cats is diagnosed by microscopic examination of the blood for microfilaria (immature larval forms).

-PCR

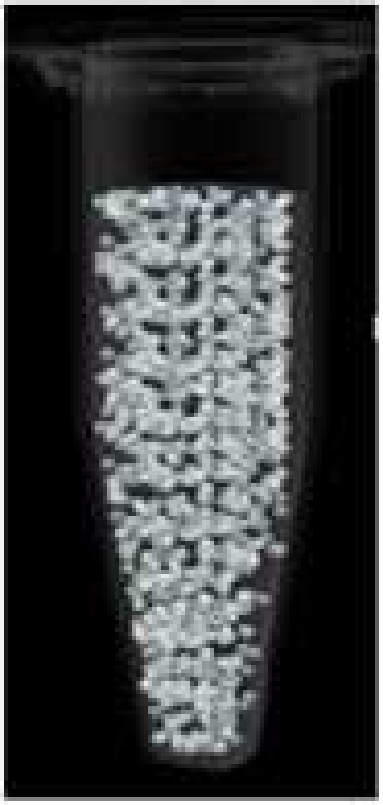
-Snap tests

-Blood smear, etc.


A mosquito picks up some microfilaria when if feeds from an infected dog. Cats rarely have circulating microfilaria.
This is microfilaria magnified about 1000 times.
Microfilaria are tiny! They circulate in the infected animals blood stream.
Adults give birth to live offspring called microfilaria. (Like a Guppy fish)
Microfilaria live about 2 weeks in mosquito salivary gland and then become infective.
The Start
The mosquito bites a dog or cat releasing heartworm larvae into the wound.
Heartworm Preventatives Kill the Larvae Here!
Adult Heartworms (About the size of spaghetti strands)
Larvae molt and grow as they migrate through tissue heading to the heart and lung vessels.
Larvae finally reach the right heart where they impede blood flow and cause damage to the heart and lung vessels.
Heartworm Life Cycle

One measurement

Nanodroplet PCR reactions
are independent, single
amplification events



Many thousands
of discrete measurements

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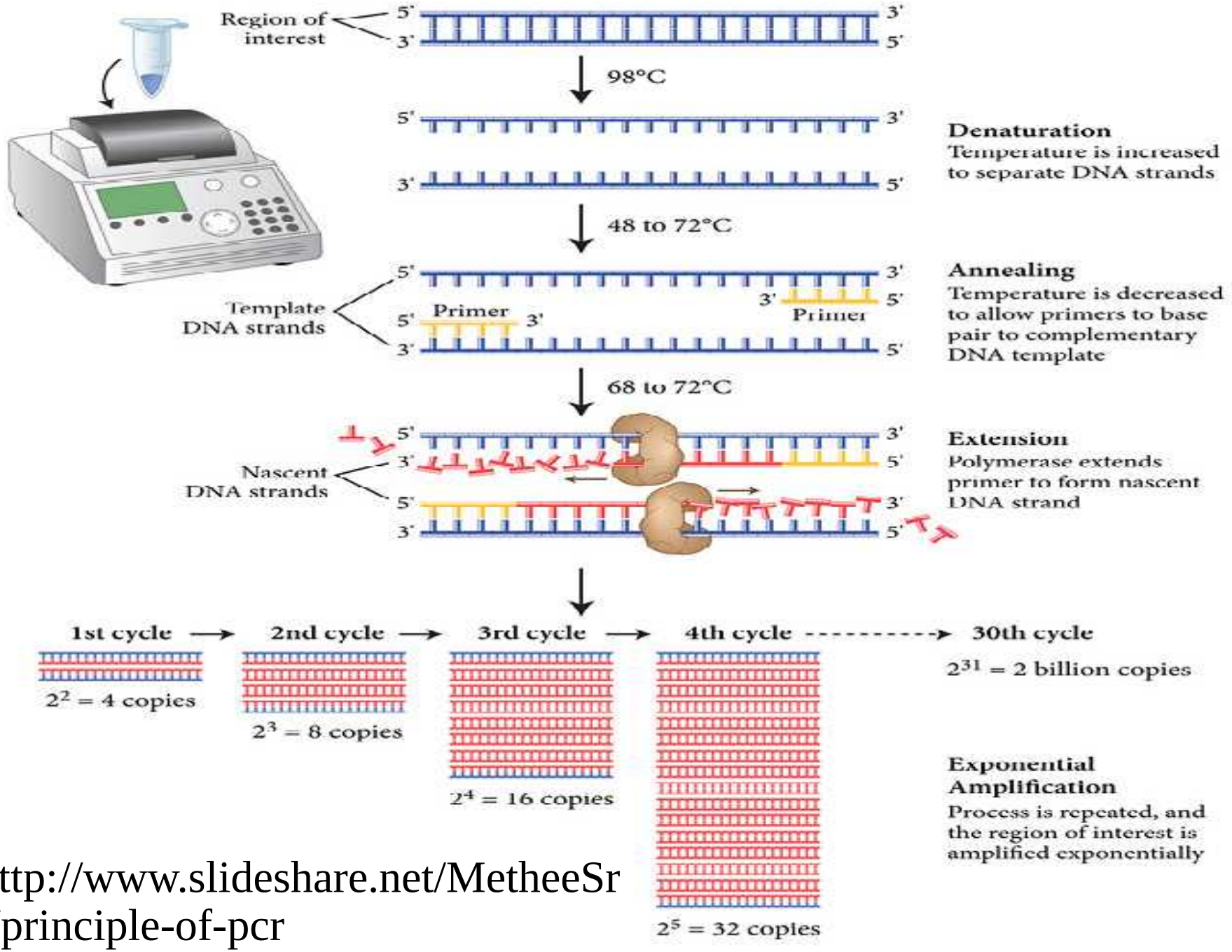

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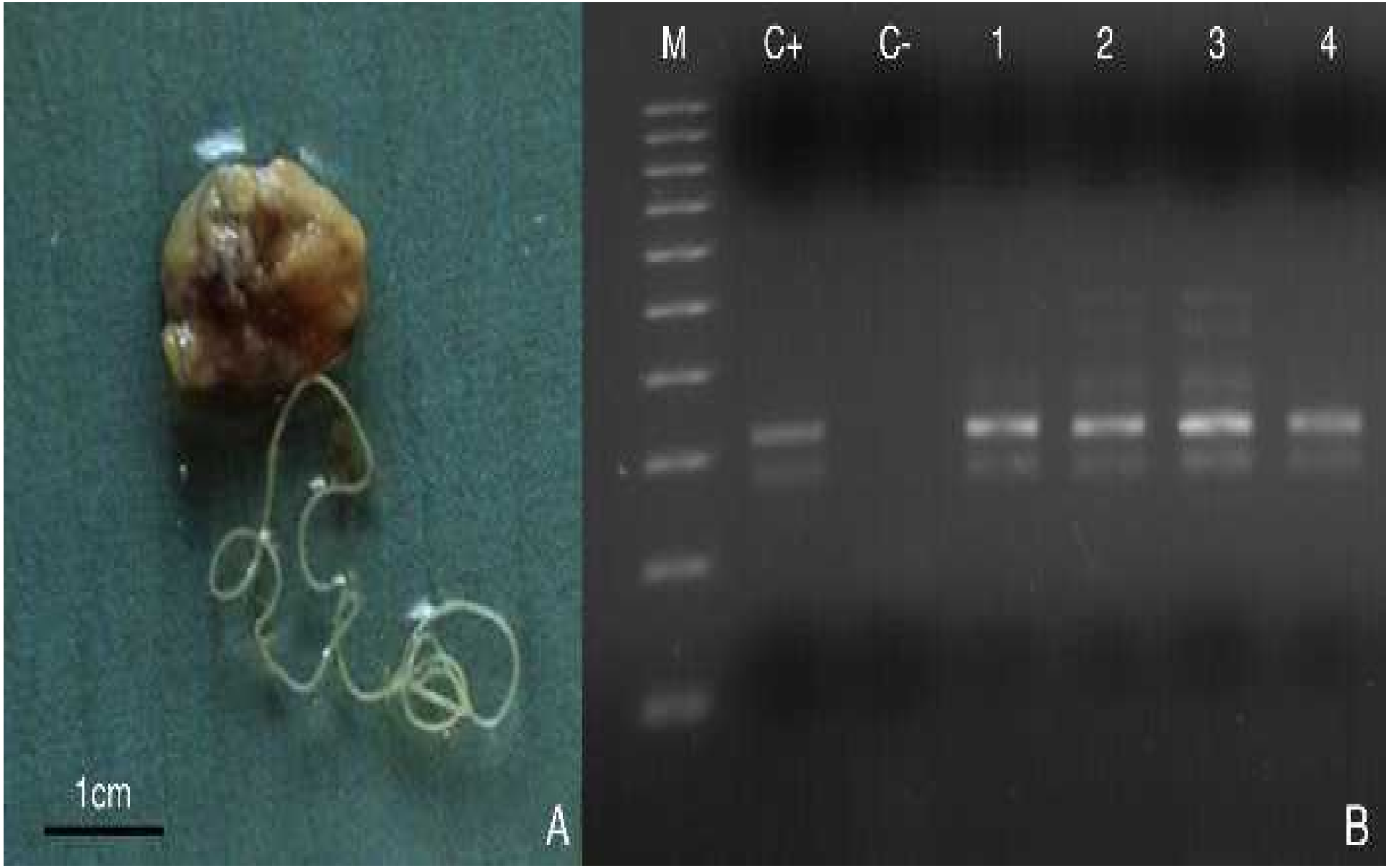

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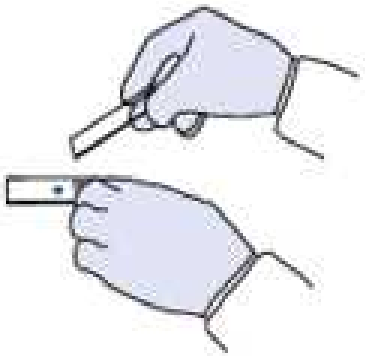
Heartworms: Testing

- Snap Tests
  - Antigen test
  - Only the female heartworm has the antigen.
  - Positive is positive! Strong or Weak is irrelevant
- Blood Smear
  - Circulating microfilaria

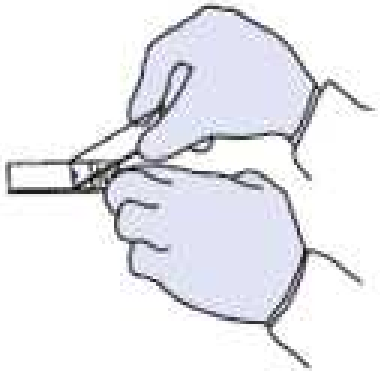
FIGURE A-2. Preparation of thin and thick blood films

1
Whenever possible, use separate slides for thick and thin films.

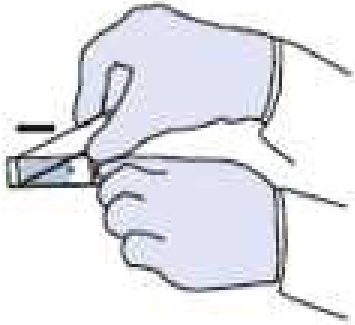
2
Thin film (a): Bring a clean spreader slide, held at a 45-degree angle, toward the drop of blood on the specimen slide.



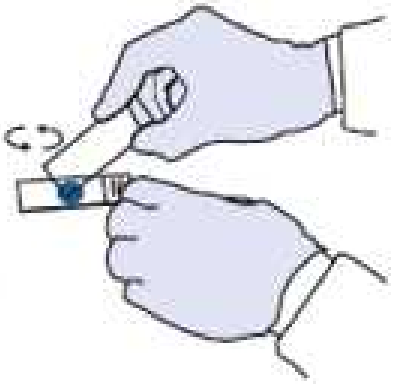
3
Thin film (b): Wait until the blood spreads along the entire width of the spreader slide.



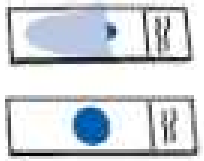
4
Thin film (c): While holding the spreader slide at the same angle, push it forward rapidly and smoothly.



5
Thick film: Using the corner of a clean spreader slide, spread the drop of blood in a circle the size of a dime (diameter 1–2 cm). Do not make the smear too thick or it will fall off the slide (you should be able to read newsprint through it).

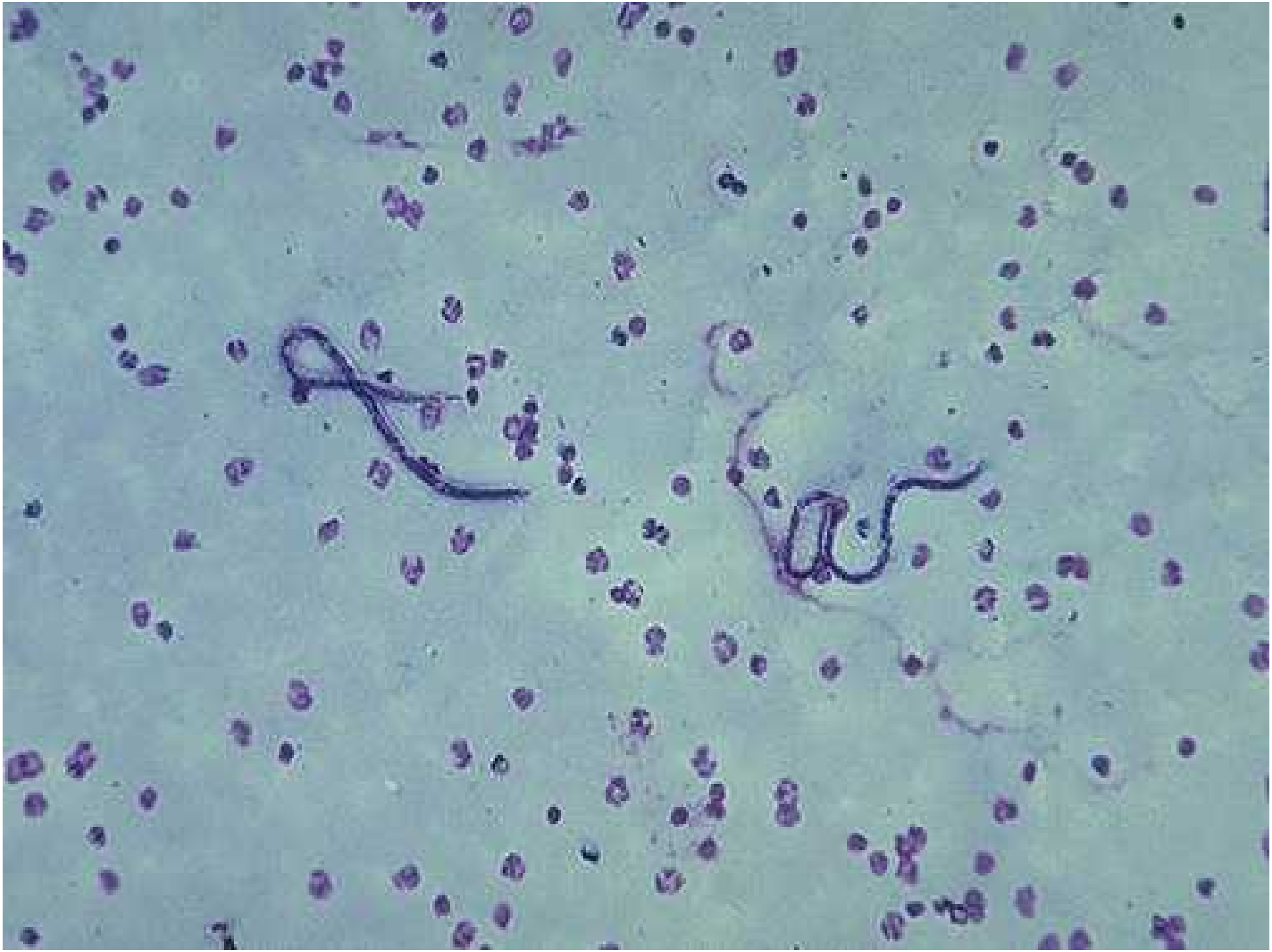


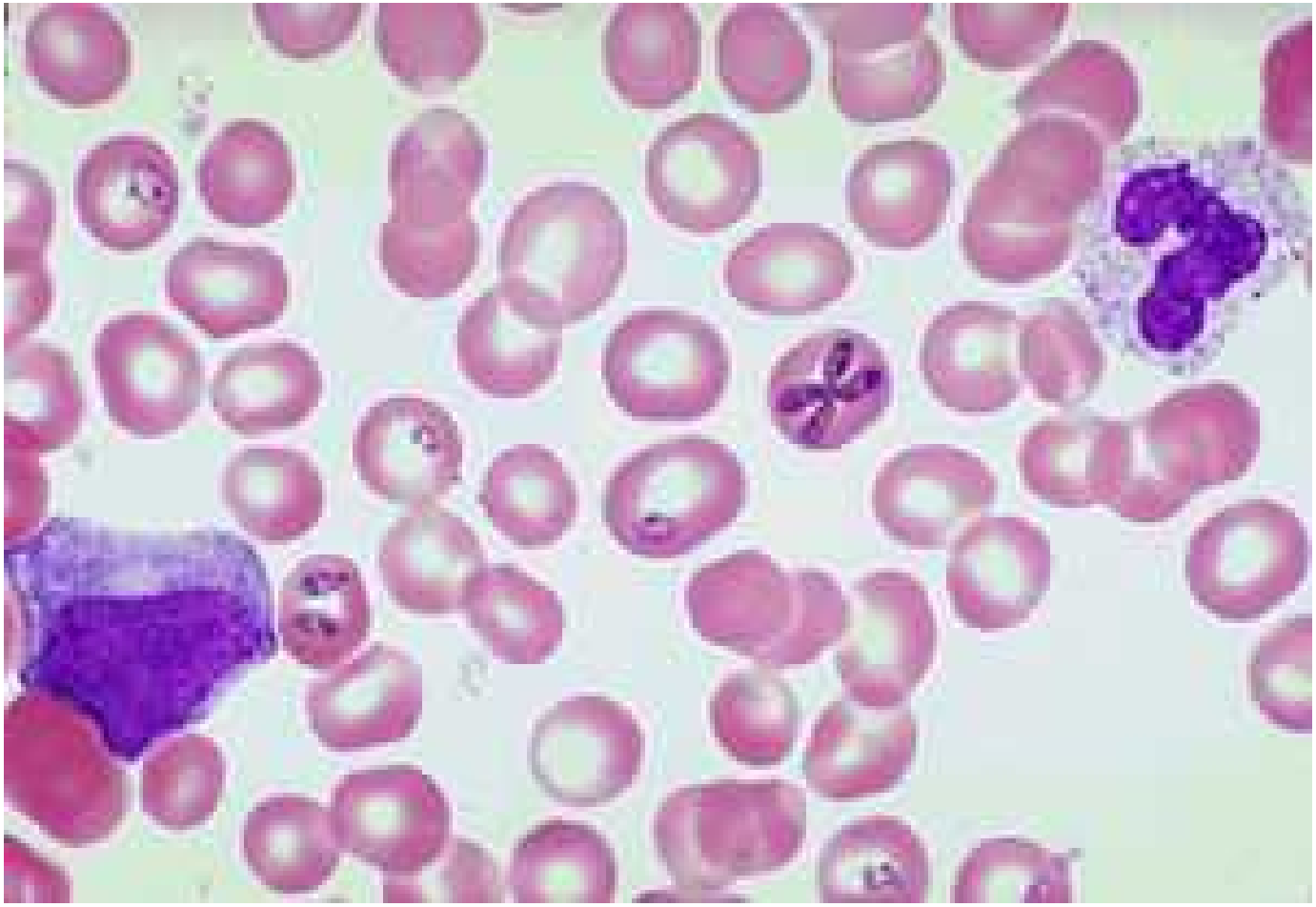
6
Wait until the thin and thick films are completely dry. Fix the thin film with 100% (absolute) methanol. Do not fix the thick film.



7
If both the thin and thick films must be made on the same slide, fix only the thin film with 100% (absolute) methanol. Do not fix the thick film.

8
When the thin and thick films are completely dry, stain them. Thick smears might take ≥1–2 hours to dry. Protect unstained blood smears from excessive heat, moisture, and insects by storing in a covered box.





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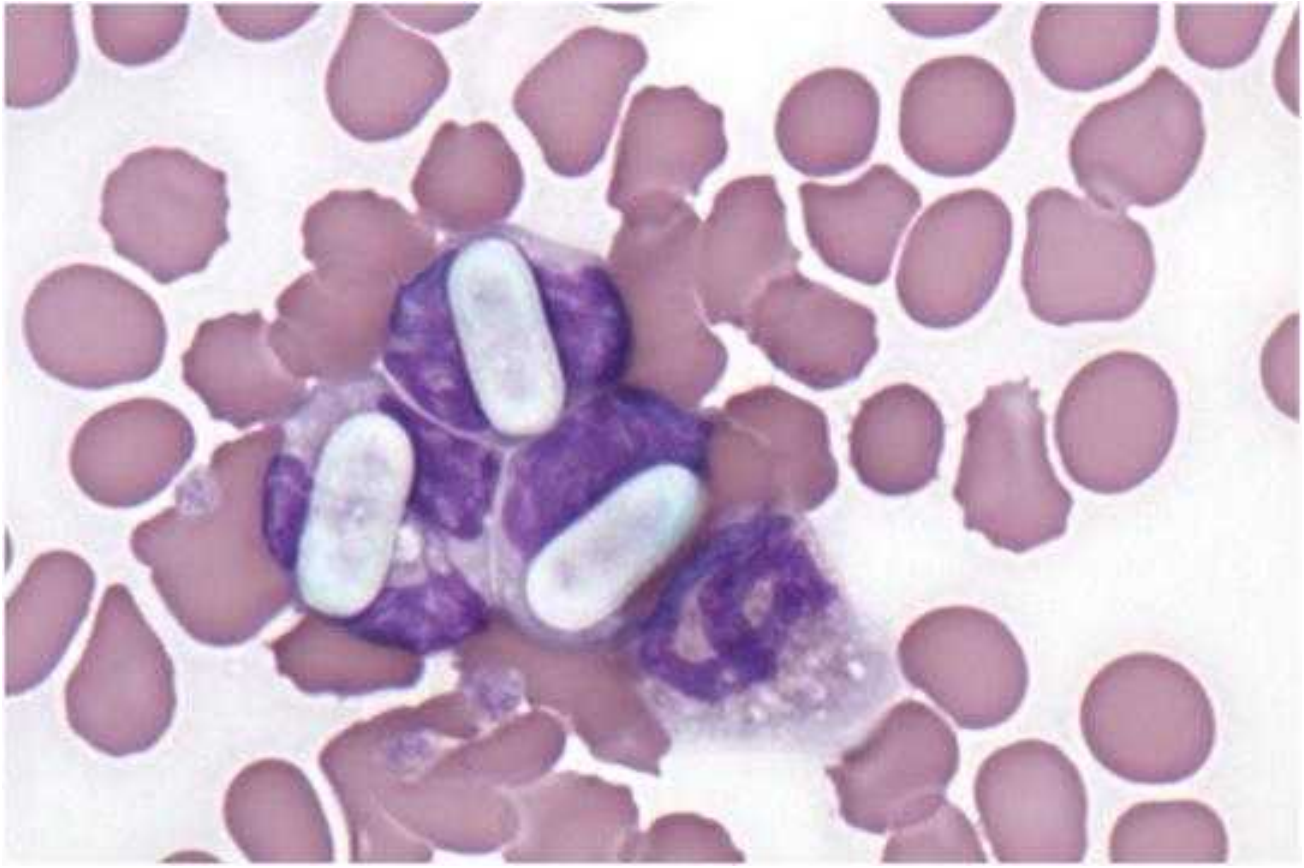
Kritnaphat Singnak
Vet-Tech KU



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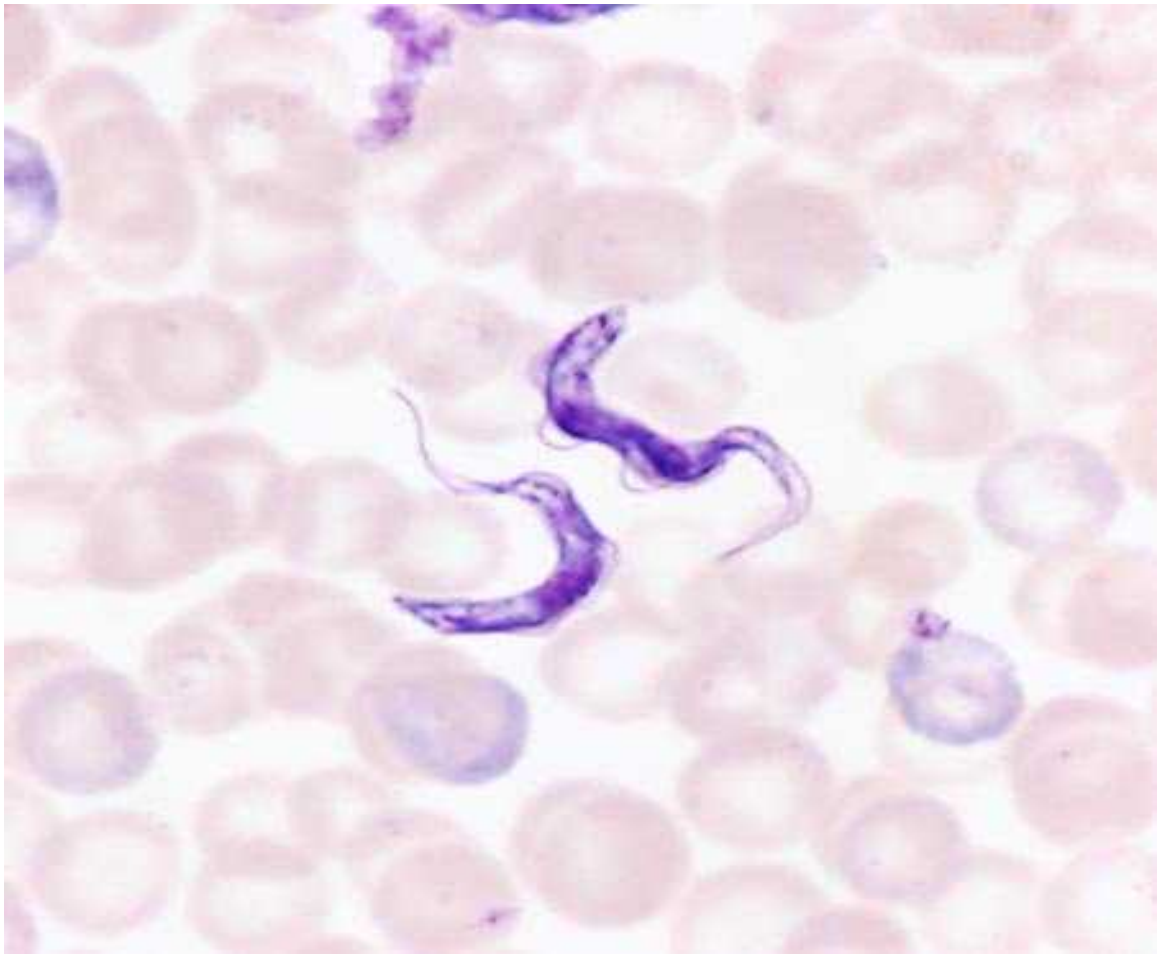


http://www.photomazza.com/?Trypanosoma-gambiense

- Fecal Examination

-Ideally, feces should be processed as soon after passage from the animal as possible.

-If the processing of a fecal specimen must be delayed, it may be:

  - refrigerated (but not frozen) for several days (not recommended for samples with live larvae that you intend to examine using the Baermann technique).
  - fixed, e.g., 10% formalin (5% formalin-saline is better for protozoal cysts). Add fixative to feces at a ratio 3:1 (v:v) and mix well. (Not for Baermann technique.)

- If an animal has been treated with anti-diarrhea preparations containing bismuth or kaolin, mineral oil, oral contrast material (barium) for radiology (all of these materials float) or antibiotics, then parasites may be difficult or impossible to find. Therefore, repeat the fecal exam 5-10 days after treatment withdrawal.

- Fecal collected Processing

-First, examine the feces for blood and other clinical signs, then examine the inside of container for tapeworm segments (which are motile and may move away from the fecal mass).

-Many techniques have been devised to increase the likelihood that parasites will be detected in a particular sample of feces. The merits and limitations of representative fecal processing techniques are summarized in the table on the next page. Step-by-step directions for performing the various methods are on the following pages.

- Repeat Fecal Exams are suggested in the following situations:

-Clinical signs suggest parasitism, but initial fecal exam was negative. Repeat in 2 or 3 days. Repeat for a total of 3 times within 7 to 10 days, if no parasites are seen it is likely the animal is not infected.

-Following specific therapy of a parasitic infection, have owner submit a fecal specimen 2 weeks following the last administration of drug. (This is late enough that all eggs and cysts will have been cleared from the gut, but , for most parasites, too early for re-infection to be showing up.)

Roundworm Ova

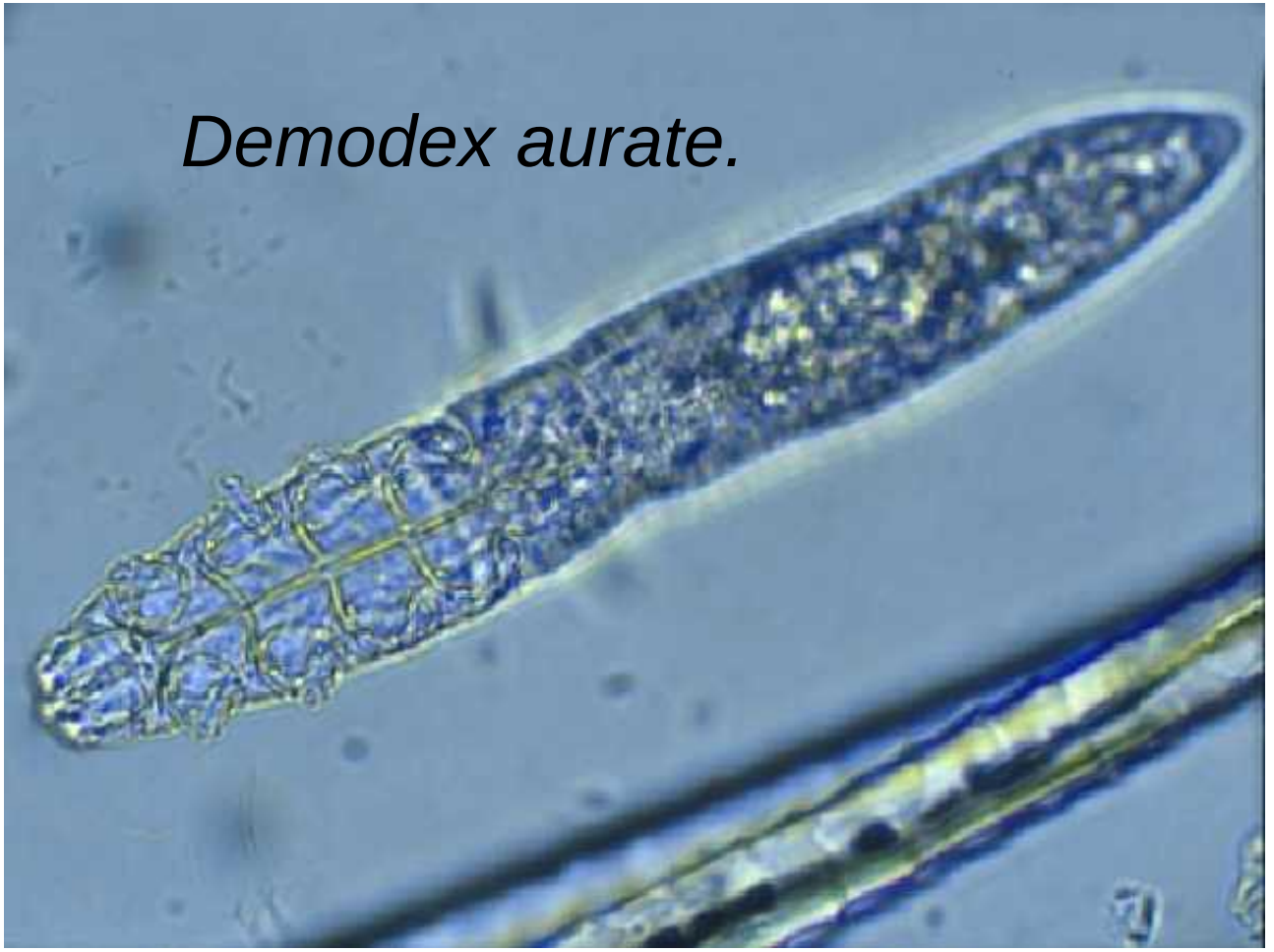
External Parasites

- External parasites, especially mites, may be identified by microscopic examination of the fur or by skin scrapings.

Kit Instructions

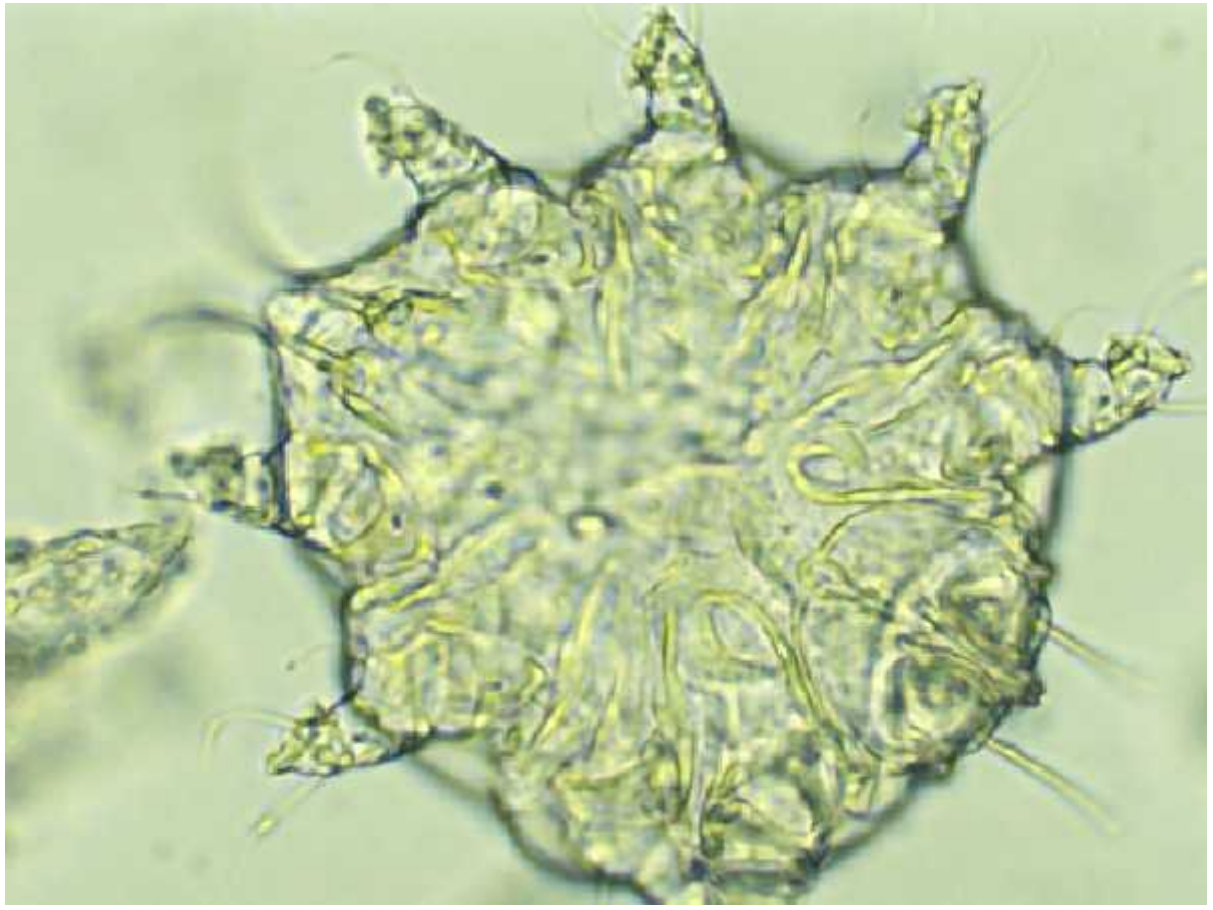
- Procedure: Skin scraping procedure
- Scrapings must be from the infected area.
  - 1. Identify the skin in the area to be scraped.
  - 2. Scrape the skin with the edge of a scalpel blade. Use the lid of the inner carrier as a work-stage.
  - 3. Transfer the material that is attached to the scalpel blade onto the glass slide by tapping gently.
  - 4. Add 1 or 2 drops of mineral oil from the pierced oil segment to the slide, and mix of skin cells and oil.
  - 5. Label the slides by writing the patient's last name, first name and date on the frosted part of the slides.
  - 6. Place the slides in the slide carrier, tape it closed, and place in a biohazard bag. The bag should be accompanied by the appropriate form with relevant patient identification, clinical history, requesting physician and location of skin that had been scraped.

Hamster Skin Mite (ventral view)



Psoregates Mange Mite (ventral view)

Isolated from Non-Human Primate



Summary

- Effective treatment can be initiated sooner if diagnostic results can be made quickly available to the clinician treating a disease outbreak.
- The diagnostic techniques discussed above are equally important in determining the true health status of normal-appearing animals, since subclinical infections can have devastating effects on research results.

- It is through the combined use of these techniques, coupled with clinical examination and daily observations by laboratory animal technicians, that the health status of both individual animals and entire colonies can be accurately defined.

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