



Diagnosis of blood protozoan diseases

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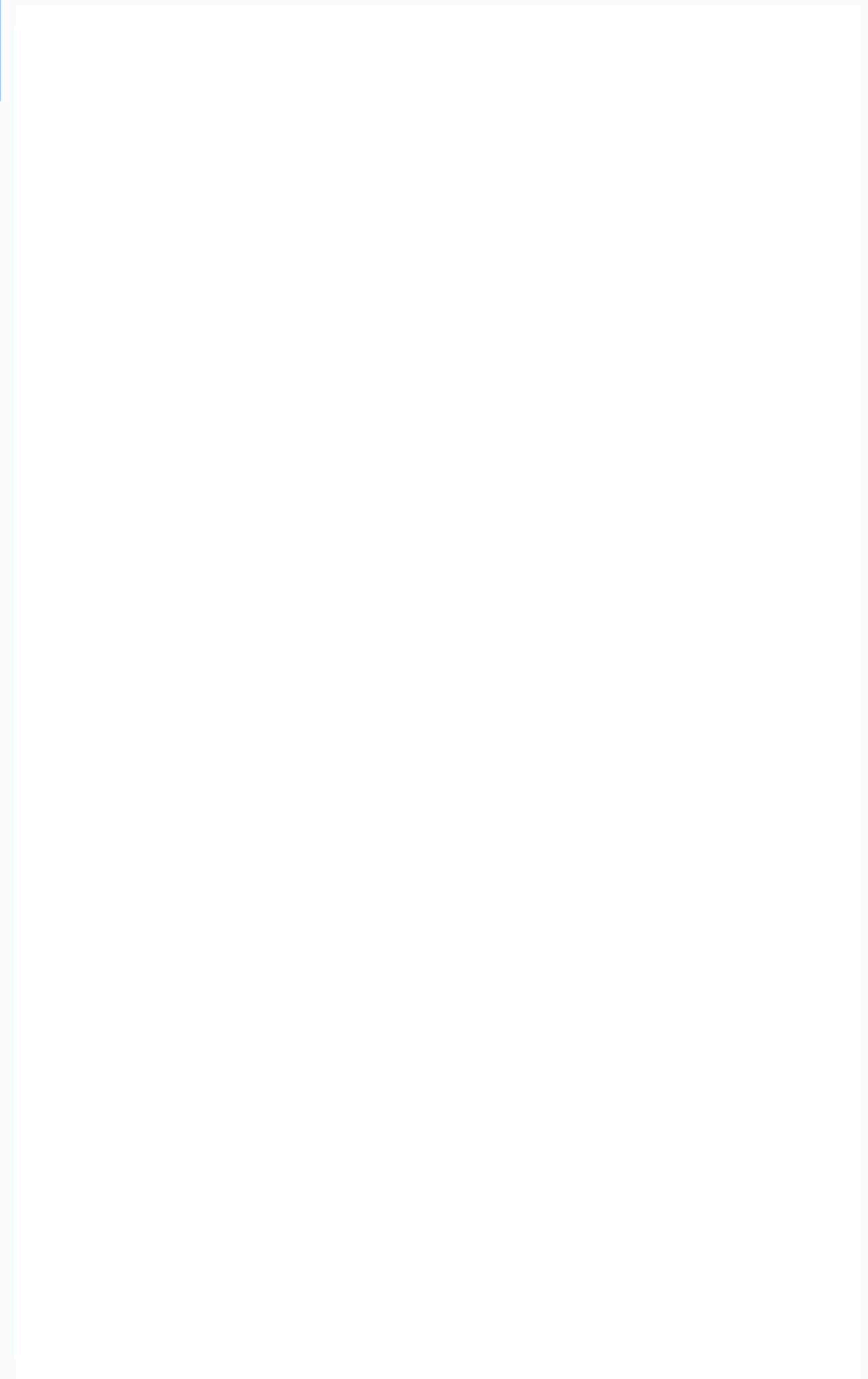
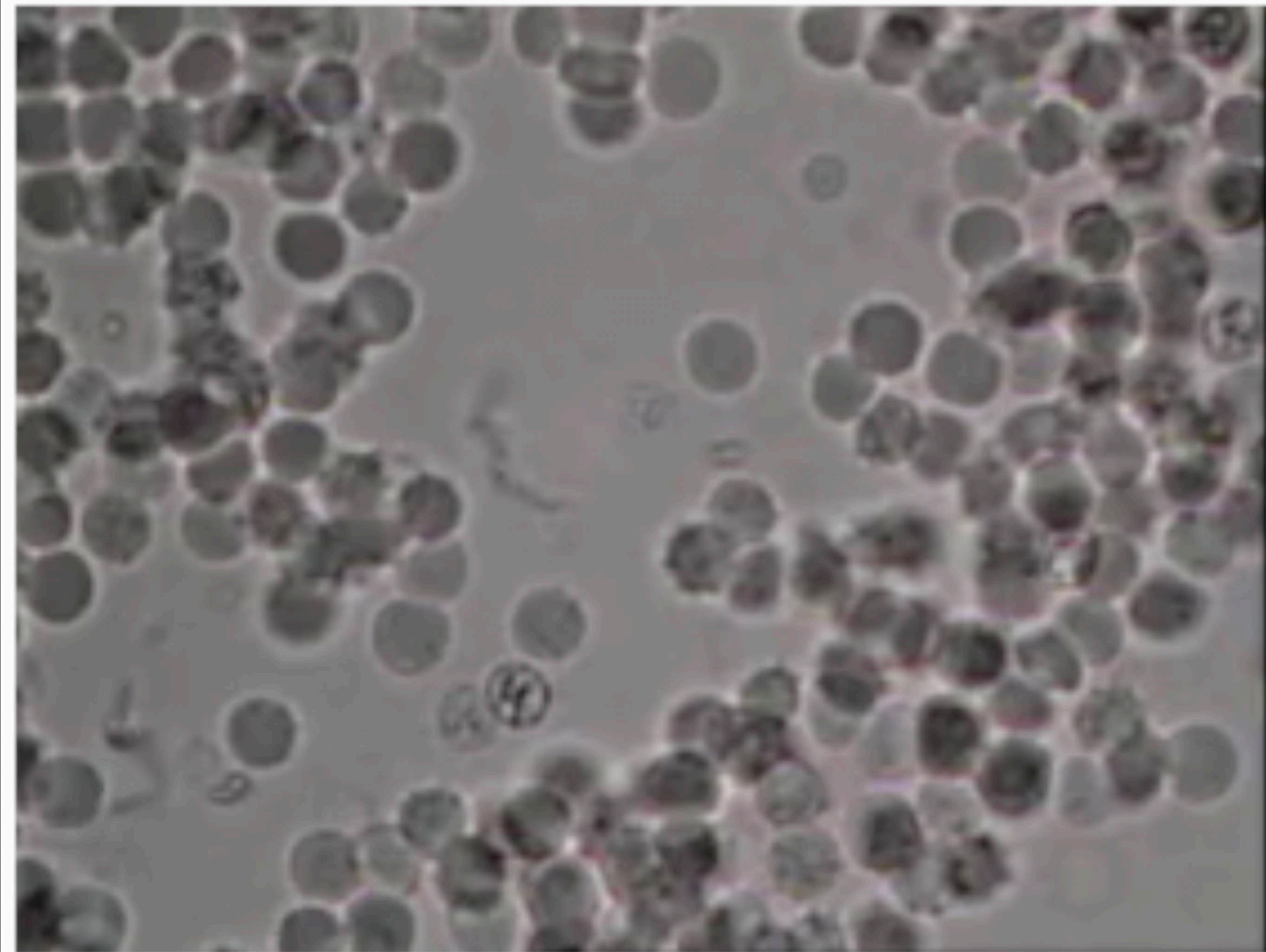


Diagnosis

- ▶ **History and clinical signs**
- ▶ **Direct blood examination**
 - Always prefer first drop from capillary bleed
 - Add a drop of blood to an equal sized drop of saline, place coverslip and examine after allowing the cells to settle. Look for motility



Diagnosis



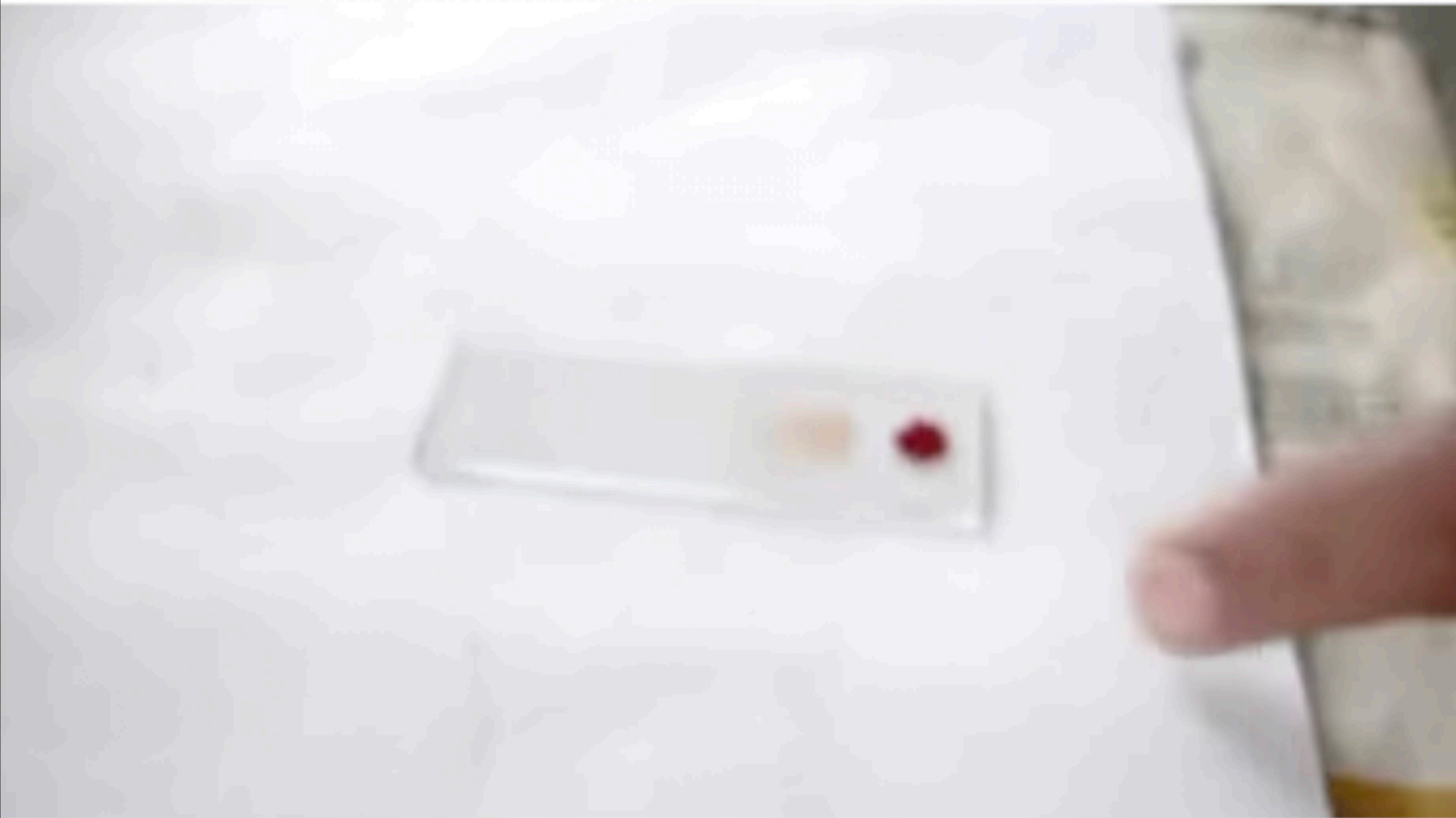


Diagnosis

- ▶ **Romanowsky's stained blood smear**
- ▶ **examination**
 - **Prepare** thin smears from first drop of blood from capillary bleed
 - **Stain** them with Leishman/Giemsa stain and
 - **look** for parasites in the fan-edge portion



Diagnosis





Diagnosis

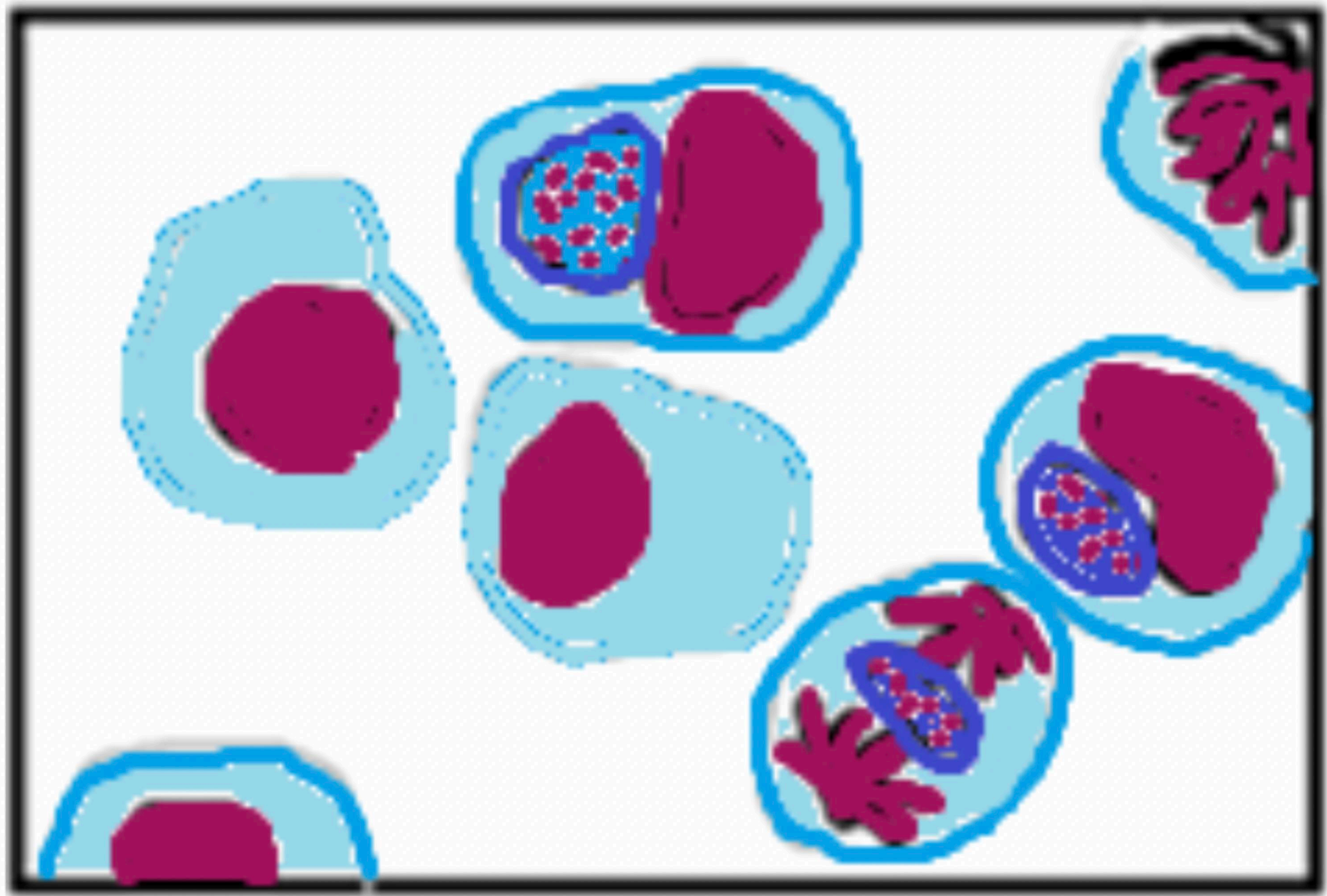
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Diagnosis

Lymph node aspirate cytology

- Secure the active, swollen lymph node with one hand and insert a 20G needle attached to a 2ml syringe vertically
- Exert suction by pulling the plunger. If fluid is drawn into the stem of the syringe, the same can be used to make a smear
- If no fluid is observed, gently withdraw the syringe (without removing it from the tissue) and direct it in a different direction and exert suction by withdrawing the plunger.
- If still no visible fluid is noticed, release the plunger, pull out the syringe, remove the needle, pull back the plunger, attach the needle again and press the plunger with force, pointing the needle on to a slide
- Any fluid collected in the needle will splash on the slide and can be used to stain
- Look for indications of cell division and /or presence of Koch's Blue Bodies





Diagnosis

- ▶ **Microhaematocrit preparation for buffy coat examination**
 - Draw blood with anticoagulant into a capillary tube.
 - Seal one end with China clay and centrifuge at 2500 RPM in a swing out centrifuge
 - Examine the interphase for **motile** trypanosomes
 - Break the tube at the interphase, tap the buffy coat on to a slide, make smear and stain



Diagnosis





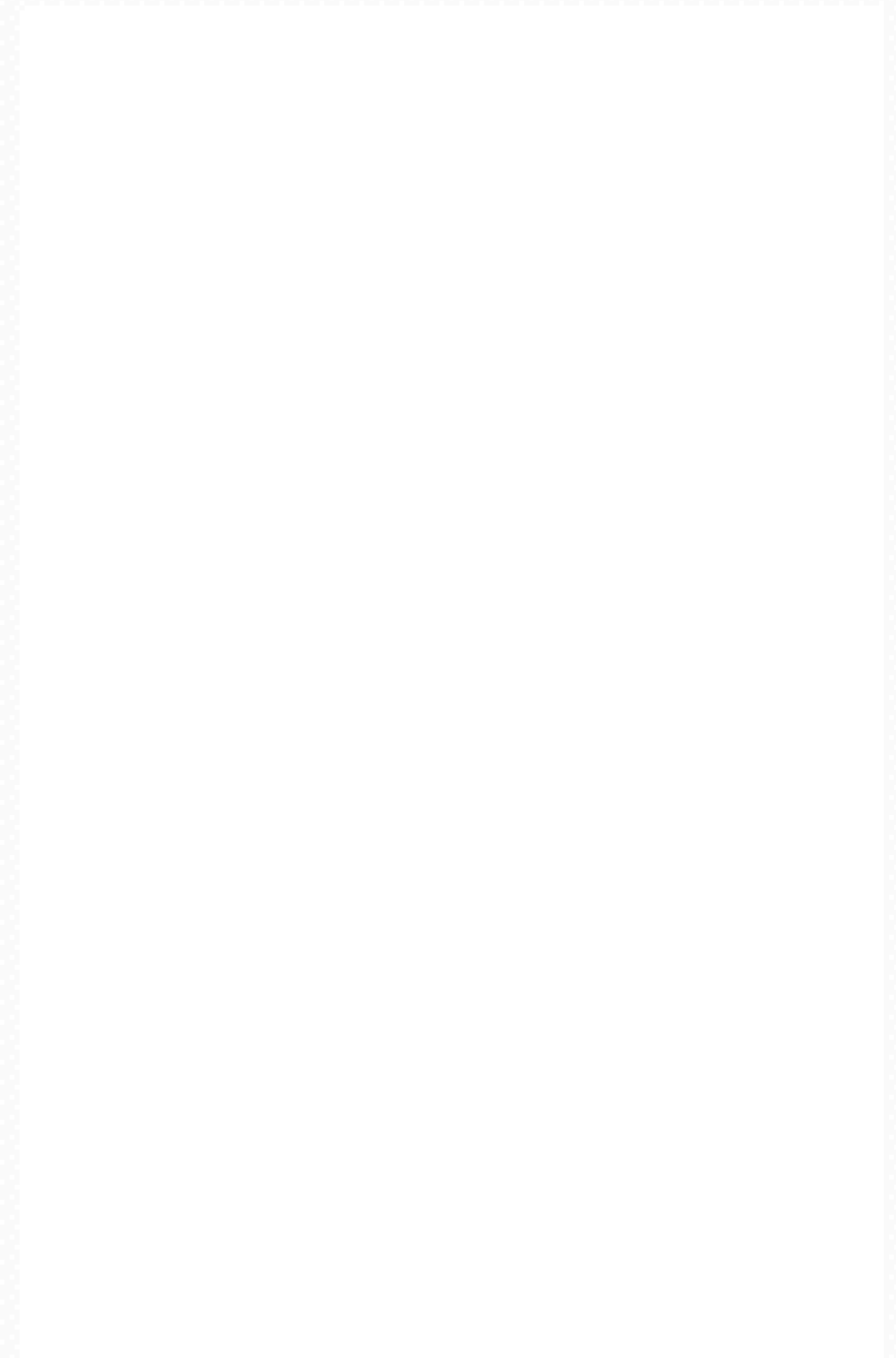
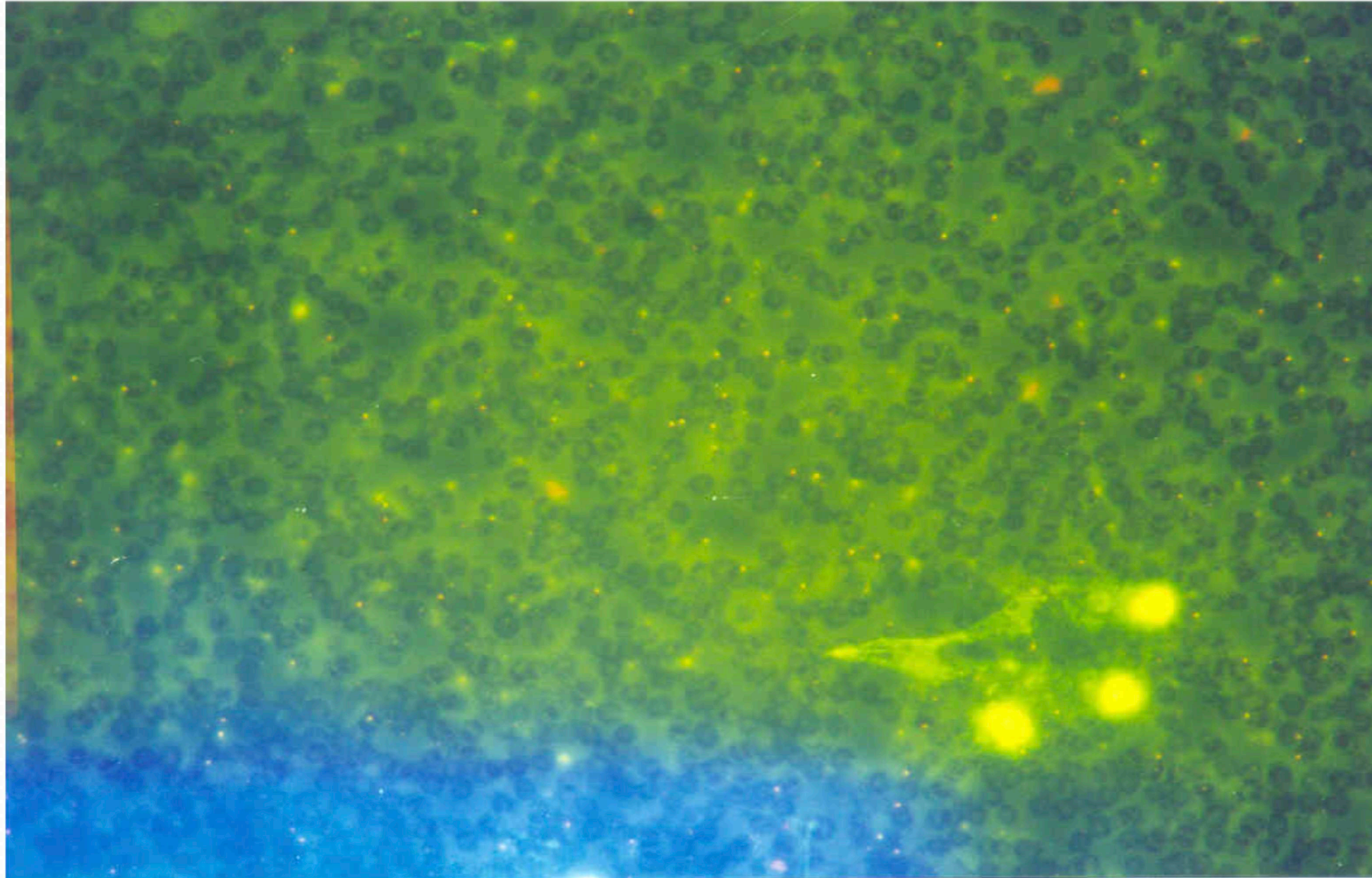
Diagnosis

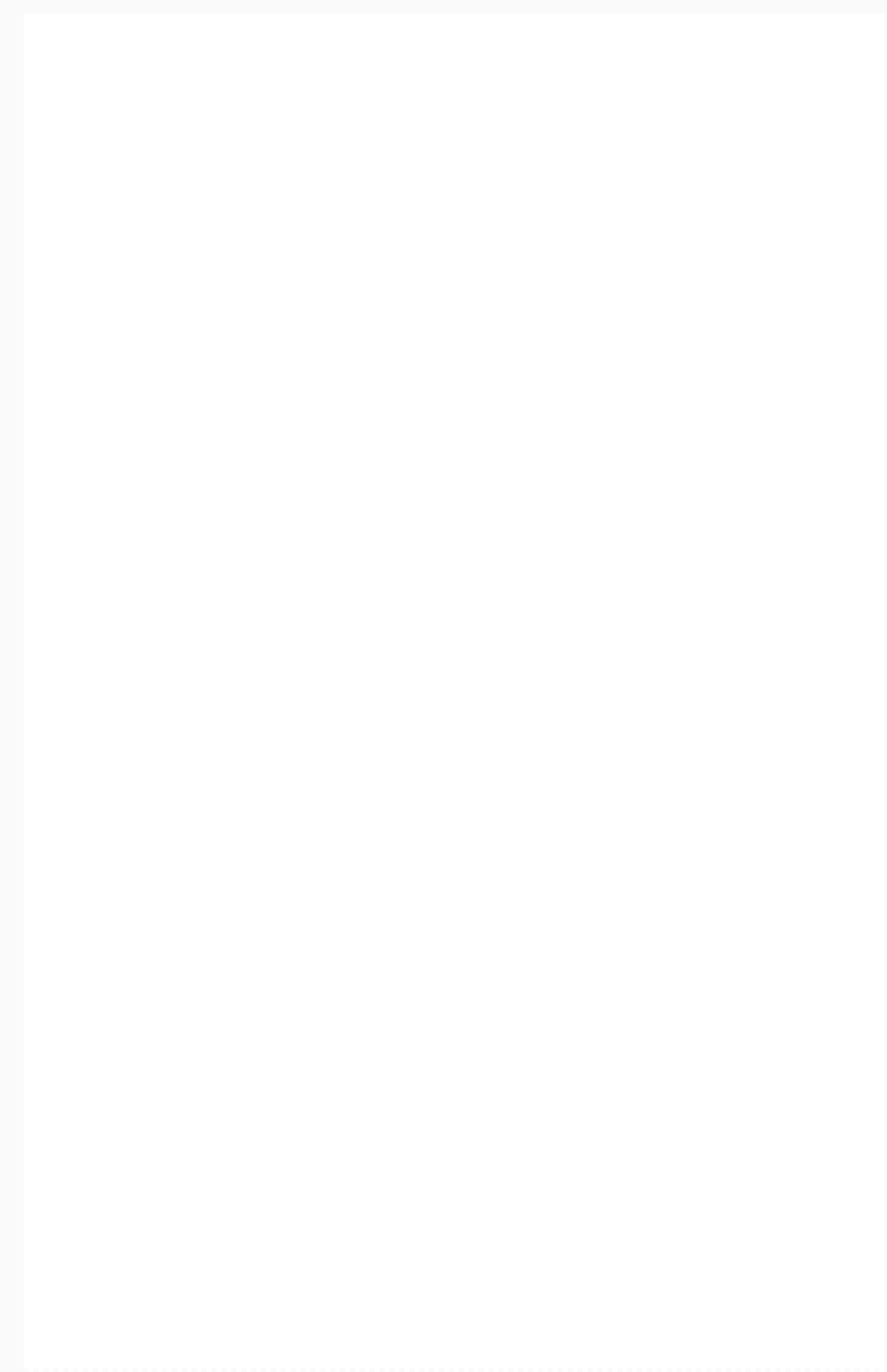
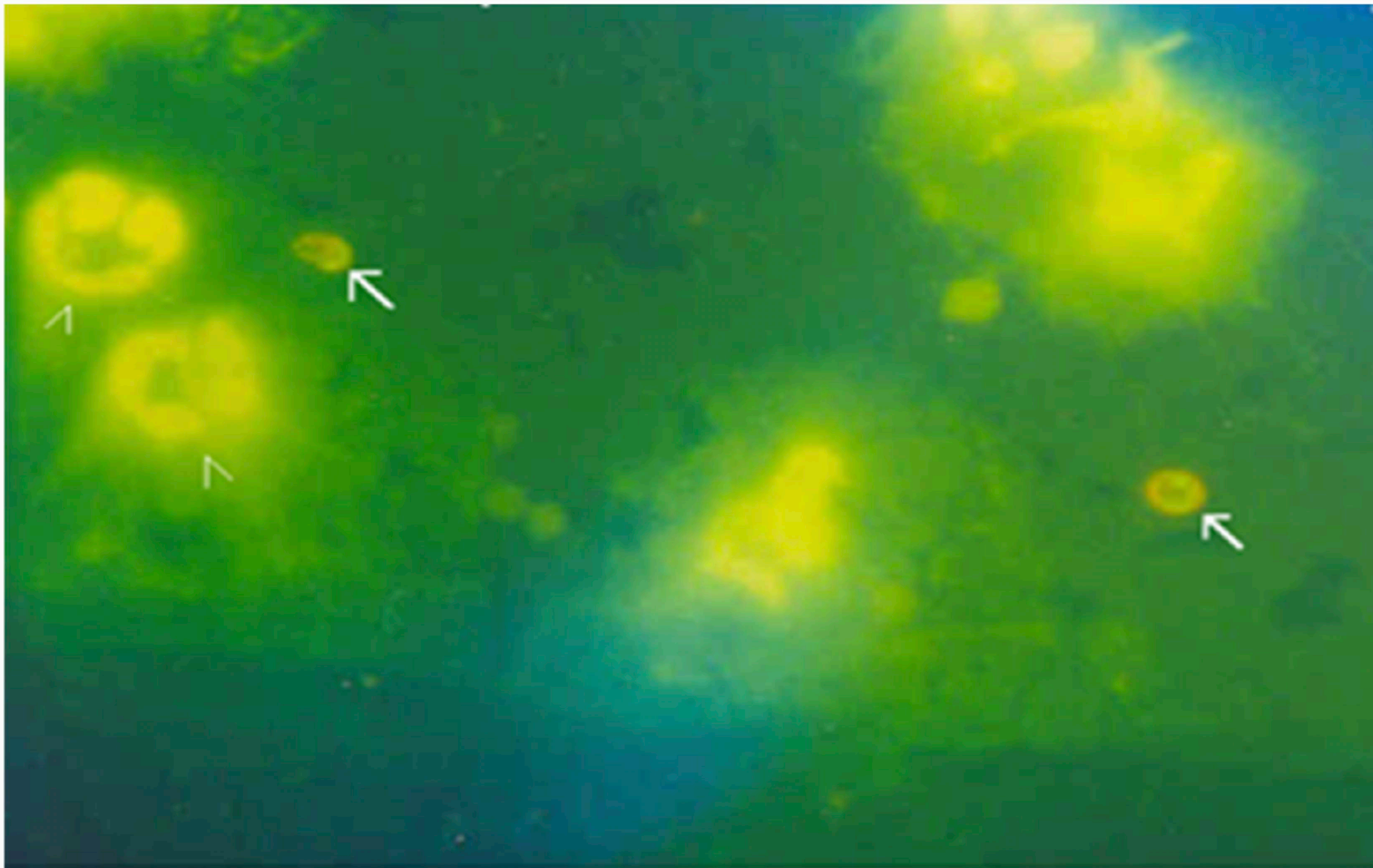
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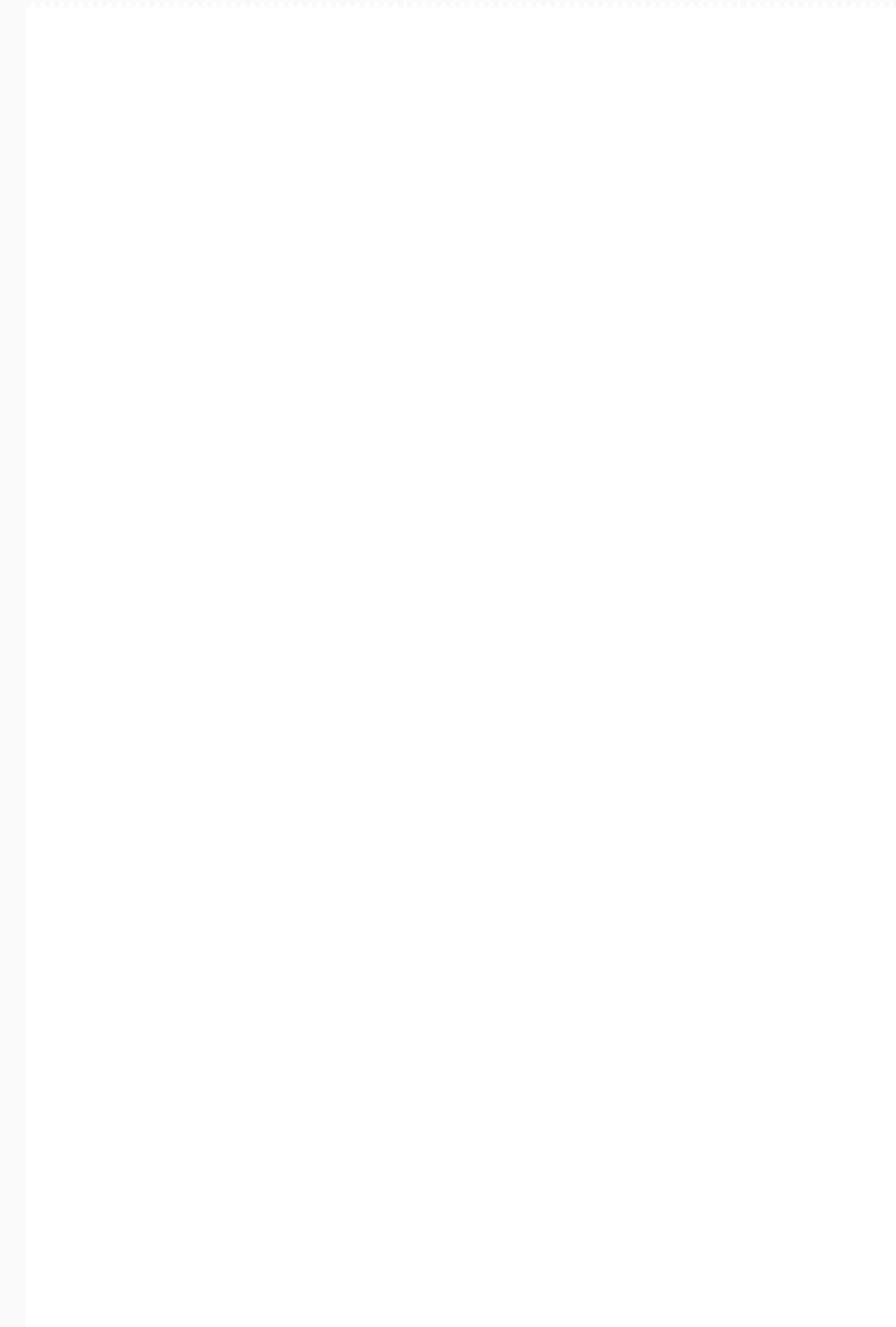
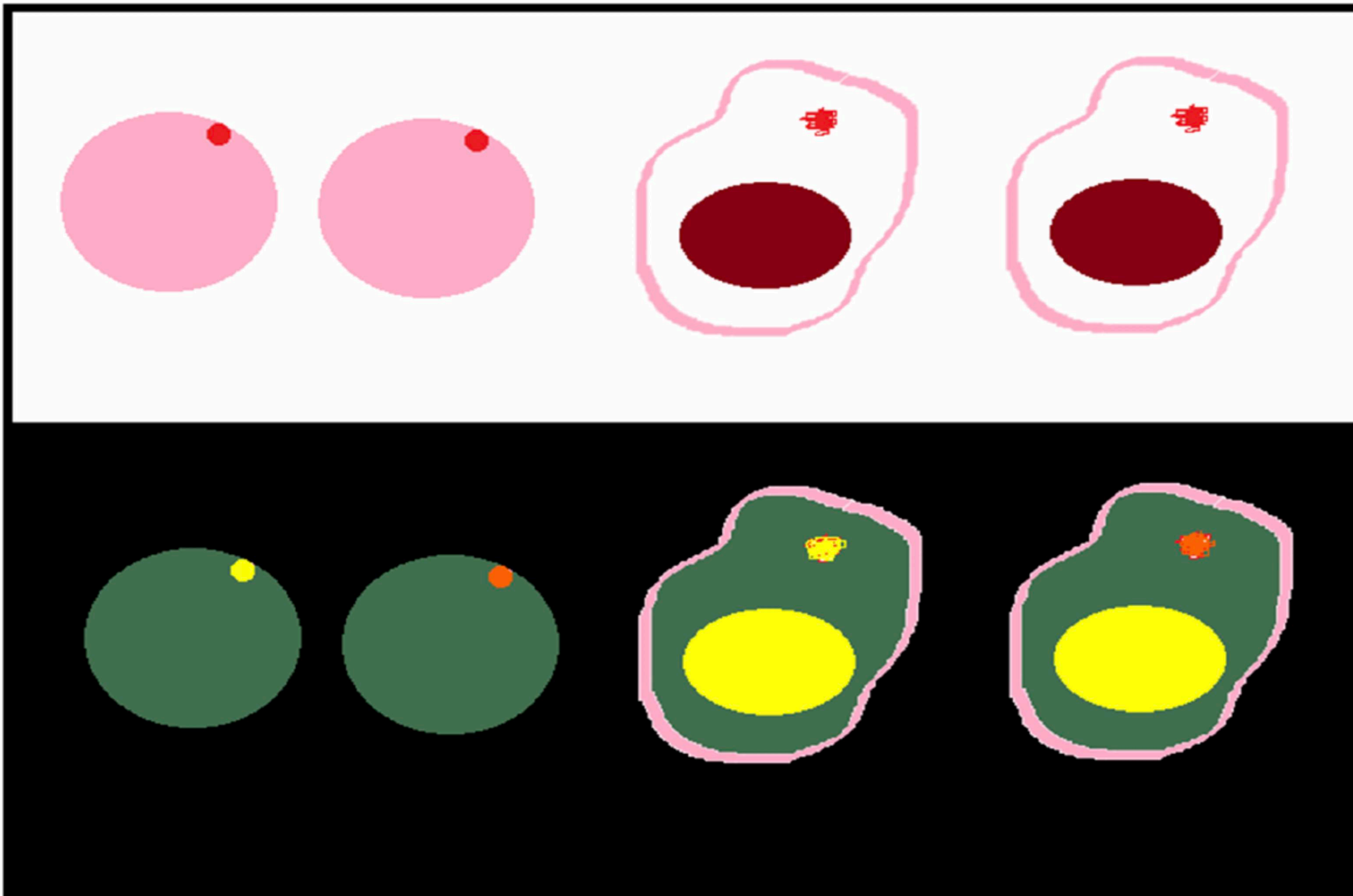


Diagnosis

- ▶ **Use of DNA staining technique will improve the efficiency and rapidity of diagnosis, as false positives and false negatives are drastically reduced.**
- ▶ **DNA staining using acridine orange takes 2 minutes and reduces false positives and false negatives**



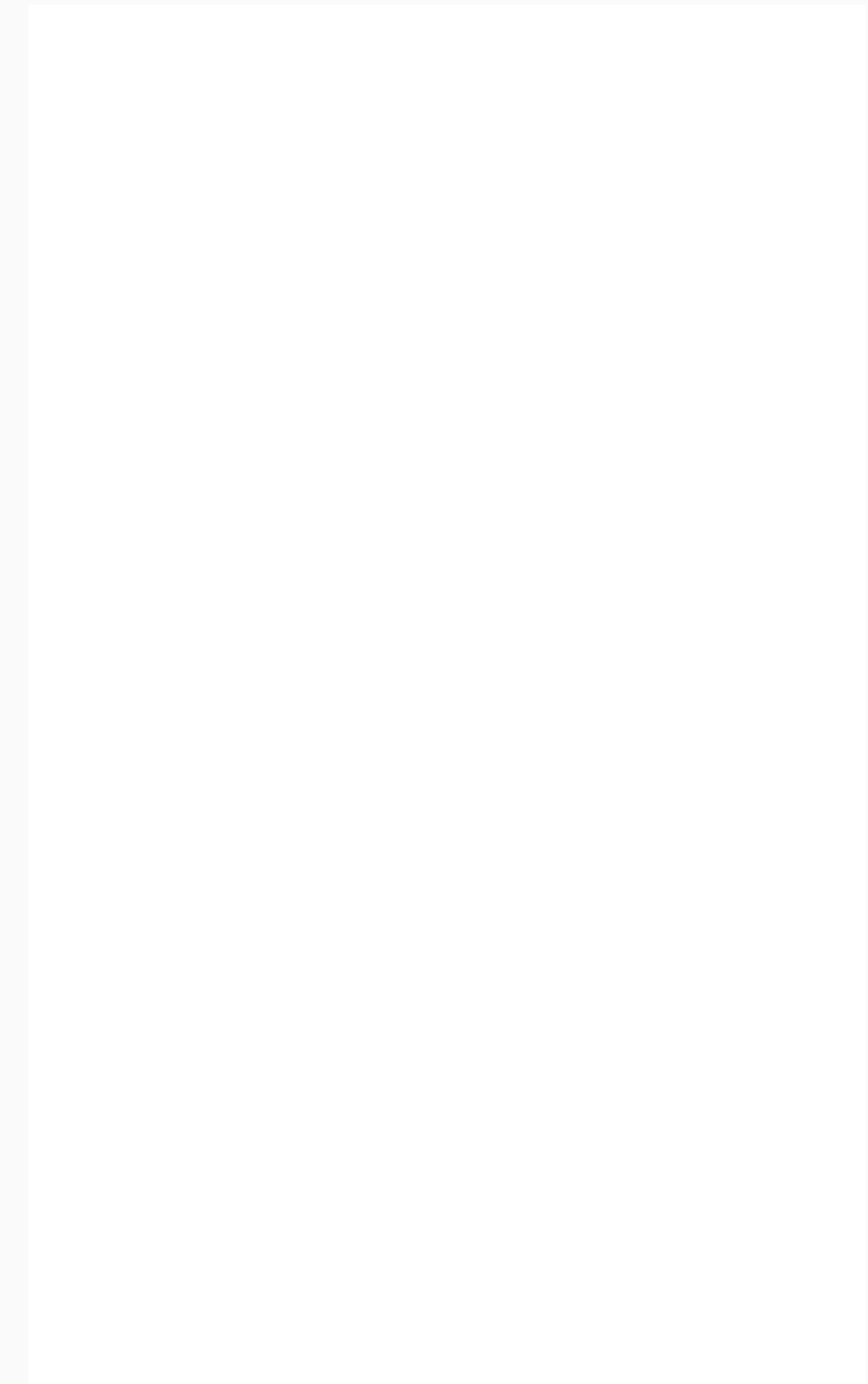
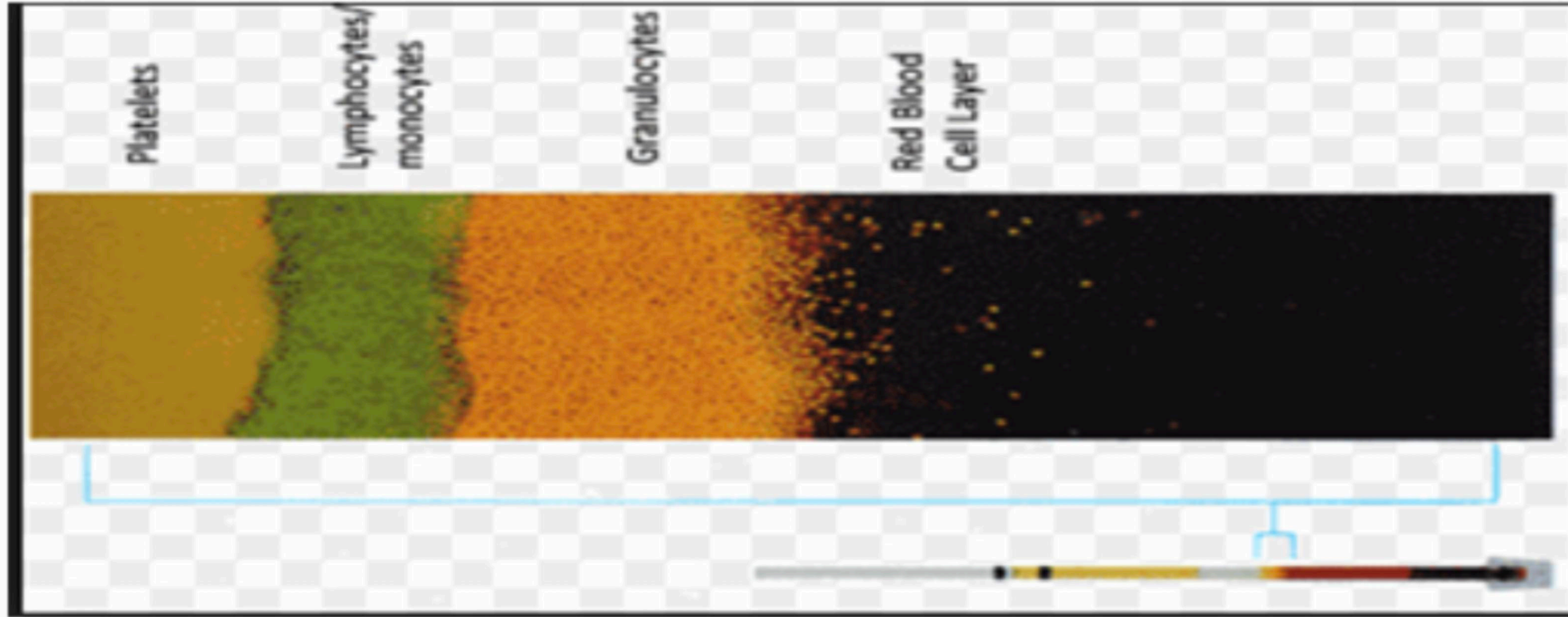






Diagnosis







Diagnosis

- ▶ **Serological tests**
 - ELISA, Dot-ELISA, CAT, IFAT, etc.
- ▶ **Molecular detection using PCR**
 - Primers available for amplification of selected parasite-specific fragments. Sequencing can further confirm diagnosis
- ▶ **Culture isolation**
 - Can be used for *Theileria* (SIL) and *Babesia* (MASP)
- ▶ **Animal inoculation**
 - *Trypanosoma* can be isolated by mouse inoculation, especially cryptic cases



Thank you