Laboratory Diagnosis of Parasitic Infections

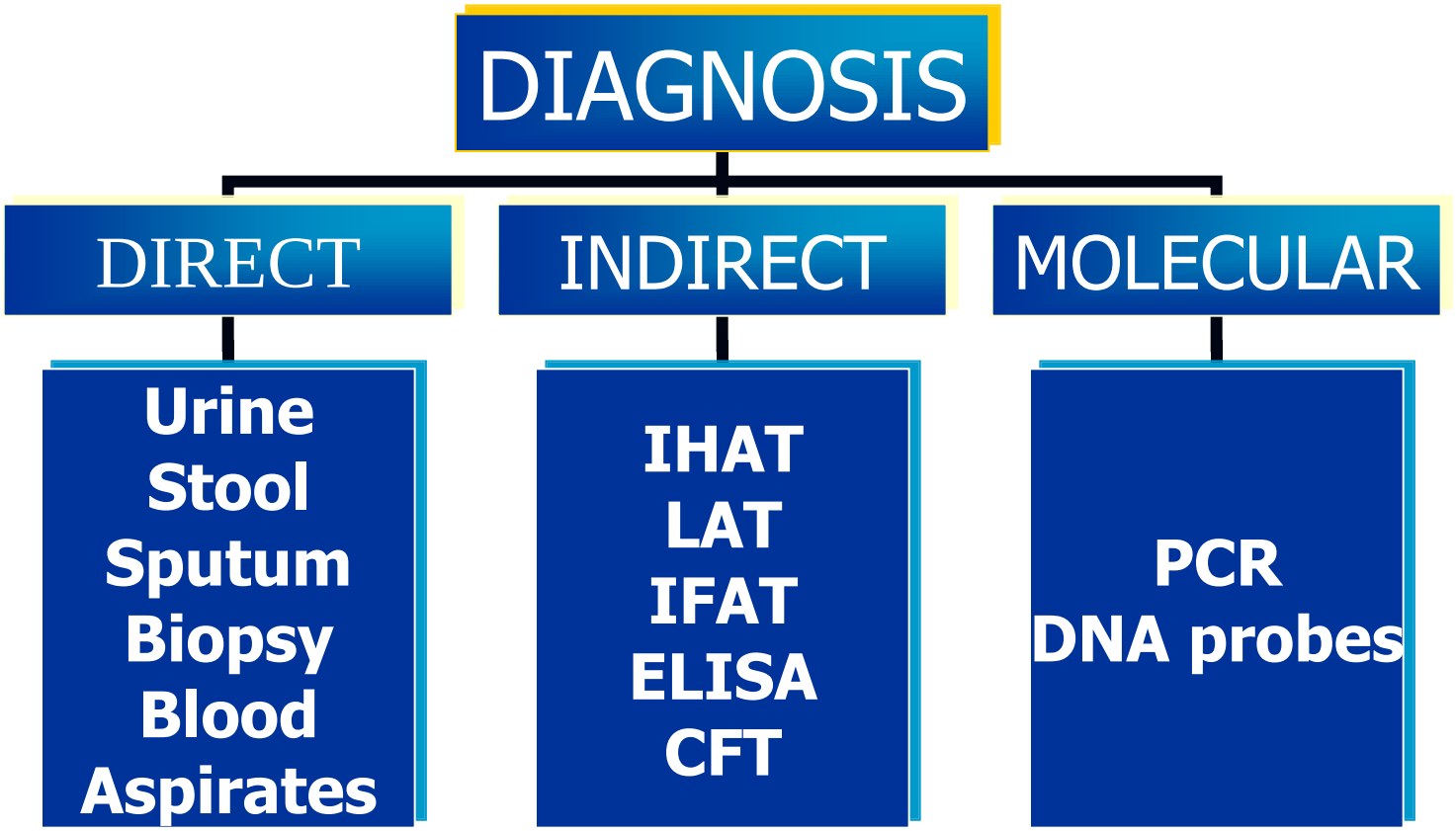
-Confirmation of clinical suspicion

-Identification of unsuspected infection

-Methods same as used in Bacteriology & Virology but significance of different methods varies.

-Isolation least important, morphological identification very important.

-Serology relatively less important


DIAGNOSIS
DIRECT
INDIRECT
MOLECULAR
Urine
Stool
Sputum
Biopsy
Blood
Aspirates
IHAT
LAT
IFAT
ELISA
CFT
PCR
DNA probes

Fecal (stool sample)

Specimen Collection

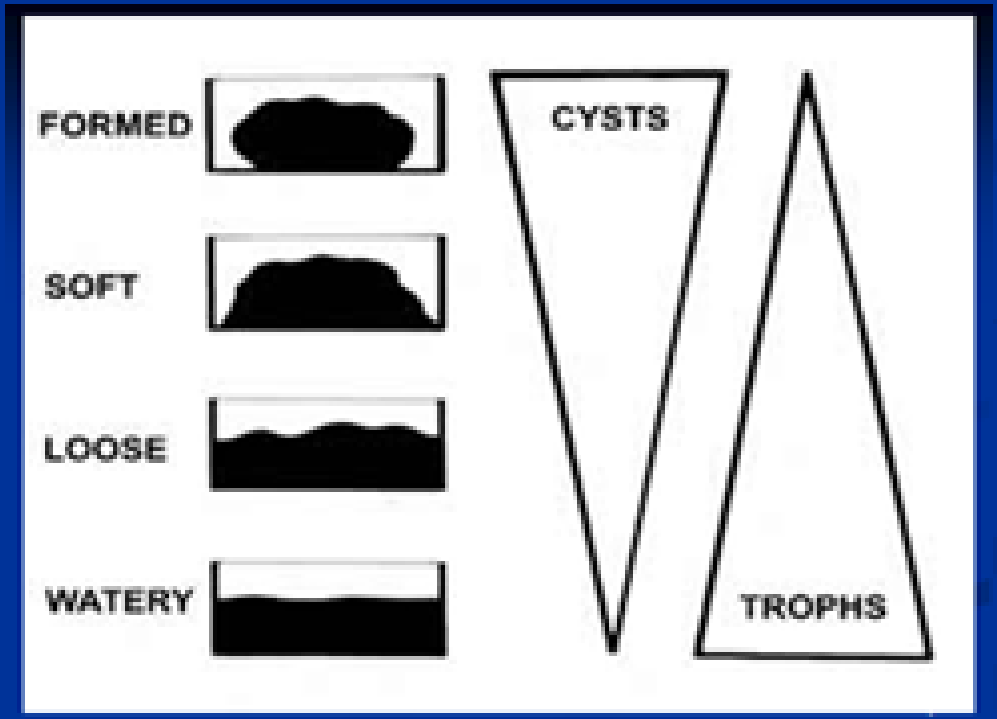
- Collect the stool in a dry, clean, leakproof container. Make sure no urine, water, soil or other material gets in the container.
- This table demonstrates the distribution of protozoa in relation to stool consistency and should be taken into consideration when specimens are received
- Fresh stool should be examined, processed, or preserved immediately. An exception is specimens kept under refrigeration when preservatives are not available; these specimens are suitable for antigen testing only.
- Preserve the specimen as soon as possible. If using a commercial collection kit, follow the kit's instructions. If kits are not available, the specimen should be divided and stored in two different preservatives, 10% formalin and PVA (polyvinyl-alcohol), using suitable containers. Add one volume of the stool specimen to three volumes of the preservative

- Insure that the specimen is mixed well with the preservative. Formed stool needs to be well broken up.
- Insure that the specimen containers are sealed well. Reinforce with parafilm or other suitable material. Insert the container in a plastic bag.
- Certain drugs and compounds will render the stool specimens unsatisfactory for examination. The specimens should be collected before these substances are administered, or collection must be delayed until after the effects have passed.

STOOL EXAMINATION

MACROSCOPIC EXAMINATION

COLOUR	CONSISTENCY	COMPOSITION	Adult PARASITES
Pale=Steatorrhea (Giardia)	-Liquid (Troph) -Formed (Cyst) -Semi formed (Cyst)	?? Blood ?? Mucus (dysentry)	*Ascaris worm *E. vermicularis *T. saginata



Stool examination

(Microscopic examination)

- 1-Temporary : Direct smear or concentration methods. Saline, iodine, eosin
- 2- Semi permanent: Quensel solution
- 3- Permanent: Iron hematoxylin and Trichrome stain.

A. Direct smear:

1. Saline:

Helminth eggs can be detected and usually identified under low power magnification (LPM=10X) but high power magnification (HPM=40X) may aid in identification after finding under low power. Also the trophozoite and cysts of the amoeba can be detected but they must be stained for positive identification. The trophozoites of flagellates can be identified by characteristic appearance and locomotion.

The steps:

1. Place one or two drops of physiological saline on a slide.

2. With an applicator stick, select a 1-2mg sample of feces (a milligram of feces is about 1 cubic millimeter). Avoid selecting non-fecal elements unless Schistosoma eggs or amoeba are suspected, in which case select flecks of mucus and blood

3. Stir into saline and make a homogenous suspension. Remove coarse fibers, seede, etc.

4. Cover with a cover slip, if it is of a proper consistency, a newspaper will be readable through the smear after applying the cover slip.

5. A satisfactory smear as one that contains a maximum of observable fecal elements (without any objects of protozoa size "8-30 µm" that may be mistaken for protozoa). If the preparation is too diluted or too concentrated discard it and prepare another.

6. Examine the entire preparation systemically and carefully under the microscopic using LPM. A portion of the smear should be examined under HPM to prevent overlooking smaller parasite. Allow a few minutes for trophozoites to adjust and start amoeboid movement. If examined too soon, trophozoites will be rounded up.

2. Iodine:

This method stains protozoan cyst but can't identify trophozoite.

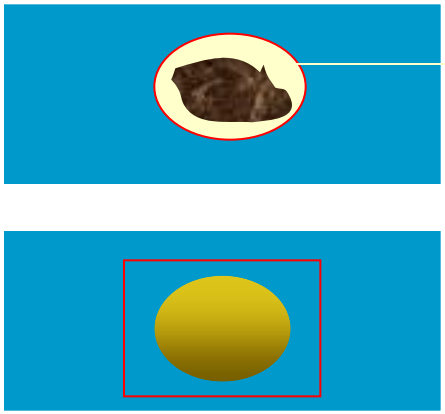
1. Place one drop of iodine on a slide.

2. The rest of steps as described above.

STOOL EXAMINATION

Temporary

Saline smear

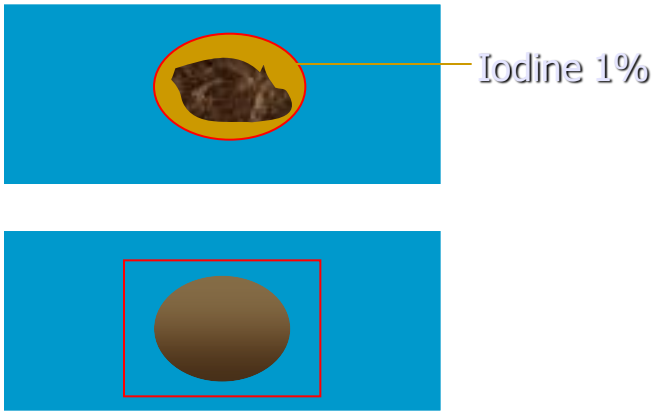


Huge number of:

- Eggs
- Protozoal troph. Motility

(Amoeba, flagellates)

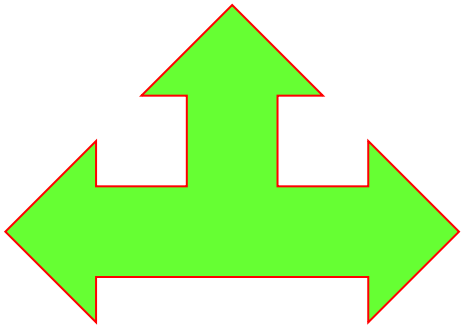
Iodine smear



Huge number of:

- Cyst morphological details

STOOL EXAMINATION
Concentration techniques



Sedimentation

- Heavy eggs (Ascaris egg)
- Operculated eggs (Trematodes)
- Larvae (Strongyloides stercoralis)
- Cysts

Floatation

Operculated eggs

Trematodes (S. mansoni)

Cestode Tape worms
Nematode(Hookworm, Round worms)

- Cysts

Concentration techniques:

1. Zinc Sulfate Floatation Concentration:

The zinc sulfate floatation concentrates protozoan cysts, most non-operculate helminth eggs and larvae. The zinc sulfate method is unsuitable for fatty stool. The formalin-ether sedimentation method is better suited for eggs of trematodes and large tape worms (Taenia & Diphyllobothrium); otherwise both methods give comparable results.

The steps:

1. Mix 1 part of feces with 10 parts of tap water.
2. Strain about 10 ml of fecal suspension through wet gauze into a Wasserman tube.
3. Centrifuge for 1 min. at 2300 rpm. Pour off the supernatant fluid. Add additional water to fill the tube.
4. Repeat step 3 once or twice until the supernatant fluid becomes clear.
5. Pour off the supernatant fluid; add 3-4ml of ($ZnSO_4$) solution and resuspend the sediment. Add additional ($ZnSO_4$) leaving 3-4 mm. space from the tube rim.
6. Centrifuge for 1 min. at 2300 rpm. And allow the tube to come to rest without interference.
7. After 1-2 min. by using a bacteriologic loop, transfer several loop-full of material from the surface film to a clean slide.
8. Add 1 drop of eosin stain and mix and cover with a cover slip. Examine under LPM (10 X).

2. Formalin- Ether Sedimentation Concentration:

The formalin-ether sedimentation method concentrates protozoan cysts, helminth eggs and larvae. Unlike the zinc sulfate floatation method, it is useful for concentrating operculate eggs. It may be used for concentrating Schistosoma eggs but apparently the sodium acid-ether technique is more efficient. It is method of choice to concentrate specimens preserved in formalin.

The steps:

1. Mix enough feces with 10-12 ml of saline.
2. Strain through 2 layers of wet gauze into a paper cup or beaker.
3. Transfer to a 15ml conical tube and centrifuge for 1 min. at 1500-2000 rpm .
4. decant the supernatant fluid and re-suspend the sediment in fresh saline, centrifuge and decant as before.
5. Mix the fecal sediment with 10ml 10% formalin and allow to stand for 10 min. or more to ensure fixation.
6. add 3ml of ether, stopper the tube and shake vigorously.
7. Centrifuge at about 1500rpm for 2min. four layers should result :
   - (A).An ether layer on top.
   - (B). A plug of fecal detritus.
   - (C). A layer of formalin.
   - (D).The bottom layer of sediment containing protozoa and helminth eggs.
8. Free the plug of fecal detritus from the sides of the tube by ringing with an applicator stick, pour off the supernatant fluid.
9. Mix the remaining sediment with the small amount of fluid that drains back from the sides of the tube. Place a drop of sediment on a clean slide, add a drop of iodine and cover with cover slip and examine under LPM.

Note: if the specimen has been previously washed, strained and fixed in the formalin, take 10ml of the thoroughly mixed specimens and start at step2.

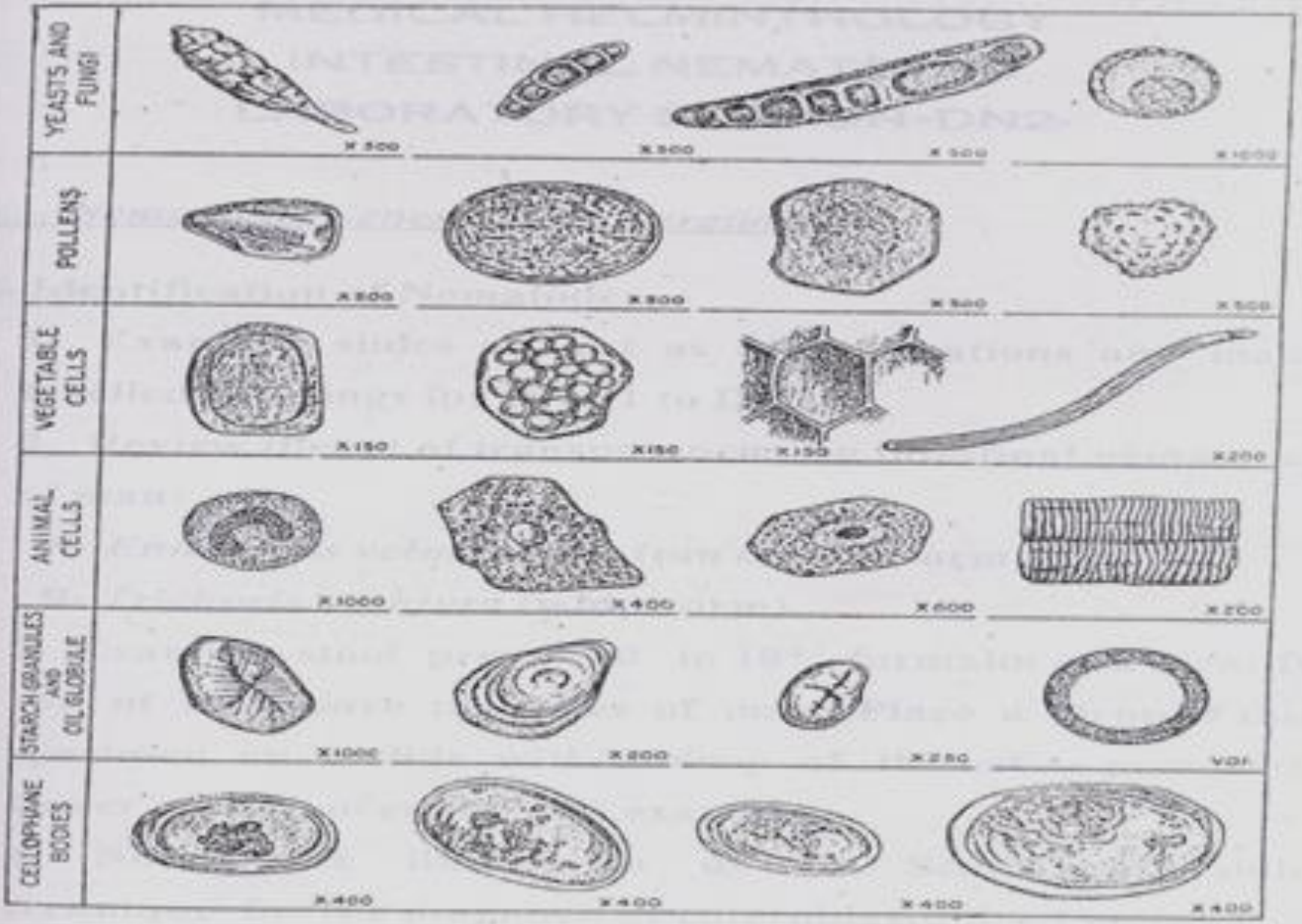


FIGURE-1-: OBJECTS IN FECES SOMETIMES MISTAKEN FOR PROTOZOAN CYSTS AND HELMINTHIC OVA.

Cultivation of parasites

culture methods

Amoeba

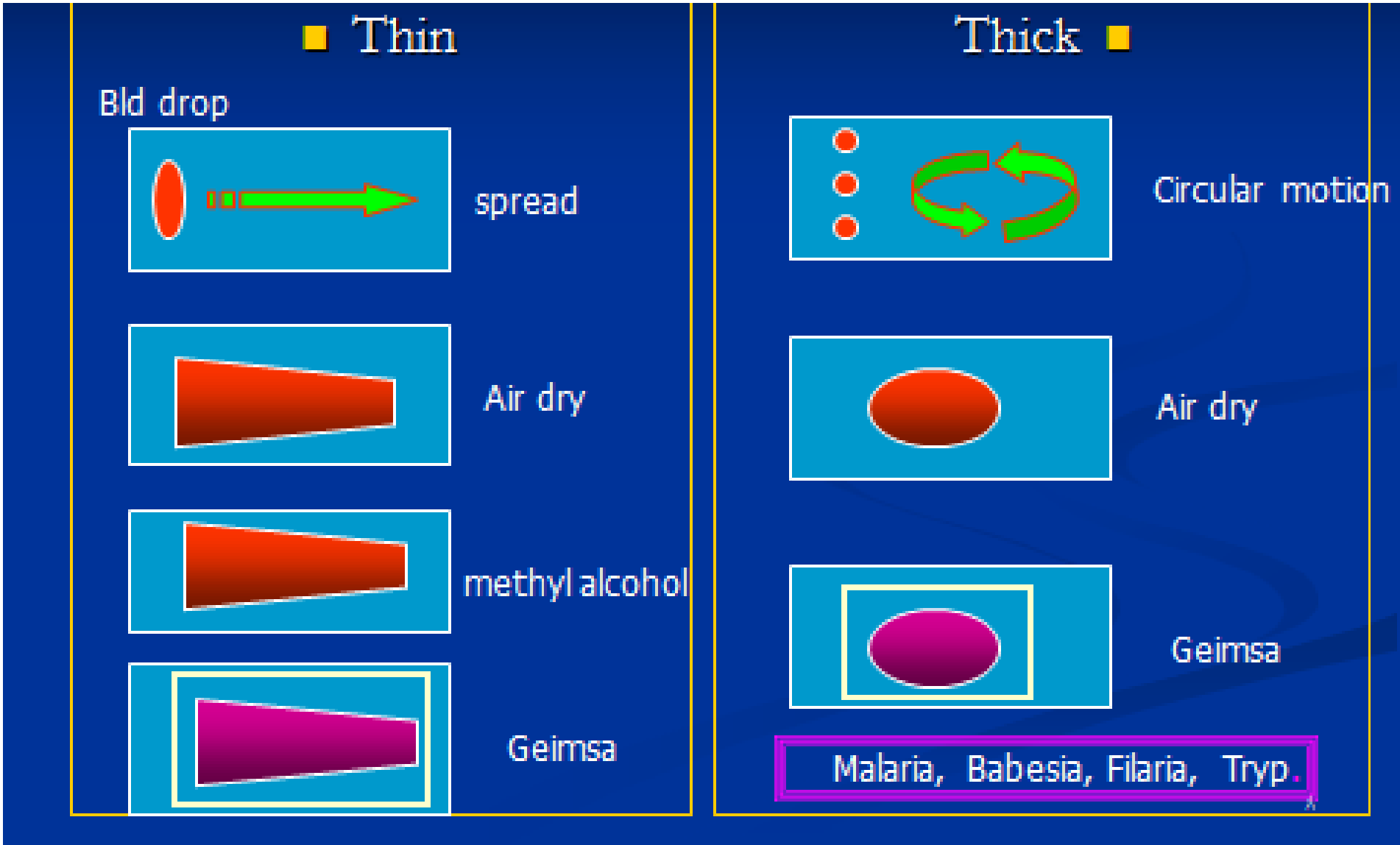
Leishmania & Trypanosoma

Malaria

Animal inoculation –not practical

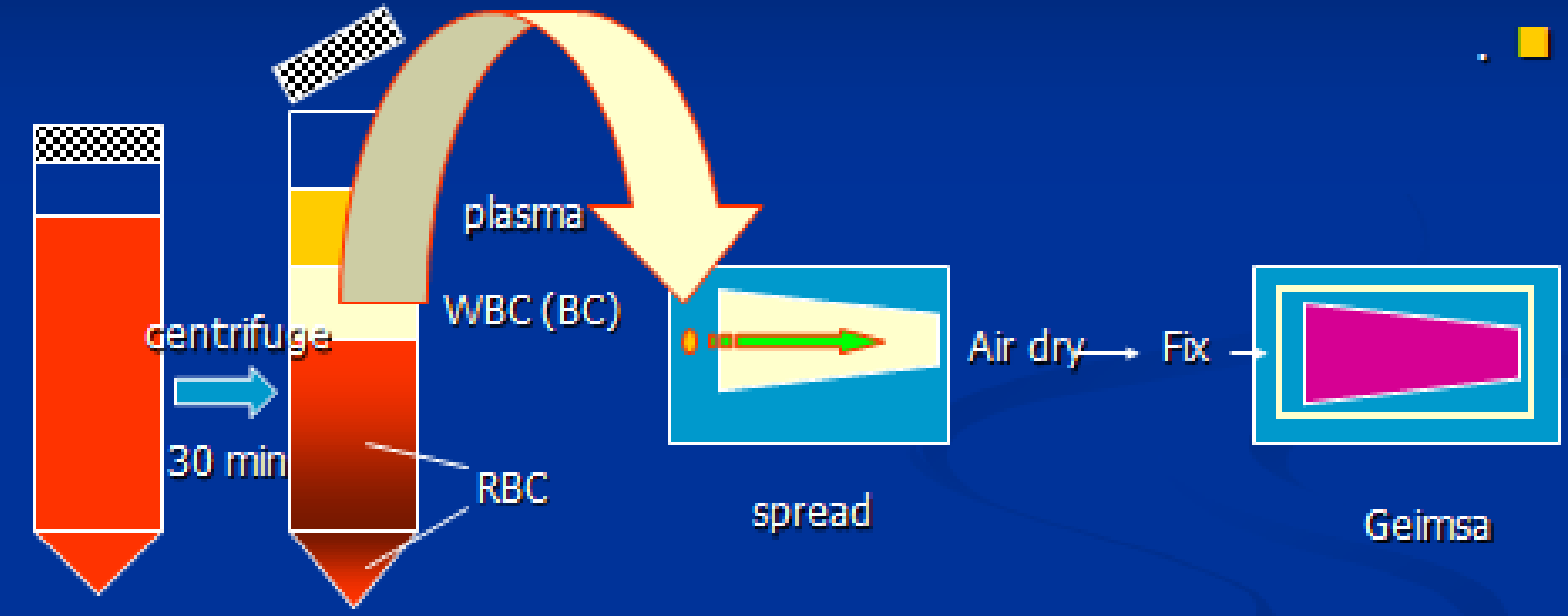
Xenodiagnosis-vector infected

Examination of blood: thin smear thick smear wet mount for microfilaria stains used –Leishman/Giemsa

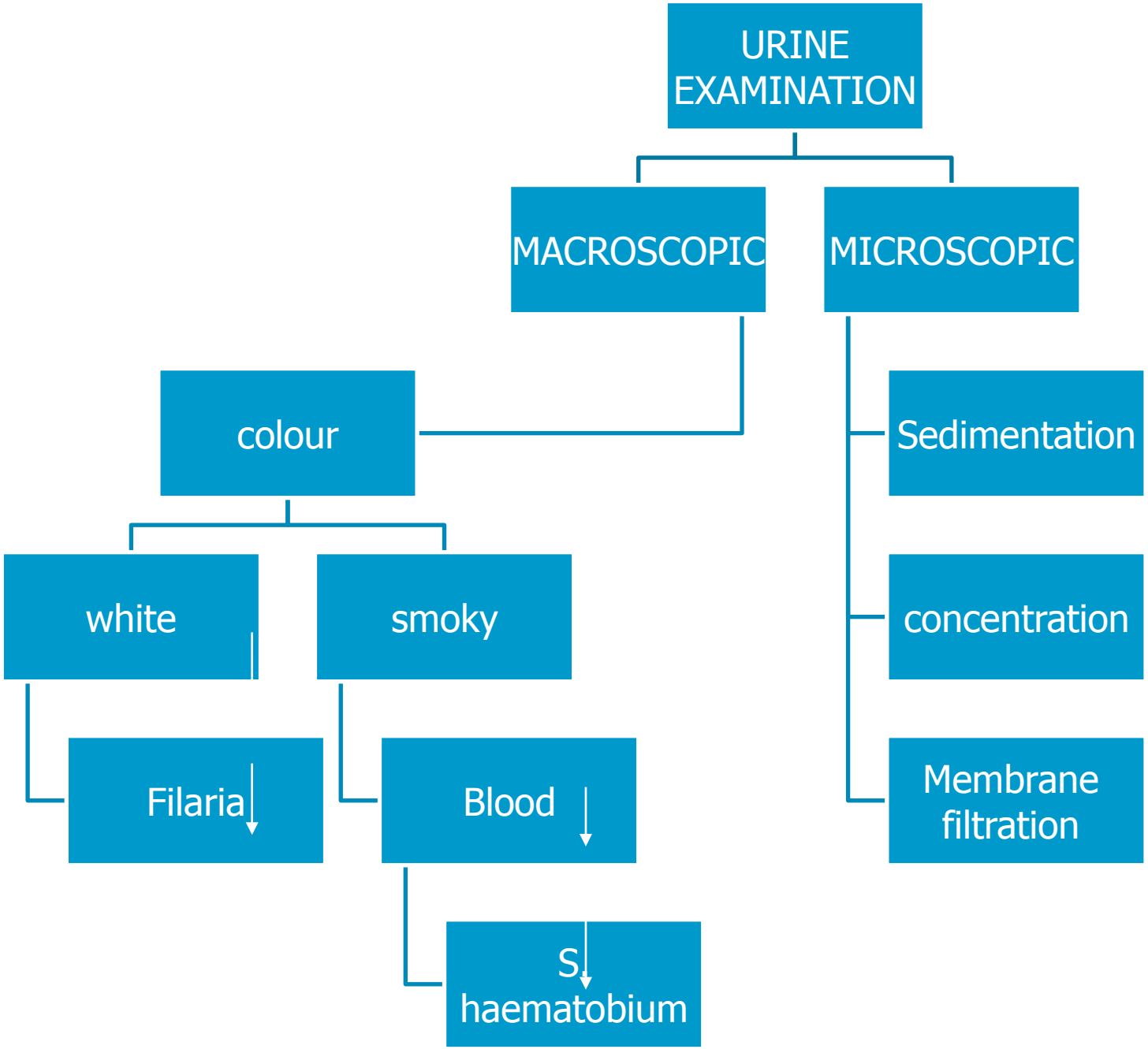


BLOOD EXAMINATION

Buffy coat film



Trypanosoma., L. donovani


URINE EXAMINATION
MACROSCOPIC
MICROSCOPIC
colour
Sedimentation
white
smoky
concentration
Filaria
Blood
Membrane filtration
S. haematobium

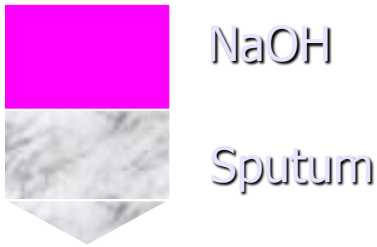
SPUTUM EXAMINATION

MACROSCOPIC

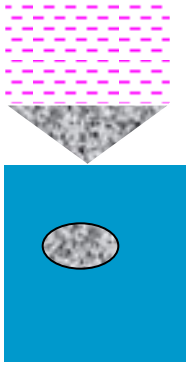
- Appearance
  - Bloody (Paragonymous)
  - Rusty brown (Paragonymous)

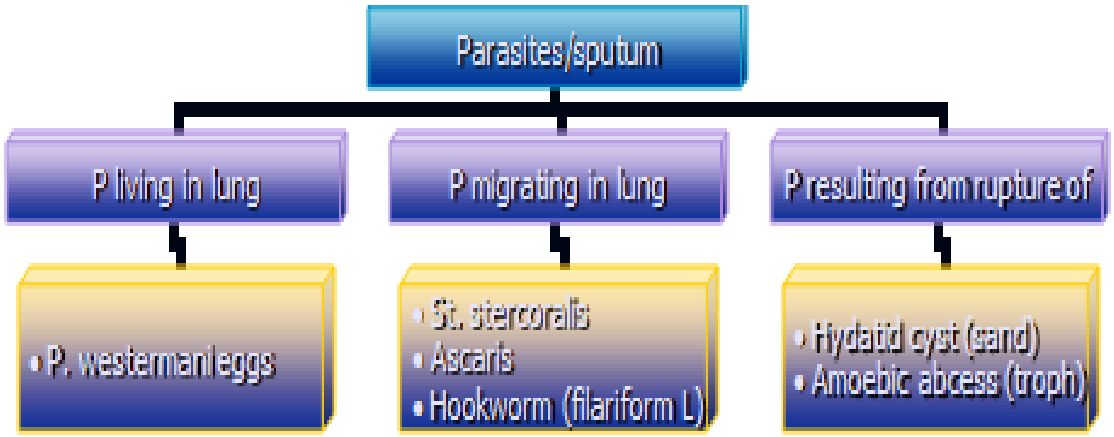
MICROSCOPIC

- Concentration



Centrifuge





BIOPSY SPECIMEN

SKIN SNIP

MUSCLE BIOPSY

RECTAL BIOPSY

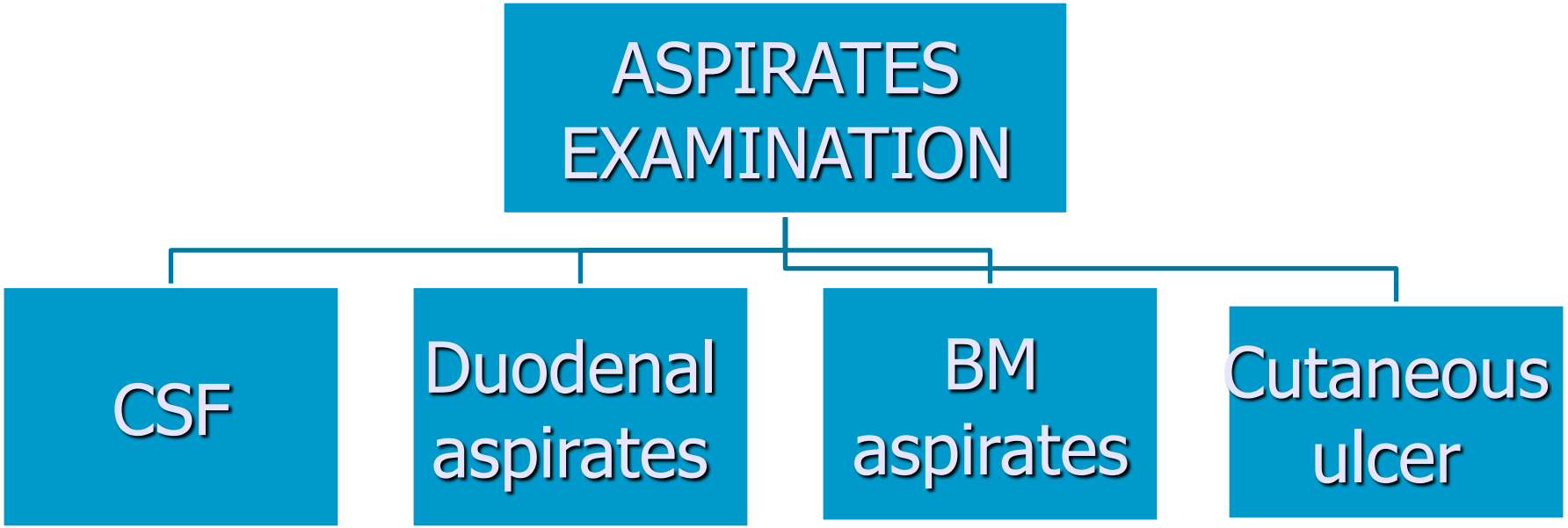
O. volvulus mf

T. spiralis larvae

Schistosoma egg

- Raise skin by needle
- Slice by scissors
- Put snip in normal saline
- Examine

Muscle digestion with HCl + pepsin


ASPIRATES EXAMINATION
CSF
Duodenal aspirates
BM aspirates
Cutaneous ulcer

Immunological diagnosis

Serology – All tests available

- IHA
- ELISA
- CIEP
- IF
- CFT

More useful in

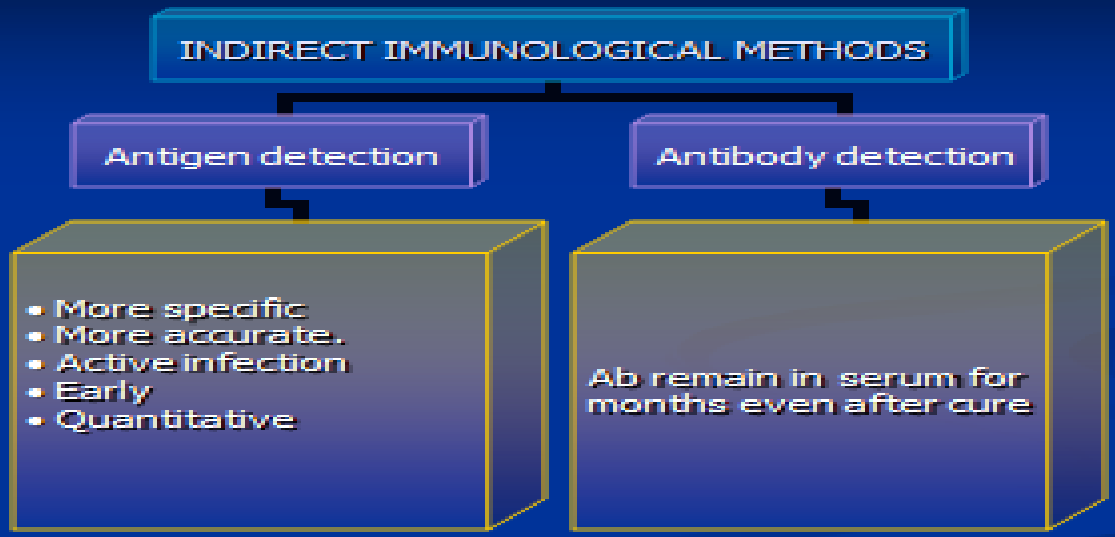
- Amoebiasis
- Leishmaniasis
- Malaria
- Toxoplasmosis
- Trichinosis
- Filariasis
- Echinococcosis

Skin Tests – Specificity low, cross reactions common

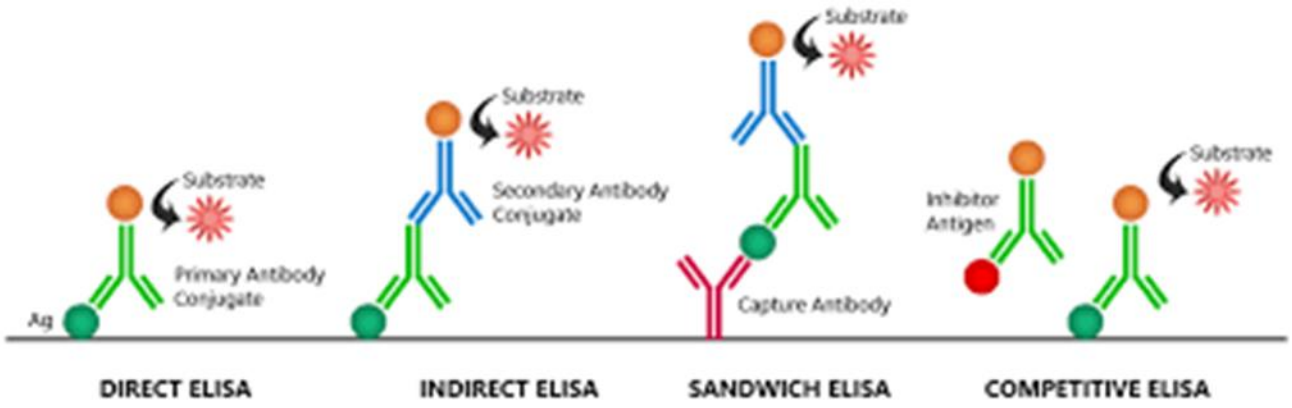
- Casoni's test
- Leishmanin test

IMMUNOLOGICAL METHODS

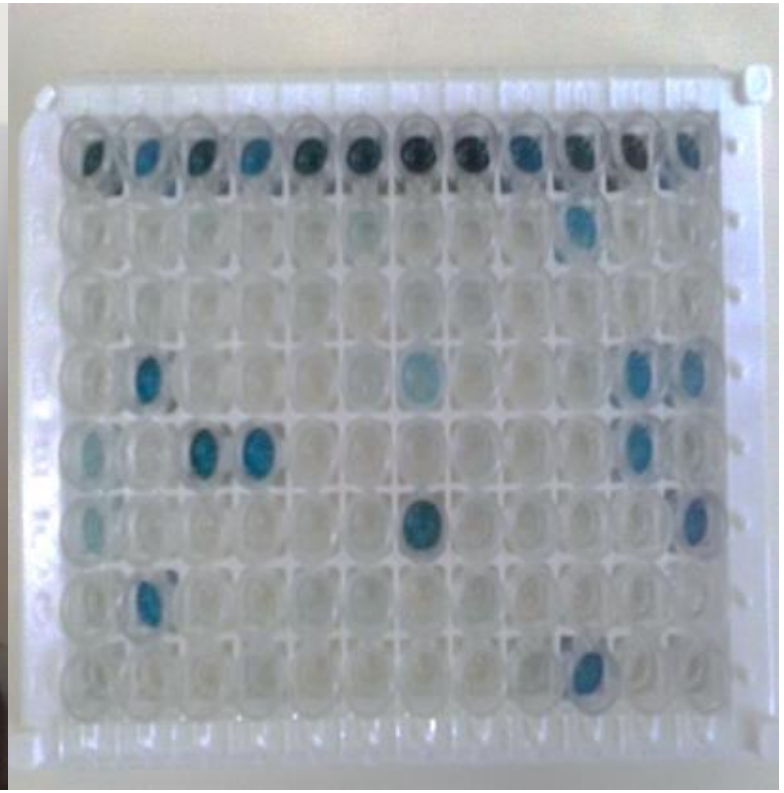
- Scanty infection.
- Tissue parasite no portal of exit (Hydatid dis.)
- Migratory stage (Fasciola)
- Chronic infection fibrosis (Bilharziasis)



- Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. ELISAs can provide a useful measurement of antigen or antibody concentration. There are two main variations on this method: The ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen.
- There are many different types of ELISAs.



The RIDASCREEN® *Entamoeba* test (ELISA)



IMMUNOLOGICAL METHODS

Detection of Entamoeba spp. antigen by RIDASCREEN® Entamoeba

- Principle of test

In the RIDASCREEN® Entamoeba test, specific antibodies are used in a sandwich-type tool. Entamoeba-specific antibodies against the antigens of E. histolytica sensu lato are applied to the surface of the well in the microwell plate. A suspension of the fecal sample to be tested and the controls are pipetted into the wells of the microwell plate. Then, antibodies conjugated with peroxidase against the antigens of E. histolytica sensu lato are added and the plate incubated at room temperature (20 – 25) °C. In the presence of E. histolytica sensu lato antigens, sandwich complexes including of immobilized anti-bodies, Entamoeba histolytica sensu lato antigens and conjugated antibodies form in the sample.

Unattached enzyme-labelled antibodies are taken away during the washing phase. After adding the substrate, the attached enzyme changes the color of the colorless solution in the wells of the microwell plate to blue if the test is positive. On adding the stop reagent, the color changes to yellow. The absorbance is proportional to the concentration of E. histolytica sensu lato antigens present in the specimen.

-Procedure

1.The micro well plate and reagents were brought to room temperature.

2.Wash buffer was diluted with distilled water 1:10.

3.The required micro well strips were placed in the frame.

4. One hundred µl of positive control, negative control and stool suspension were added in the wells.

5. One hundred µl of the enzyme-conjugated antibody were added, after mixing thoroughly.

6.The well plate was incubated at room temperature for 60 minutes.

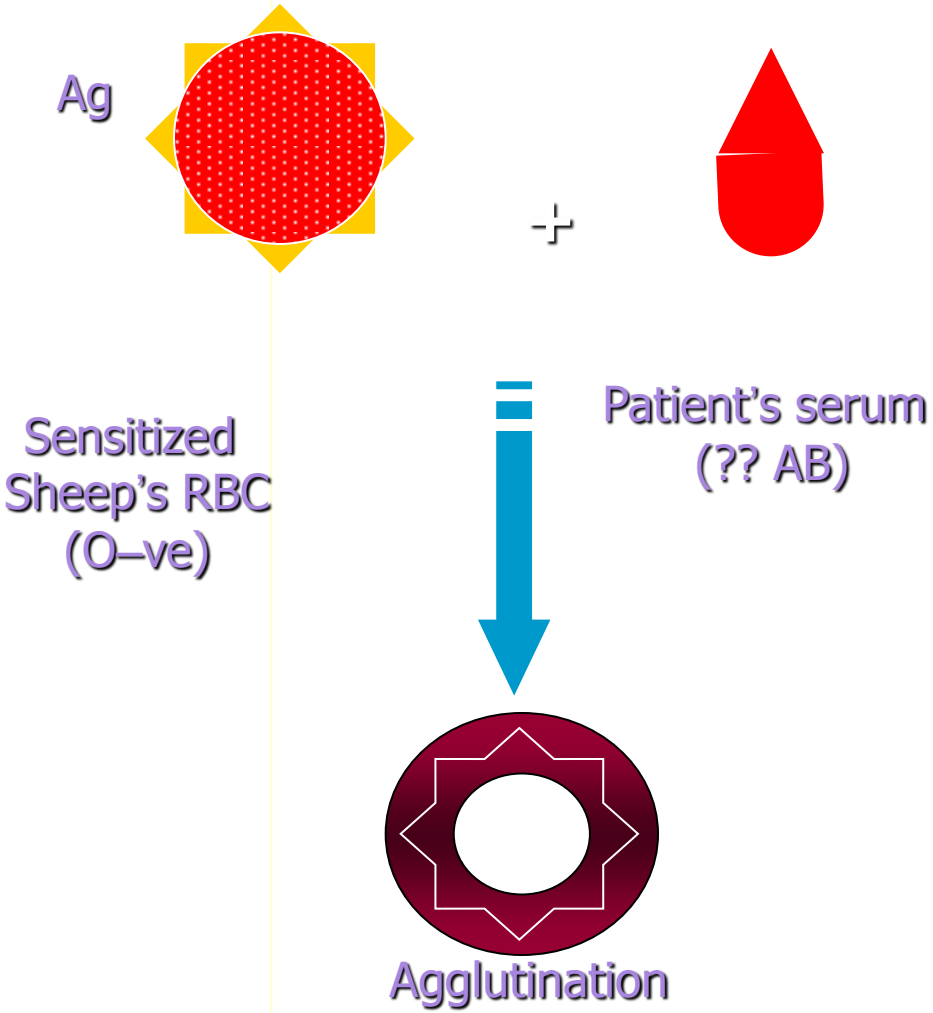
7.The well plate was washed five times using 300 µl diluted wash buffer

8. One hundred µl of substrate were added to each well. .

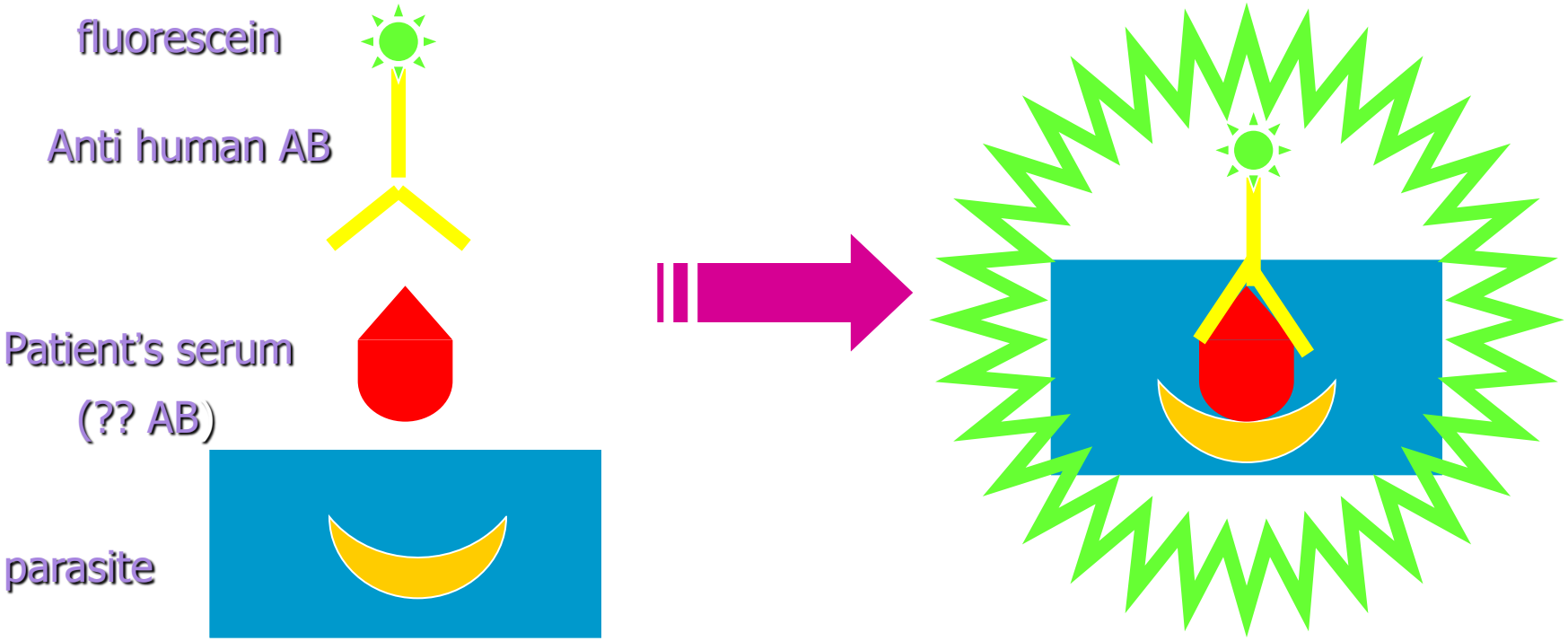
9.The well plate was incubated at room temperature for 15 minutes in the dark

IMMUNOLOGICAL METHODS

IHAT

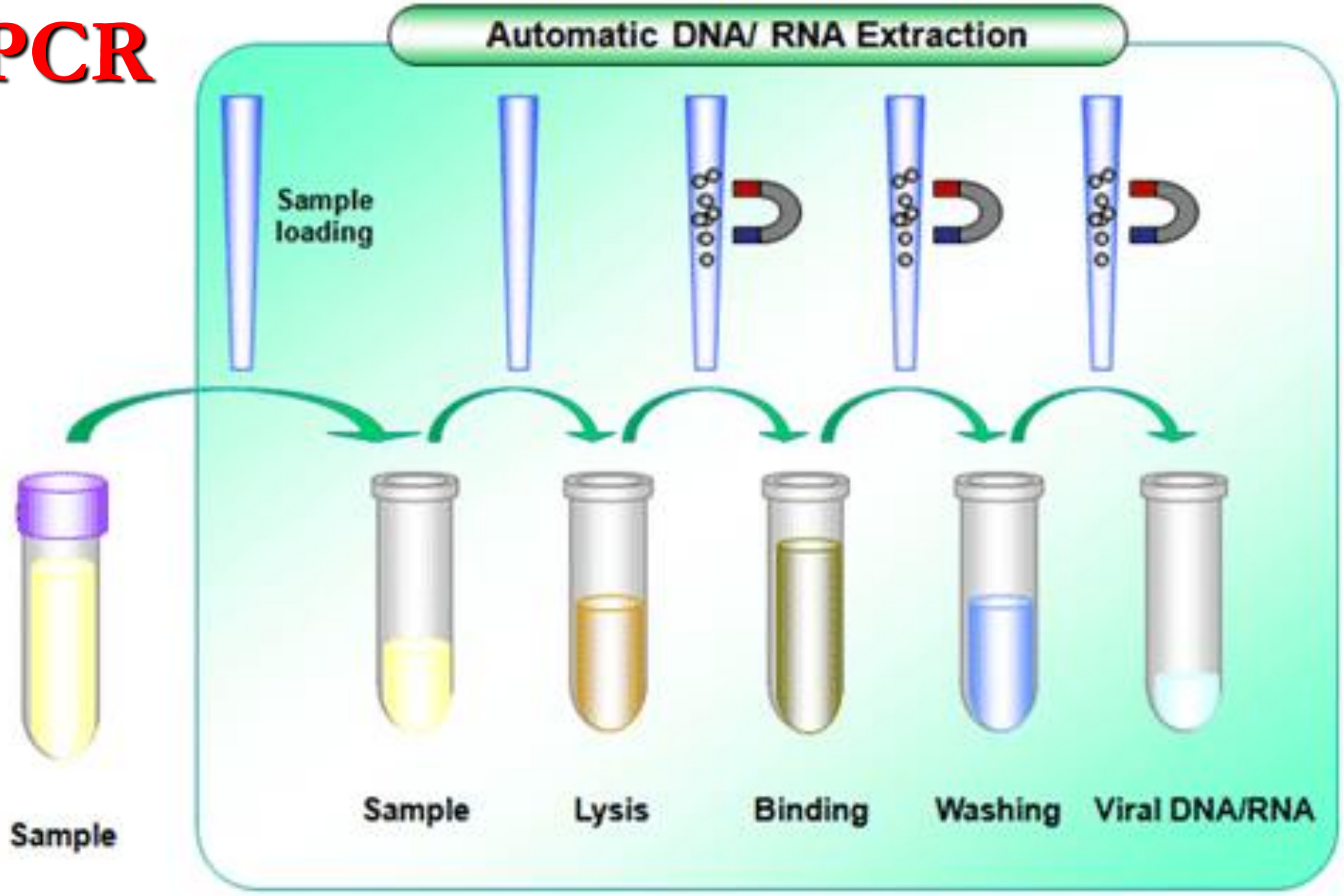


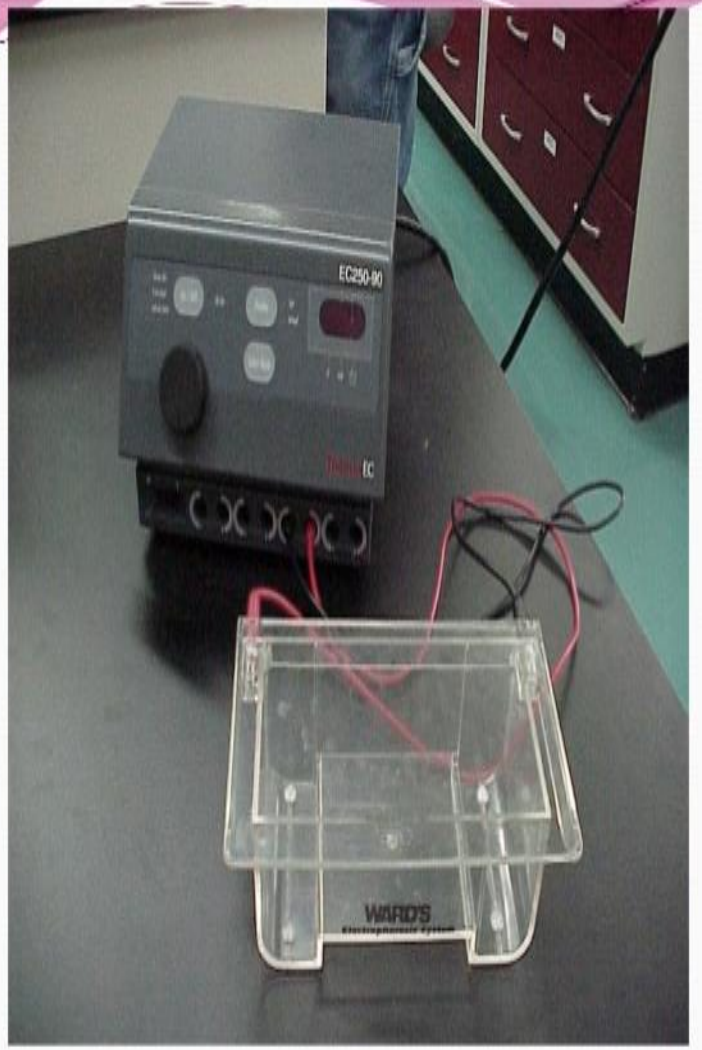
INDIRECT FLUORESCENT ANTIBODY TEST



Molecular method

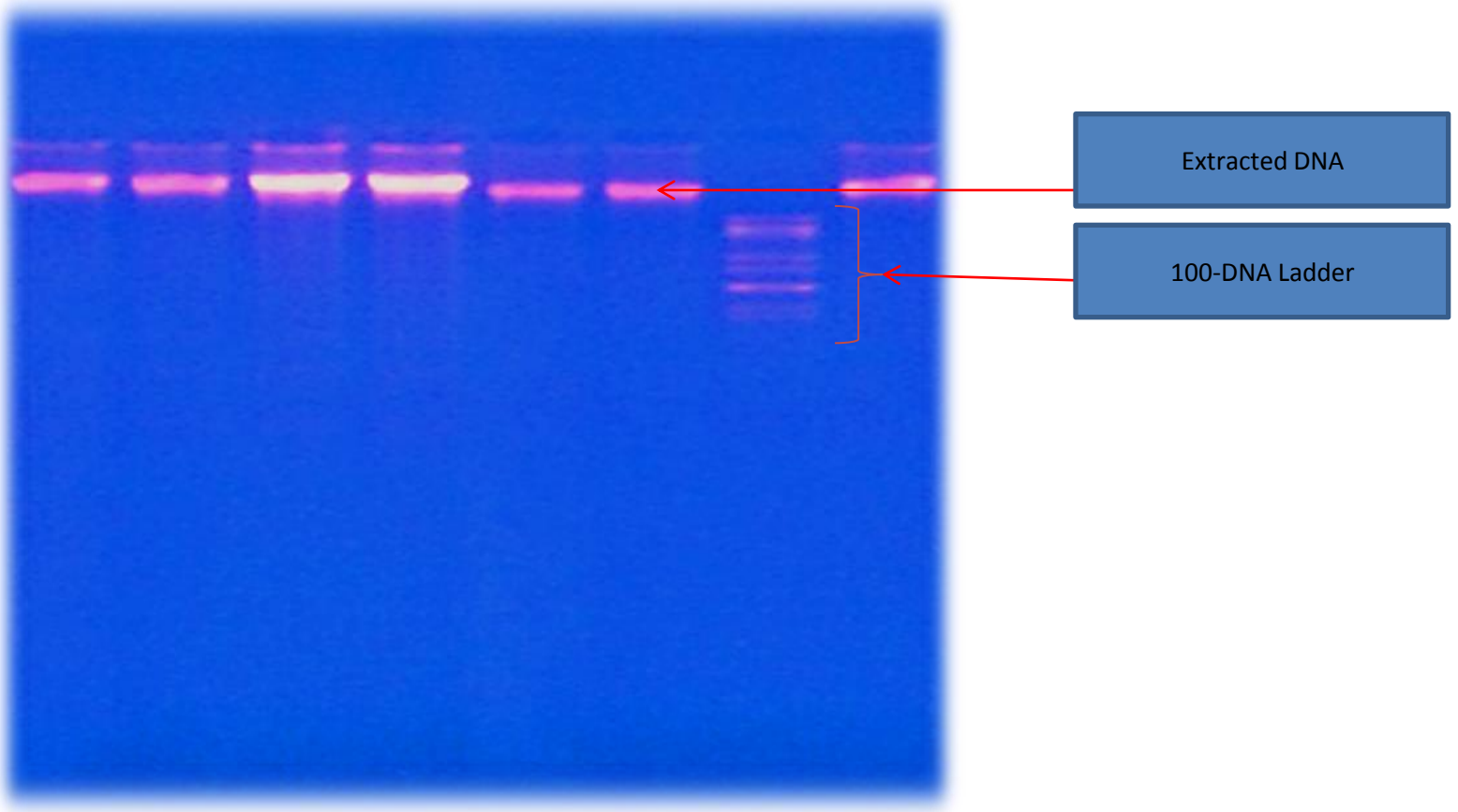
PCR



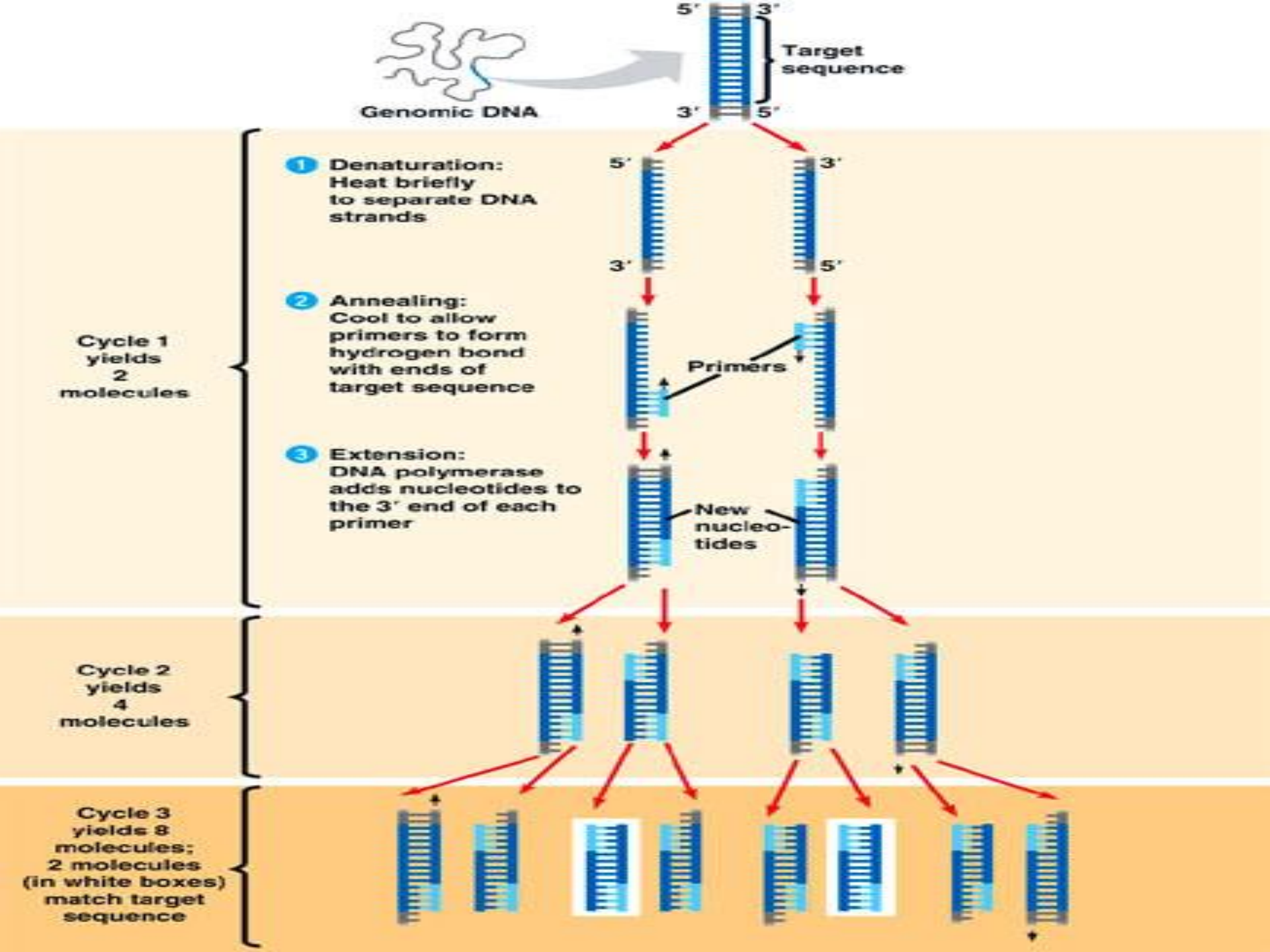


ELECTROPHORETIC TANK AND POWER PACK

Agarose electrophoresis of total DNA parasites extraction from fecal samples.



DNA extracted was measured by Nanodrop instrument


Genomic DNA
5′
3′
Target sequence
3′
5′
1 Denaturation: Heat briefly to separate DNA strands
2 Annealing: Cool to allow primers to form hydrogen bond with ends of target sequence
Primers
3 Extension: DNA polymerase adds nucleotides to the 3′ end of each primer
New nucleo-tides
Cycle 1 yields 2 molecules
Cycle 2 yields 4 molecules
Cycle 3 yields 8 molecules; 2 molecules (in white boxes) match target sequence

PCR instrument