
بسم الله الرحمن الرحيم
السلام عليكم ورحمة الله وبركاته

Recent Diagnostic Methods for Intestinal Parasitic Infections

By

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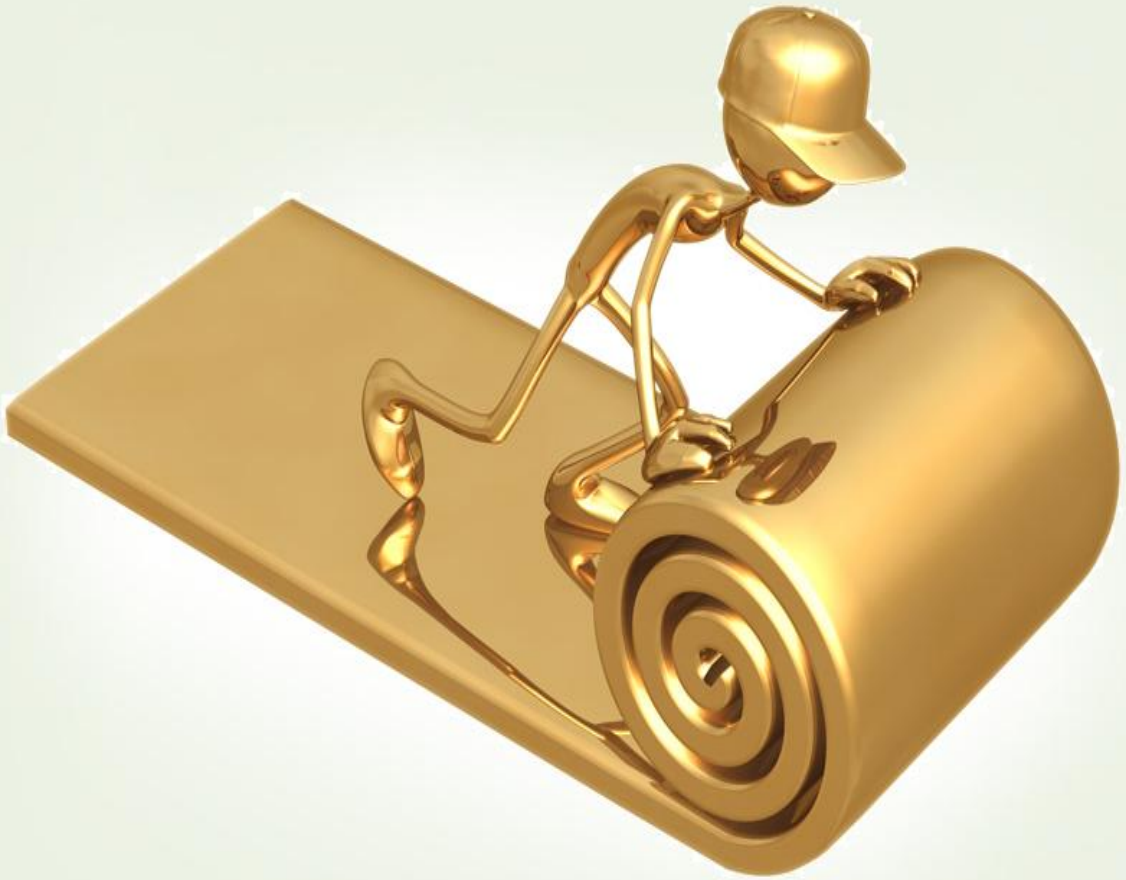
Agenda

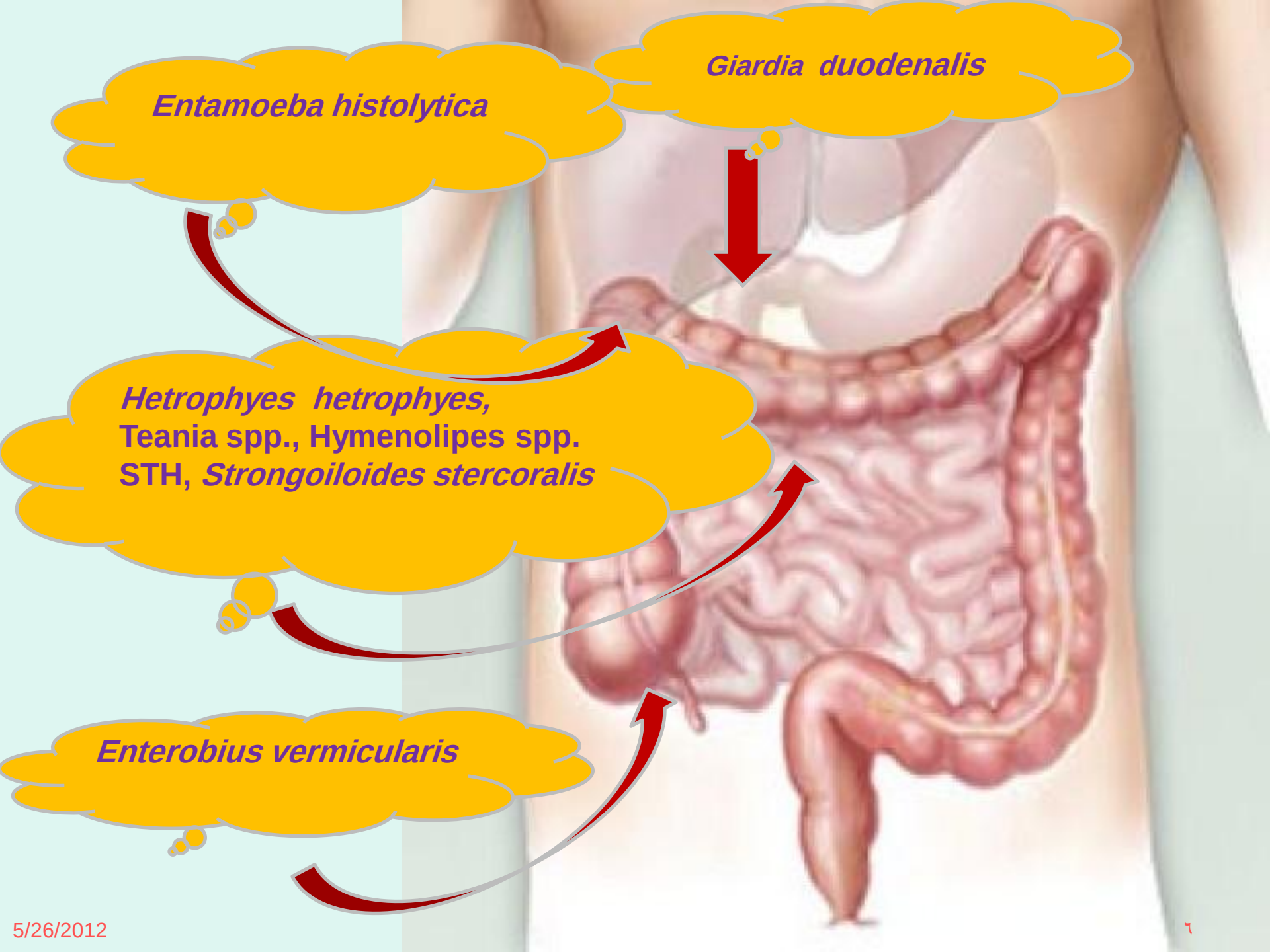
- **Intestinal parasites.**
- **Traditional Diagnostic Methods.**
- **Why we are looking for new techniques for diagnosis of intestinal parasitic tract?**
- **Commercially available techniques**
- **Hoping future techniques**
- **Summary**

Intestinal parasites



Most common Egyptian intestinal parasites

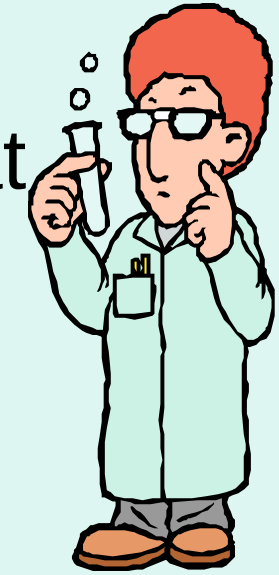
Protozoa	Helminthes		
Entamoeba histolytica	**Trematodes**	**Cectodes**	**Nematodes**
Coccidea (Cryptosporidium parvum, Isospora belli, Cyclospora *cayetanensis**)***	*Schistosoma mansoni*	Teania spp. (*T. saginata and T. solium)*	*Strongoiloides stercoralis*
Giardia duodenalis	*Hetrophyes hetrophyes*	Hymenolipes *(H. nana and H. diminuta*).	Soil transmitted helminths STH *(Ascaris lumbricoid*, *Trichuris trichiura*, Hook worm)
			Enterobius vermicularis


Entamoeba histolytica
Giardia duodenalis
Hetrophyes hetrophyes,
Teania spp., Hymenolipes spp.
STH, Strongoiloides stercoralis
Enterobius vermicularis

Mode of transmission

- IPIs are linked to lack of sanitation, lack of access to safe water and improper hygiene.
- Protozoan mainly transmitted by feco-oral (auto infection, person to person, food, water,….).
- Intestinal trematodes by fish eating, and skin penetration.
- Intestinal cectodes by ingestion of meat

  auto infection, insect ingestion.
- Intestinal nematodes by *ingestion of contaminated food, autoinfection, skin penetration*



Diagnosis of intestinal parasitic infections

CLINICAL PICTUR

LABORATORY

Clinical picture

- Diarrhea
- Dysentery
- Anemia
- Allergy
- Constipation
- Acute abdomen
- Peri-anal itching
- Bloating, dyspepsia

I-Traditional Diagnostic Methods

1-Macroscopic stool examination

Searching for:

- 1- Blood and mucus.
- 2-Some adult helminths as *Ascaris lumbricoid* or segments as Tenia spp. or Hymenolips spp.

2-Microscopic stool examination.

Relies on the detection of

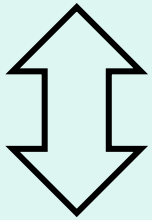
- Trophozoites
- Cysts
- Eggs
- Larvae

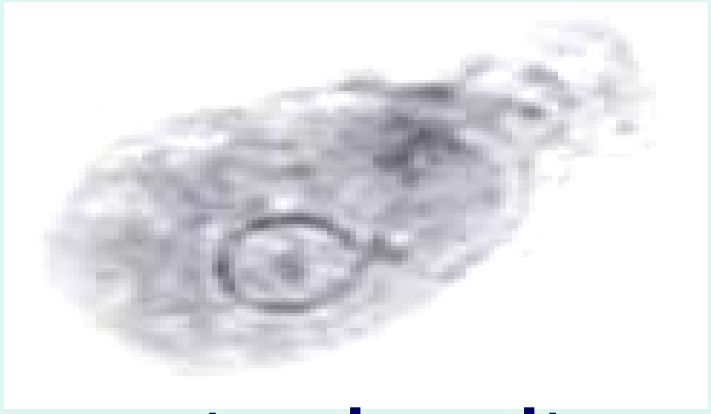
- **How to improve the sensitivity of microscopic examination?**

- Multiple stool specimens.
- Concentration procedures.
- Variety of staining methods, are needed to achieve ample sensitivity for intestinal coccidean as
- Modified ZN stain, non specific fluorescent stain, and some have the ability for auto fluorescence.

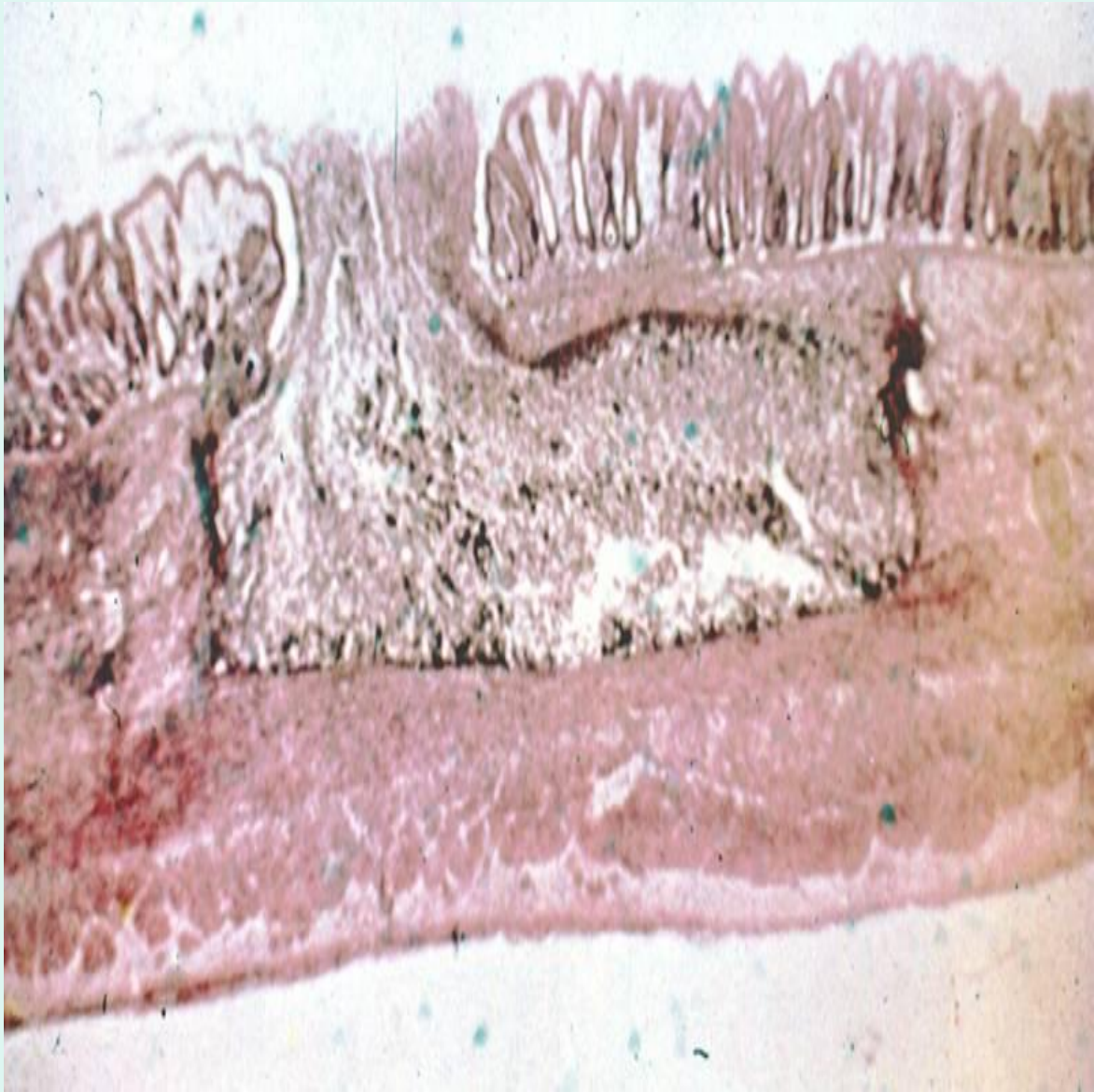
Entamoeba

cyst

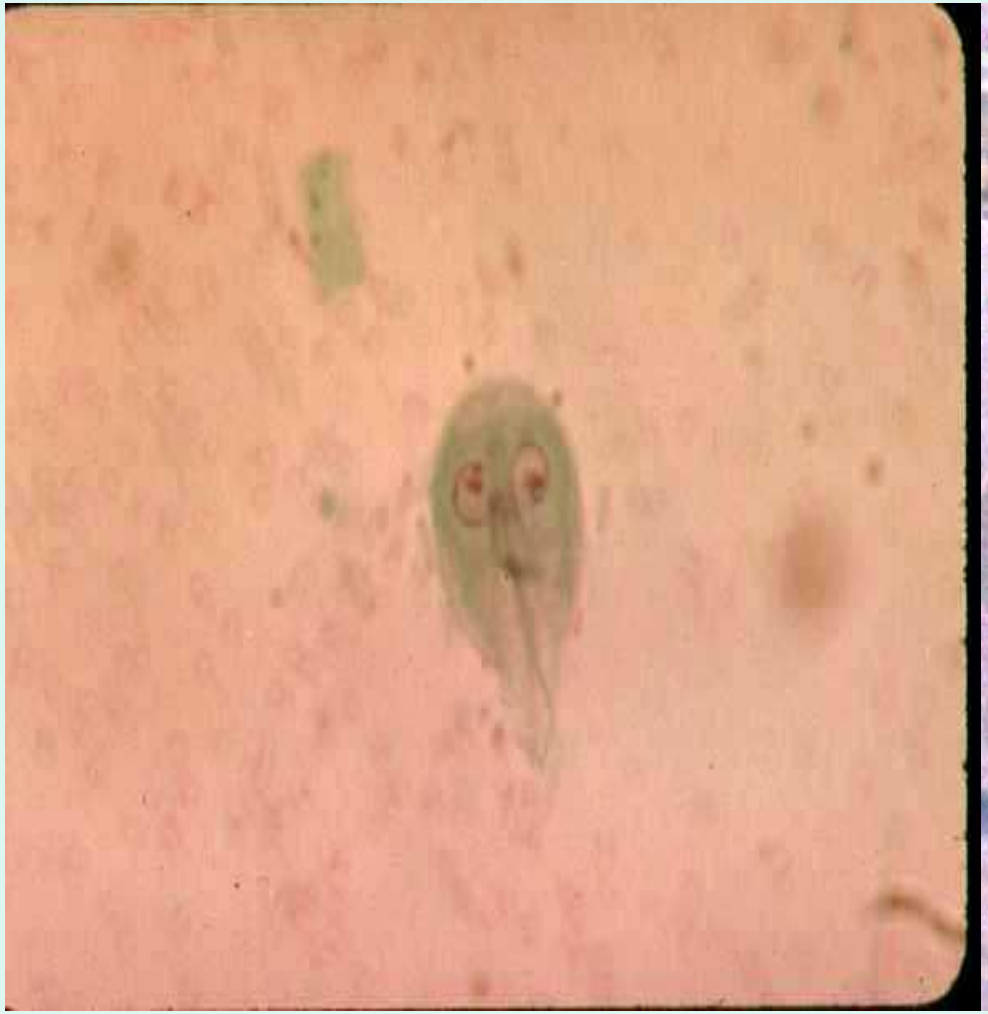


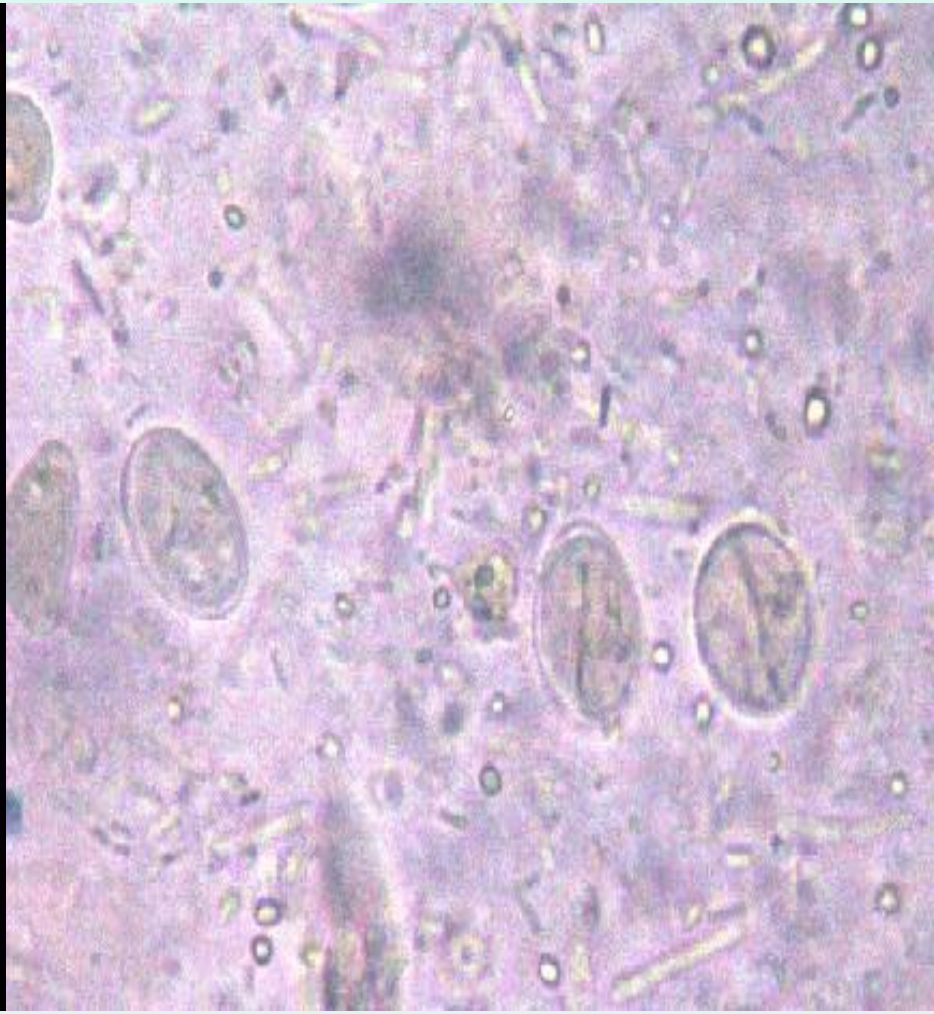


trophozoite

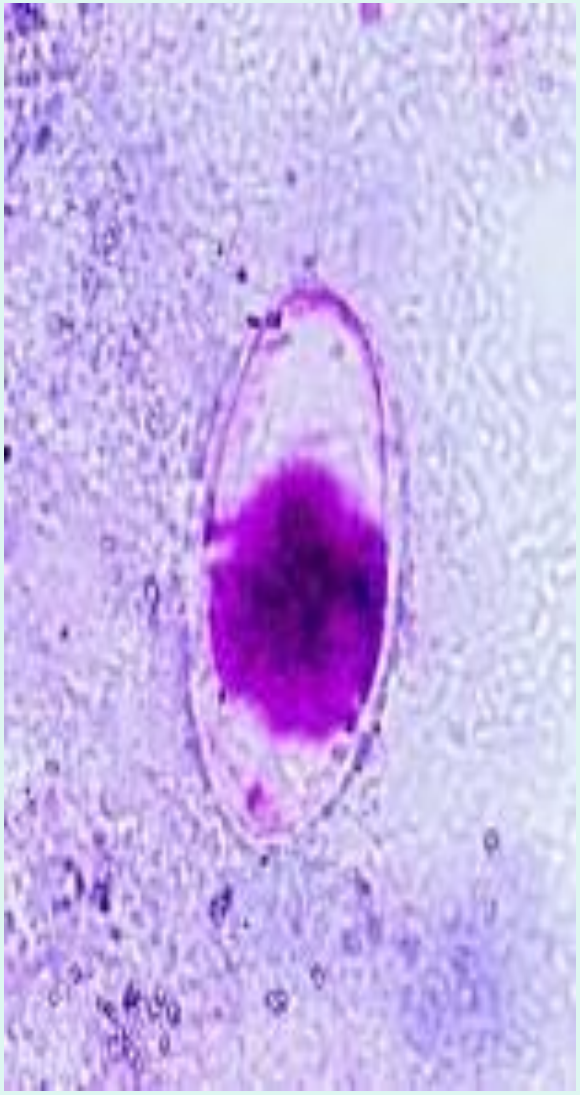


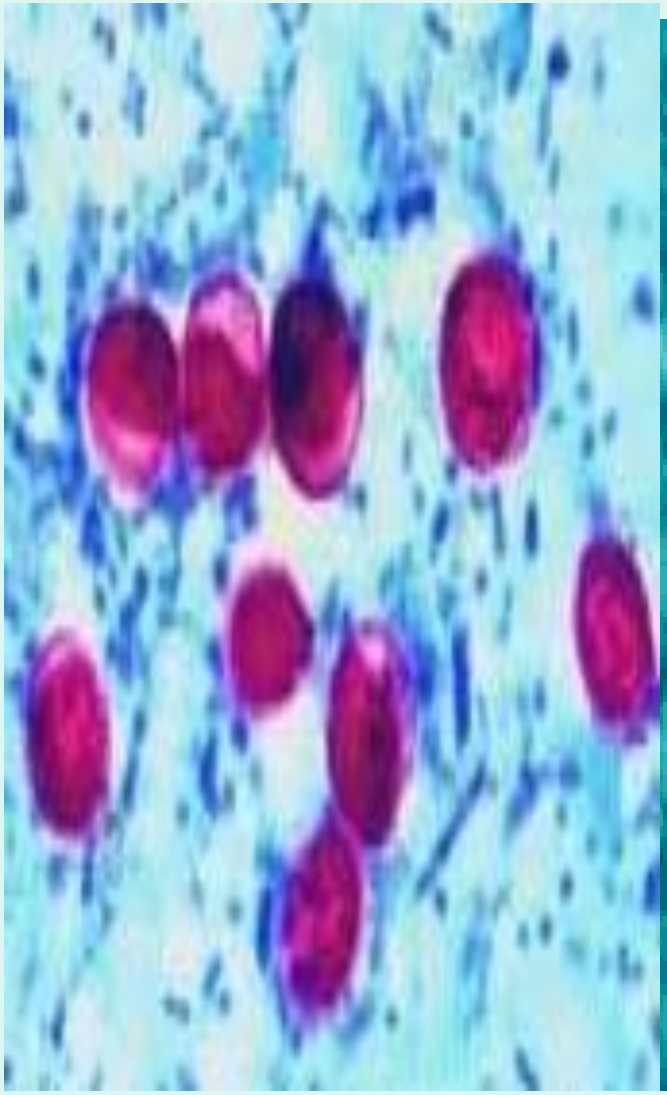
Giardia intestinalis

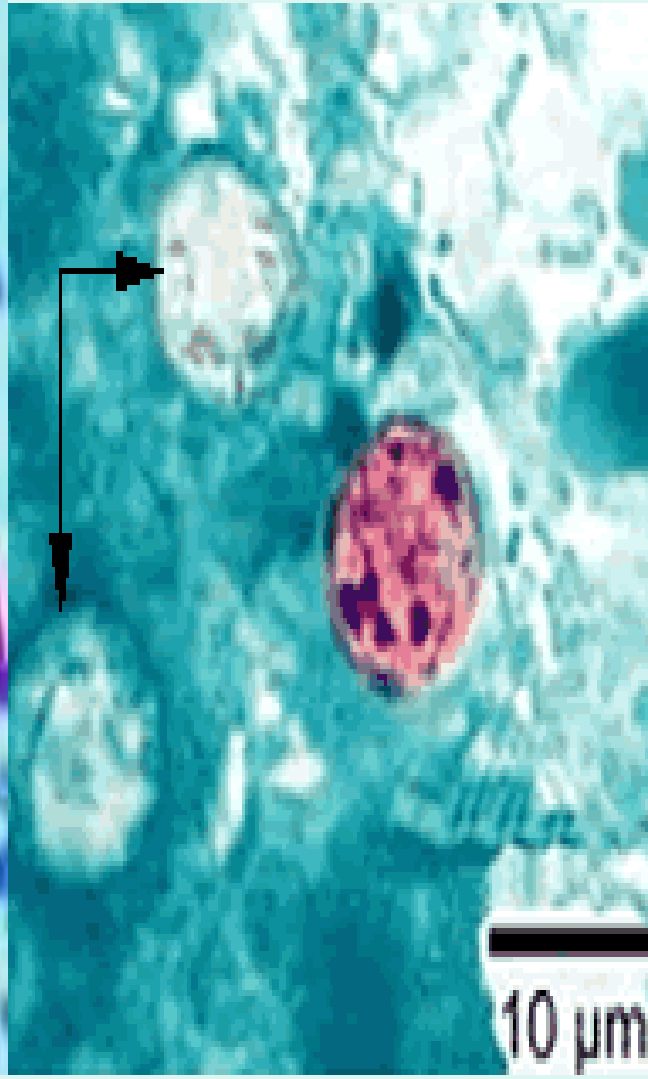




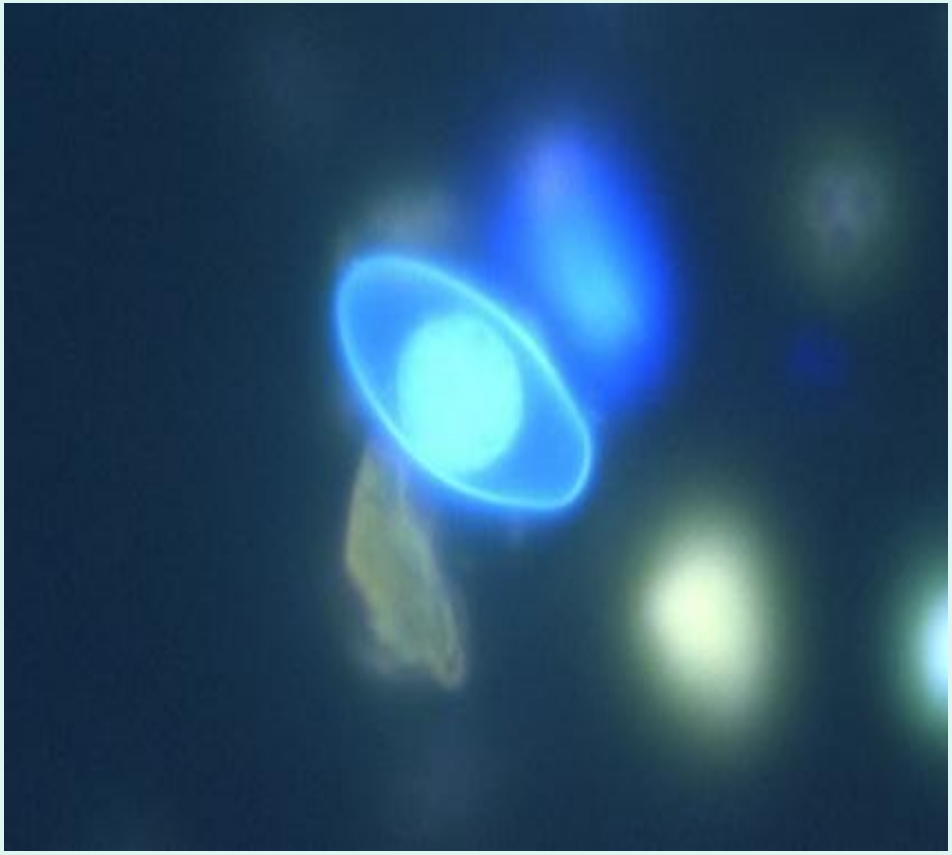
Intestinal coccidean



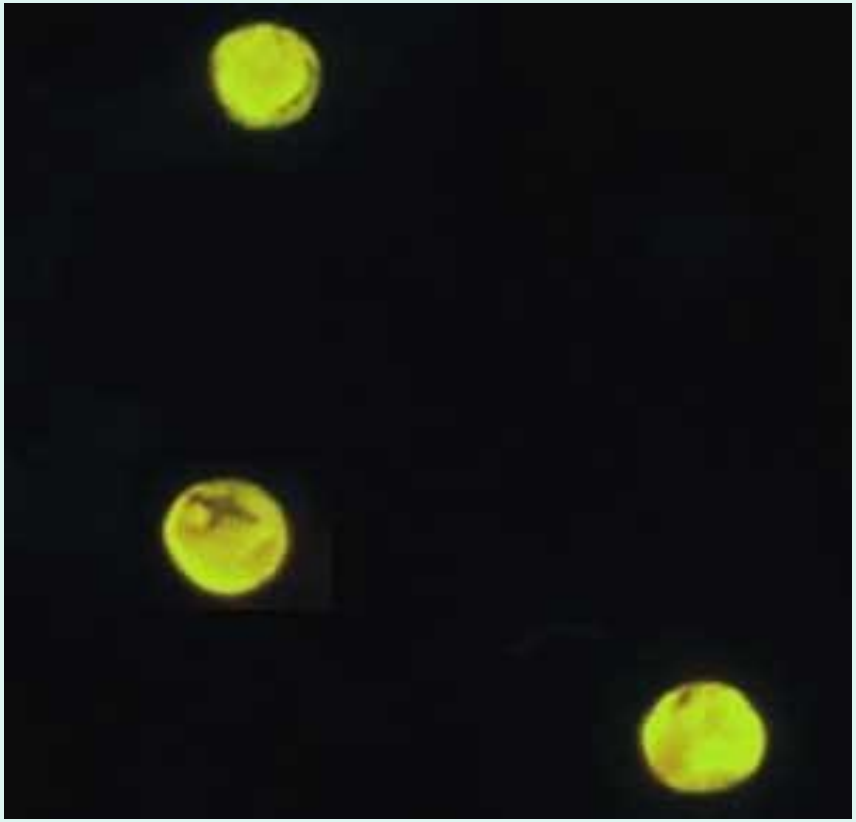




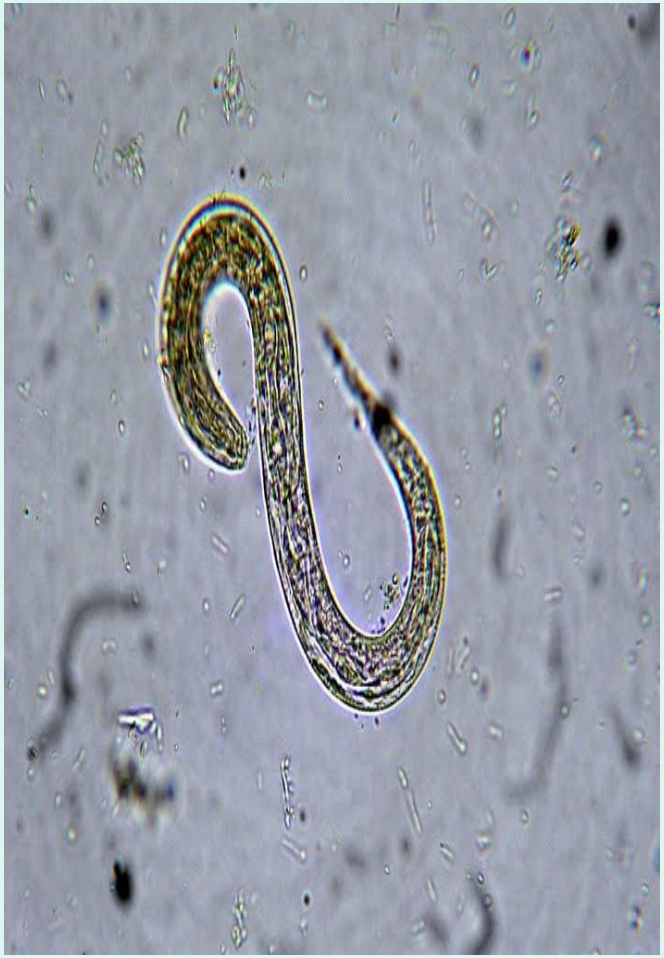
Auto fluorescence *Isospora belli*

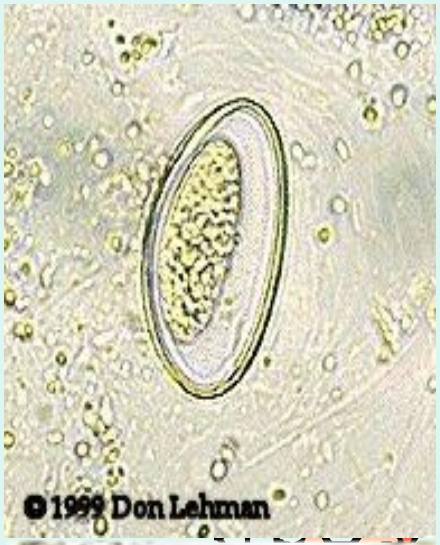


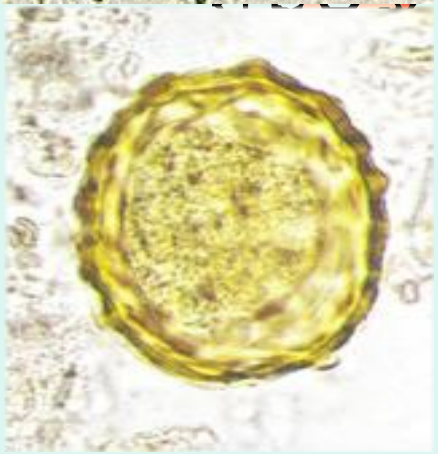
Non specific fluorescent stain for *Cryptosporidium*



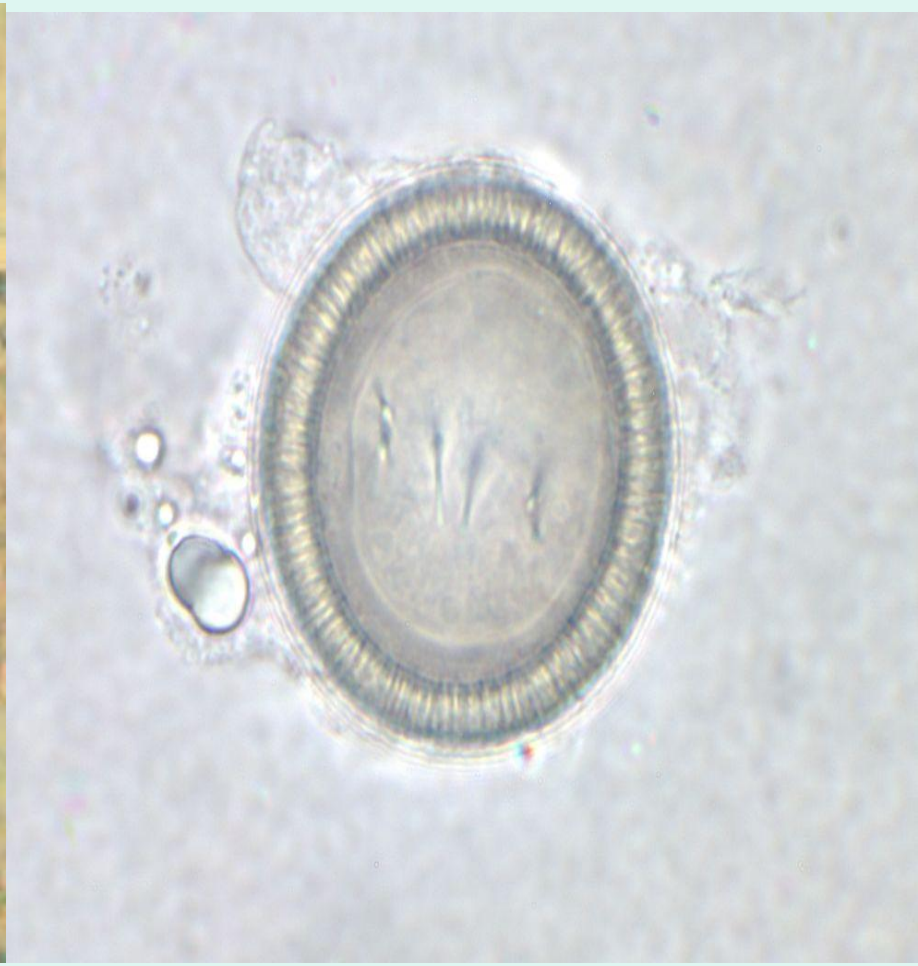
Some intestinal helminths eggs



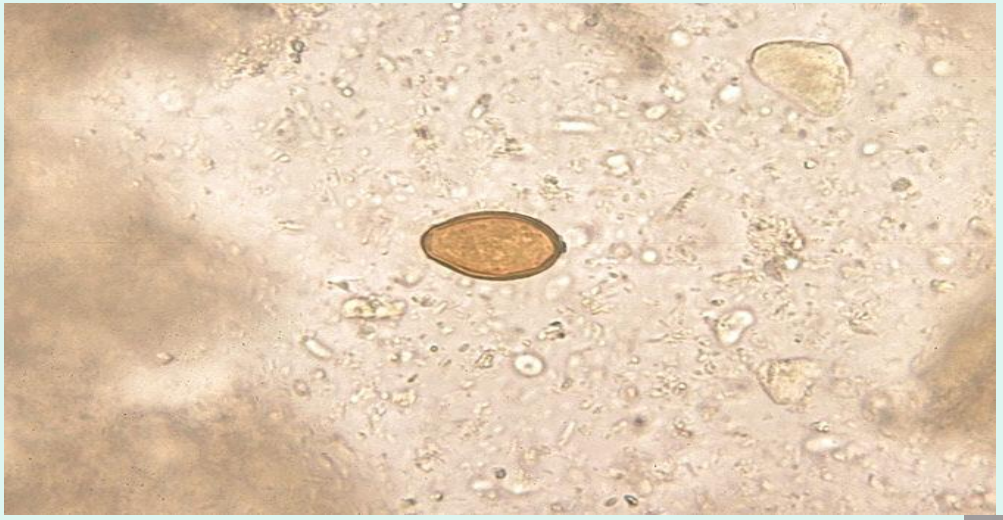




Some cectode eggs



Some trematodes eggs



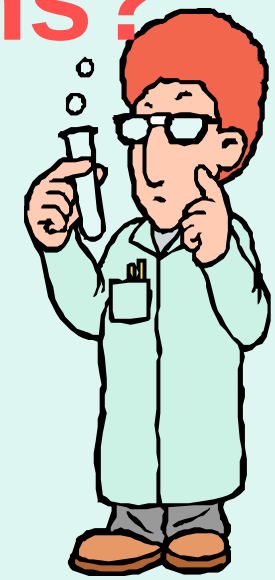
3- Antibody-detection assays

1- CFT

2- IHA

3-ELISA

Why we are searching for new methods for diagnosis of intestinal parasitic infections?



- **Although microscopy is considered to be the gold standard,**

-It is labour-intensive

-Its diagnostic performance critically depends on well-trained microscopists.

-It is not sensitive and may result in misdiagnosis leading to delayed or inadequate treatment.

-

- Diagnosis of *E. histolytica* cannot be done any longer by microscopy, since this parasite is morphologically similar to the non-pathogenic parasite *E. dispar*.
- Increase prevalence of immune compromised sectors in our locality (chronic liver disease, CKD, DM, malignancy…….).
- Empirical treatment is not preferable

Limitations for Ab detection:

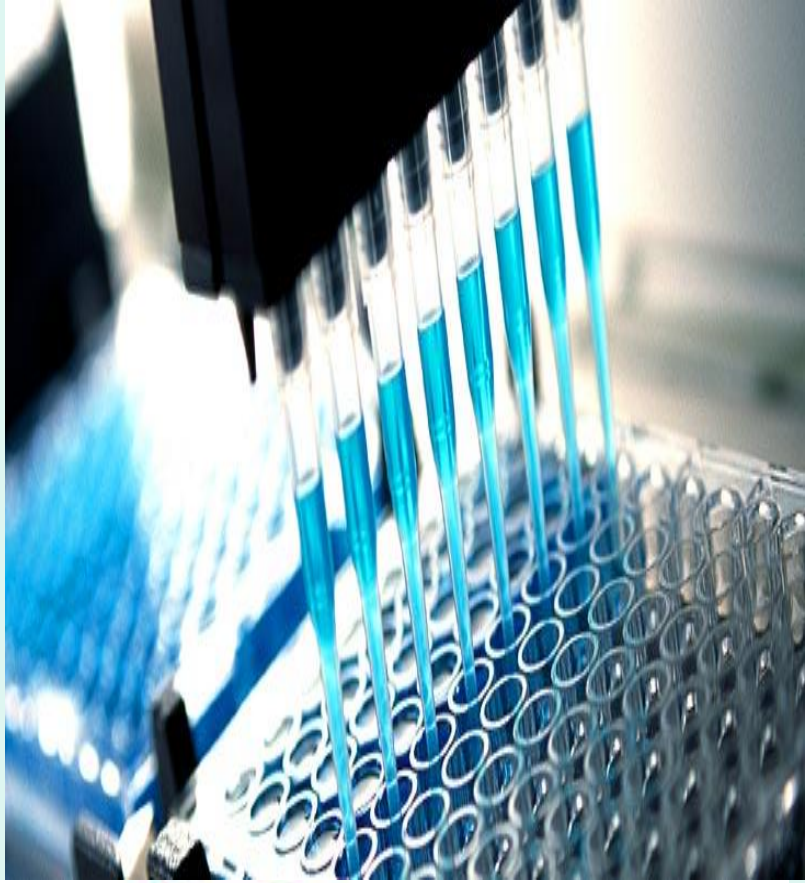
1- Some intestinal parasites do not have commercially or FDA approved antibody detection tests for their diagnosis.

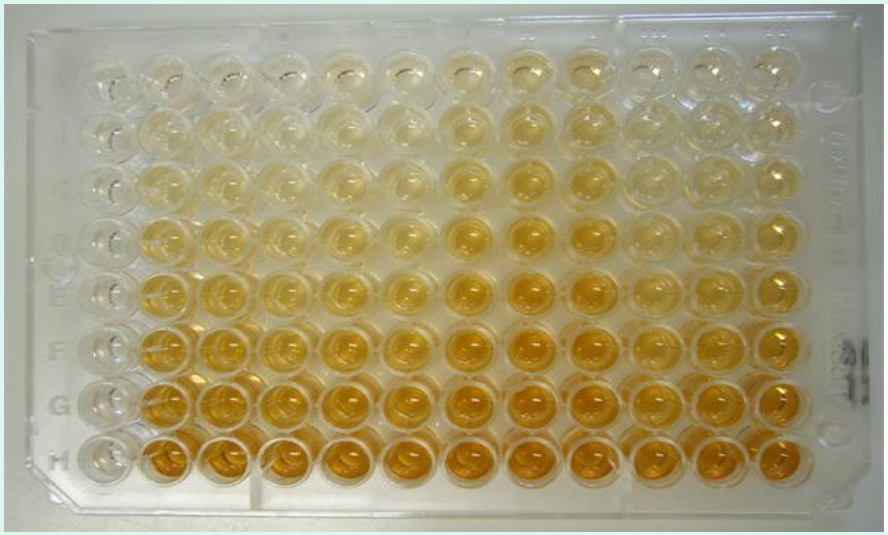
2-Experimental results have been too variable due to the type of antigen preparations used (e.g., crude, recombinant purified, adult worm, egg) and also because of the use of non standardized test procedures.

- 3- Cross-reaction leading to false-positives and misdiagnosis, especially in regions where more than one parasite is endemic. (cross-reactivity exists between filarial and other helminth antigens).
- 4- Inability of antibody-detection tests to differentiate between past and currently active infections

Commercially available techniques

I-Immunology -Based Assays





- **Immunology-based diagnosis tools can be divided into two categories:**

1-Antigen-detection assays

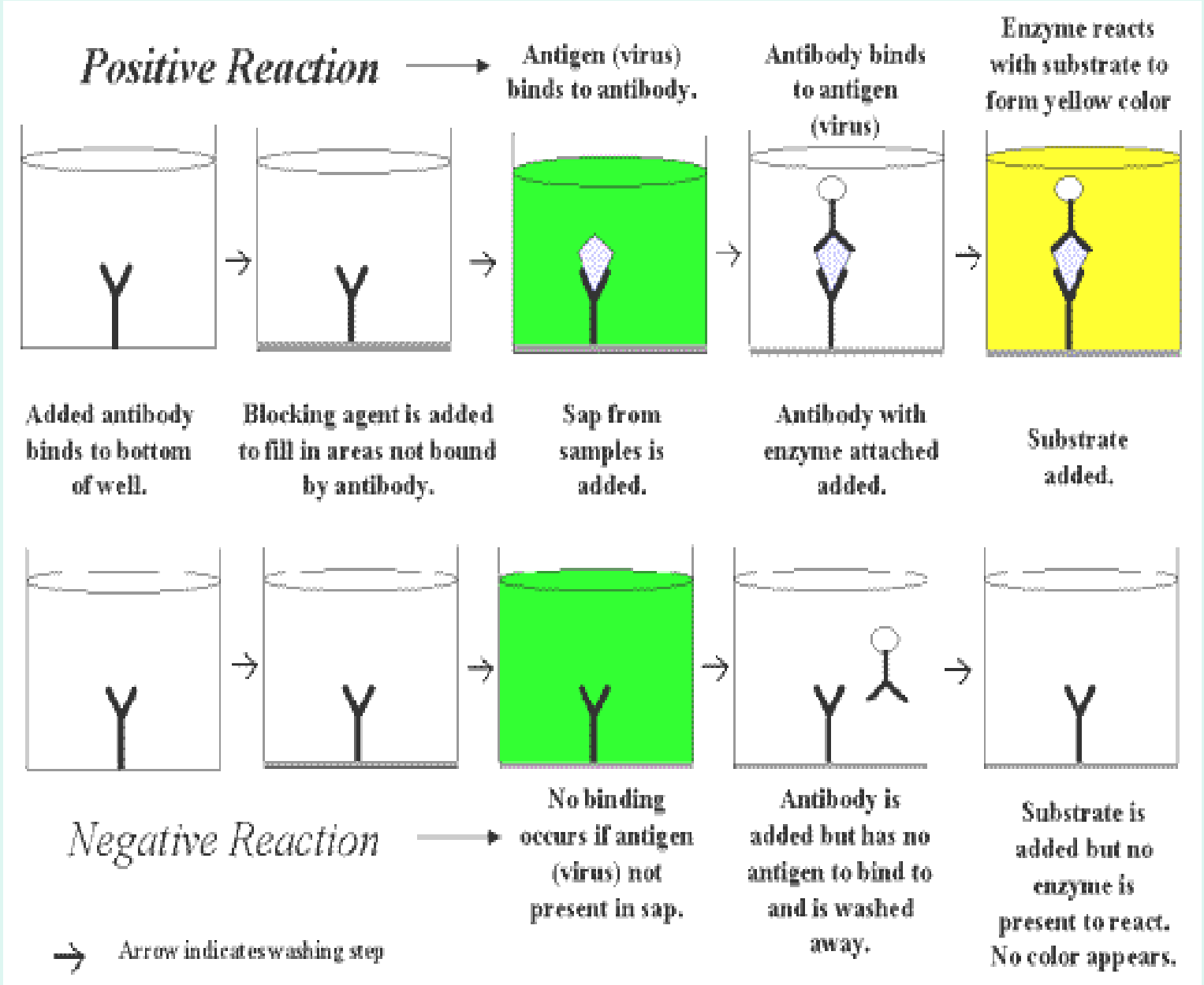
2- Antibody-detection assays.

- ELISA and direct fluorescent antibody assays have been accepted as cost-effective alternative diagnostic methods for the detection of *G. lamblia* and *Cryptosporidium* in stools.

Coproantigen ELISA:

- **Principle**:

The various components involved in ELISA are a solid phase to which specific antibody is coated, an antibody enzyme conjugate as probe and enzyme substrate. Solid supports used are polystyrene or PVC microtitre plates


Positive Reaction
Antigen (virus) binds to antibody.
Antibody binds to antigen (virus)
Enzyme reacts with substrate to form yellow color
Added antibody binds to bottom of well.
Blocking agent is added to fill in areas not bound by antibody.
Sap from samples is added.
Antibody with enzyme attached added.
Substrate added.
Negative Reaction
No binding occurs if antigen (virus) not present in sap.
Antibody is added but has no antigen to bind to and is washed away.
Substrate is added but no enzyme is present to react. No color appears.
Arrow indicateswashing step

Applications:

- For *Strongyloides*
- *Cryptosporidium*
- *Giardia*
- *E. histolytica*

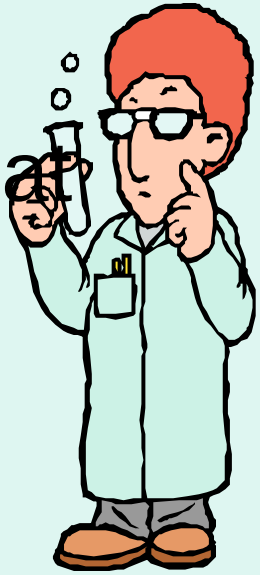
Disadvantage

- Requires multiple reagent additions
- Washing steps.
- Incubations.

Advantages

(i) Numerous samples can be screened at one time.

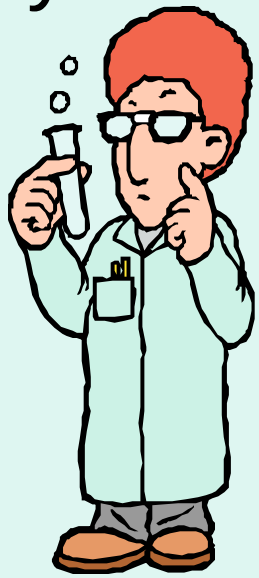
(ii) Tests can be read objectively on a spectrophotometer instead of subject.

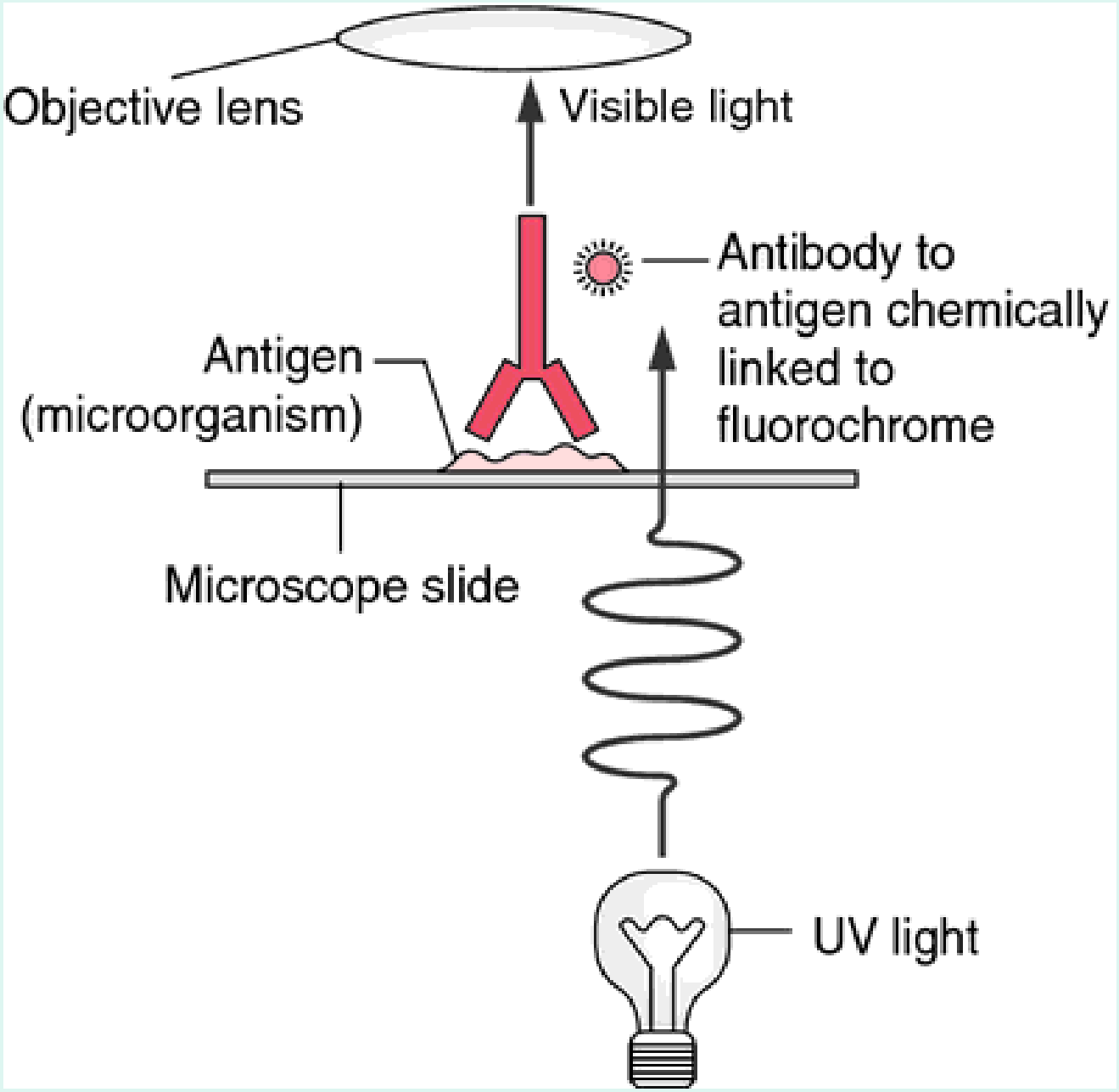


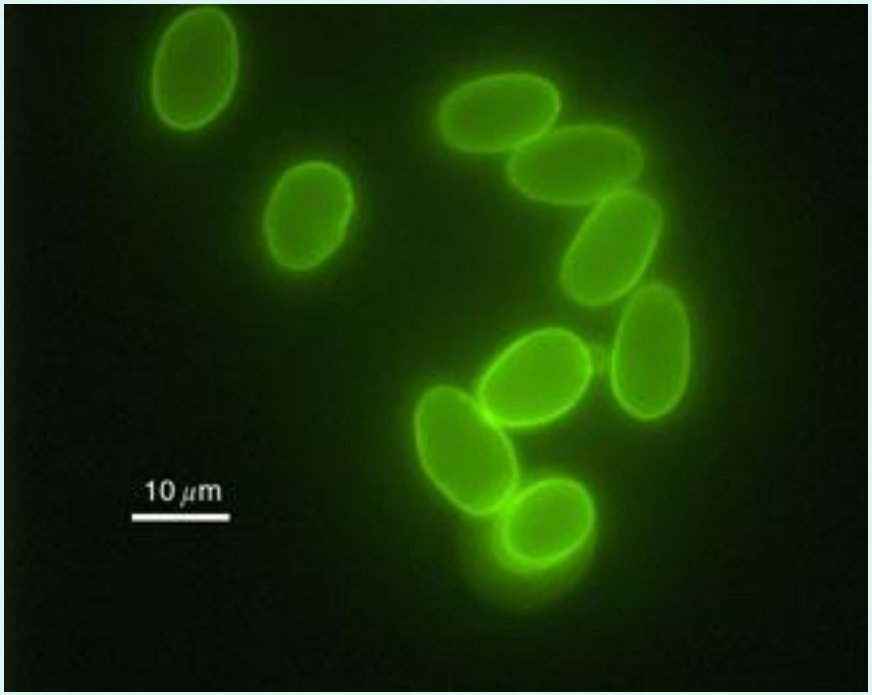
Direct fluoresce antibodies (DFA)

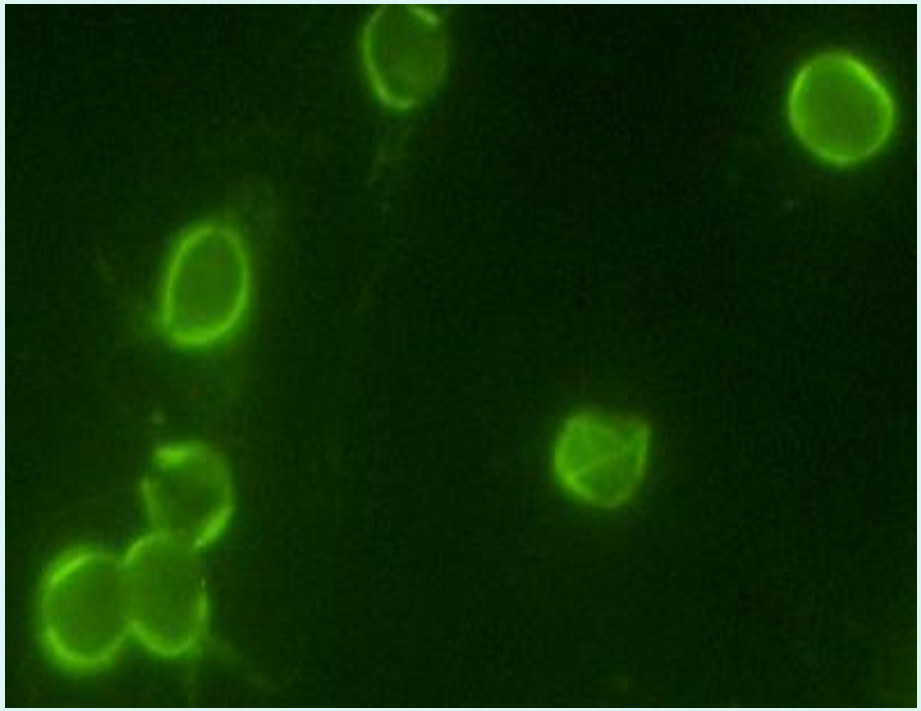
Principle:

- Utilize fluorescein-labeled antibodies directed against cell wall antigens of *Giardia* cysts and *Cryptosporidium* oocysts




Objective lens
Visible light
Antibody to antigen chemically linked to fluorochrome
Antigen (microorganism)
Microscope slide
UV light


10 μm



Advantage of DFA:

- Allow visualization of the intact parasites, providing a definitive diagnosis

Disadvantage of DFA

- Requires fluorescence microscopy

Immunecromatographic assay

Principle:

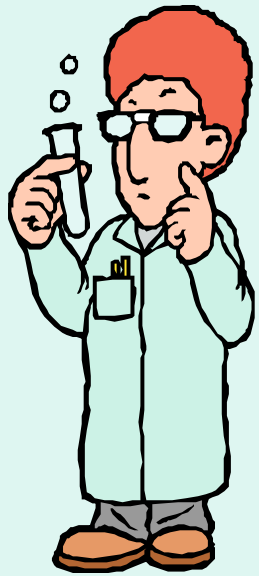
It is the same as ELISA, but differs only in that the immunological reaction carried out on cromatographic paper by capillary action.

- Two kinds of specific Ab :

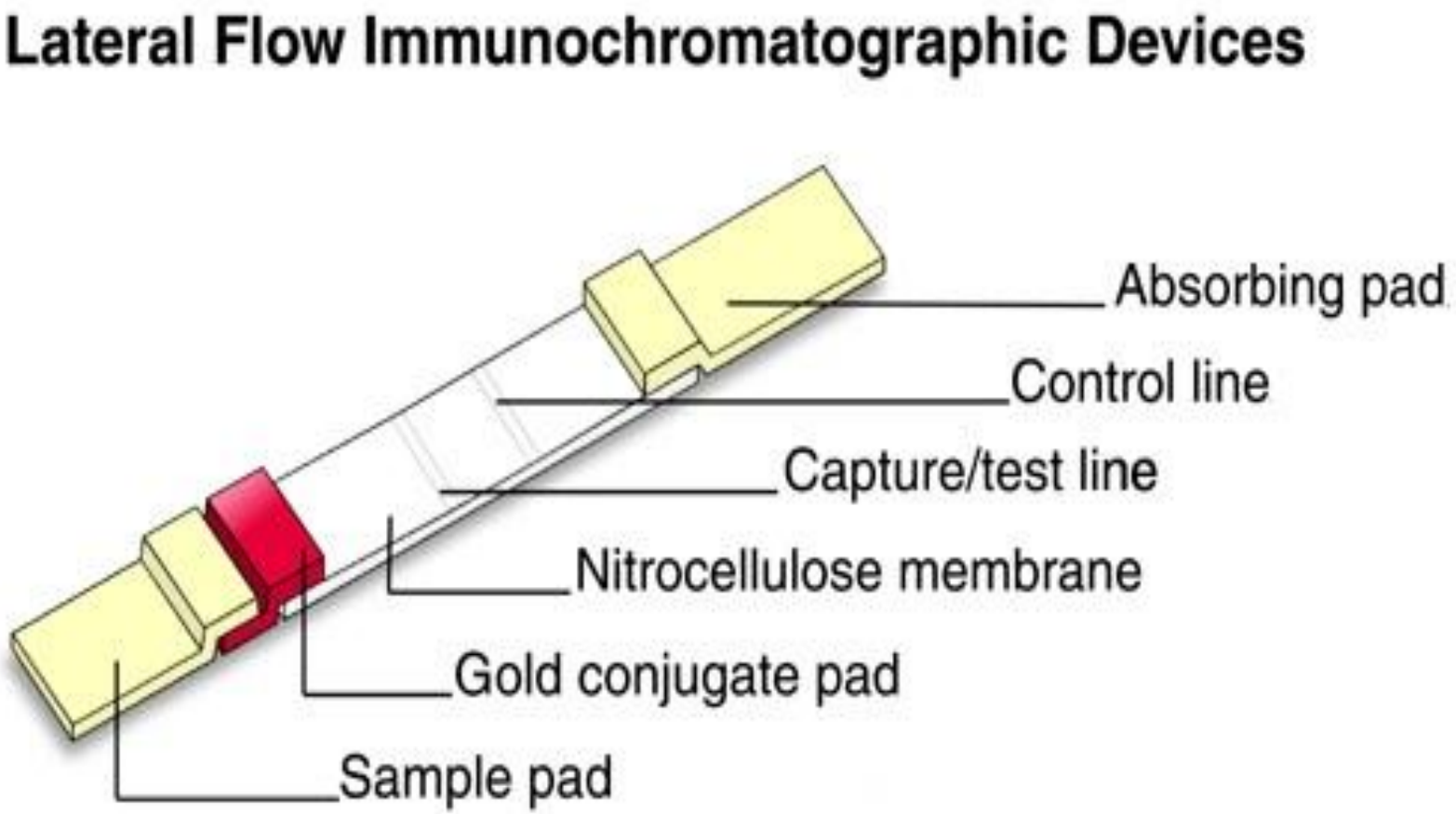
-one of the Ab (immoblised on clear defined line (test line)

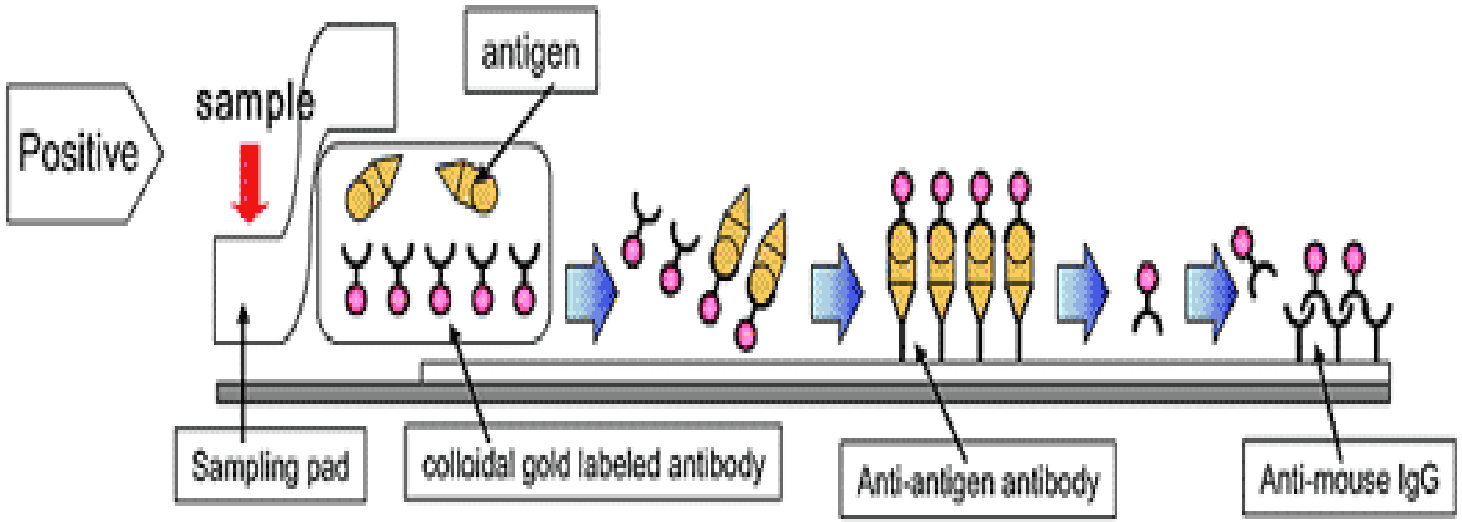
-the 2nd labled with detection agent as colloidal gold and infiltrated in congugated pad

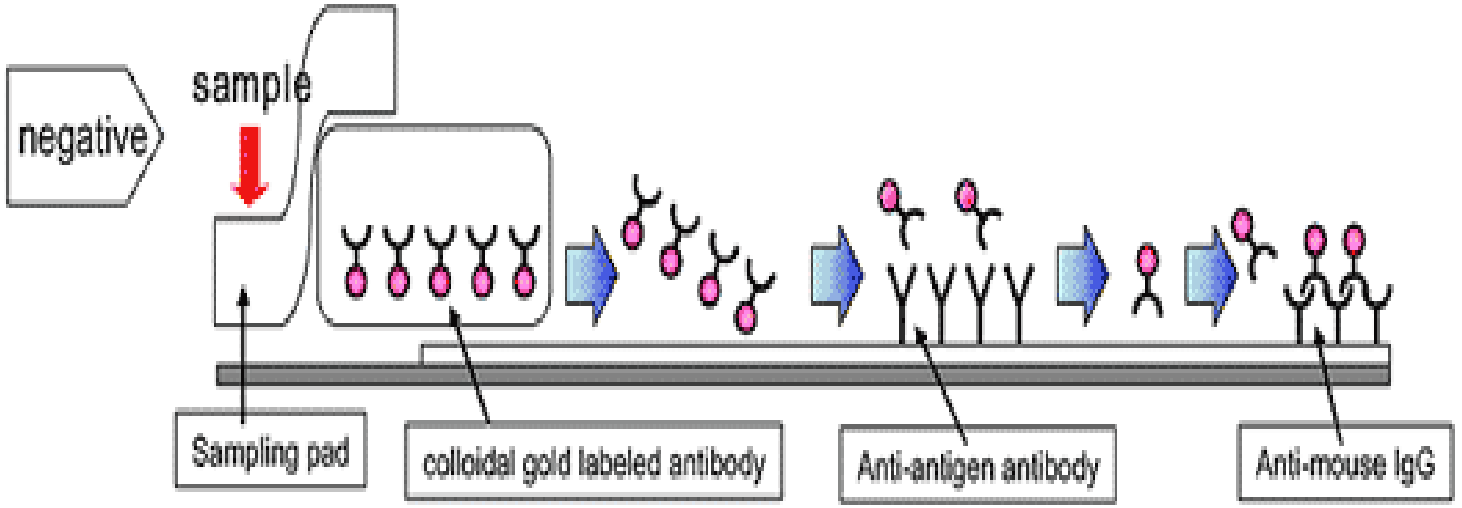
- The Ag to be detected or anti Ab deposited on the same strip in another line (control line)



Cromatographic paper structure



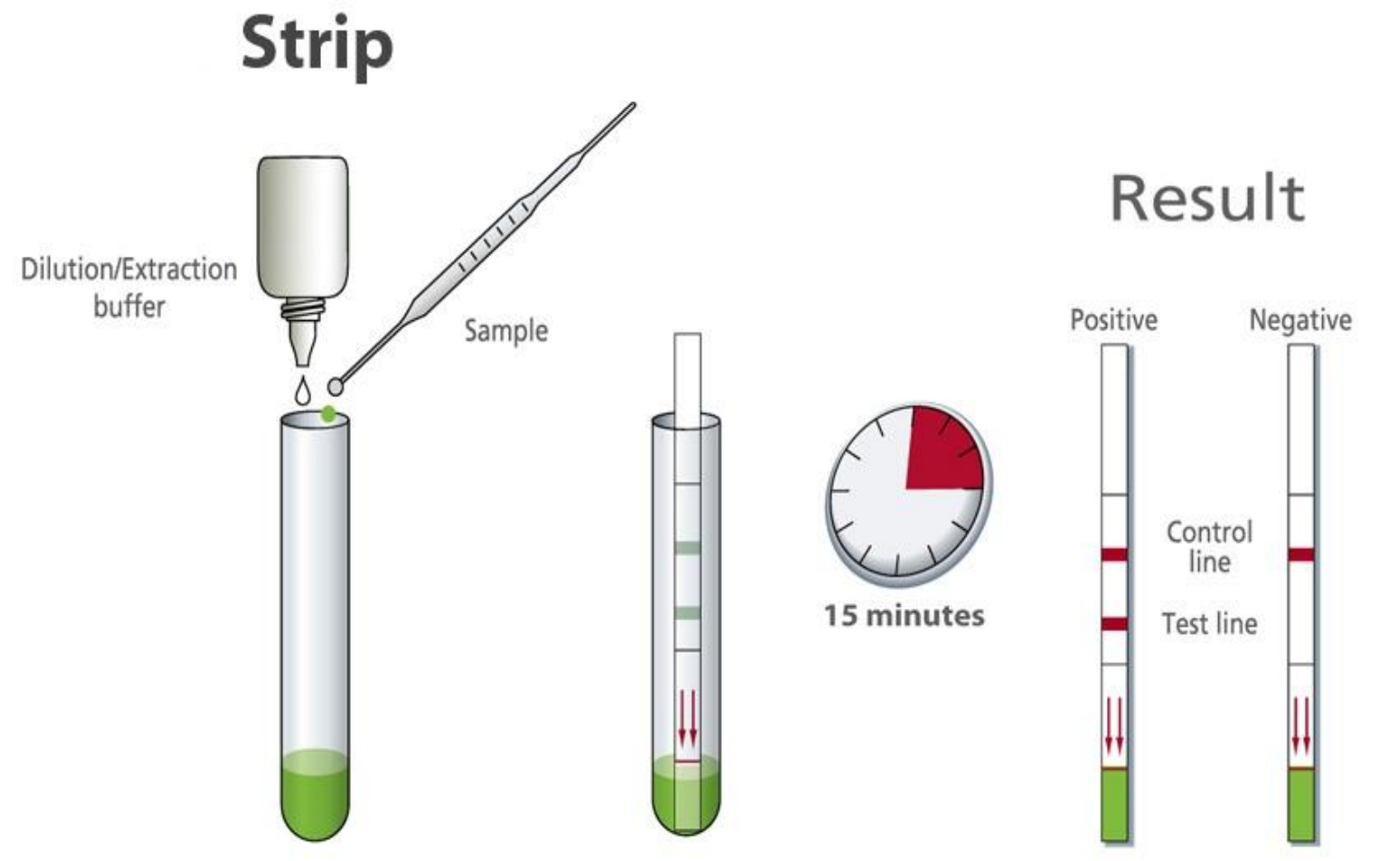

Positive
sample
antigen
Sampling pad
colloidal gold labeled antibody
Anti-antigen antibody
Anti-mouse IgG


negative
sample
Sampling pad
colloidal gold labeled antibody
Anti-antigen antibody
Anti-mouse IgG

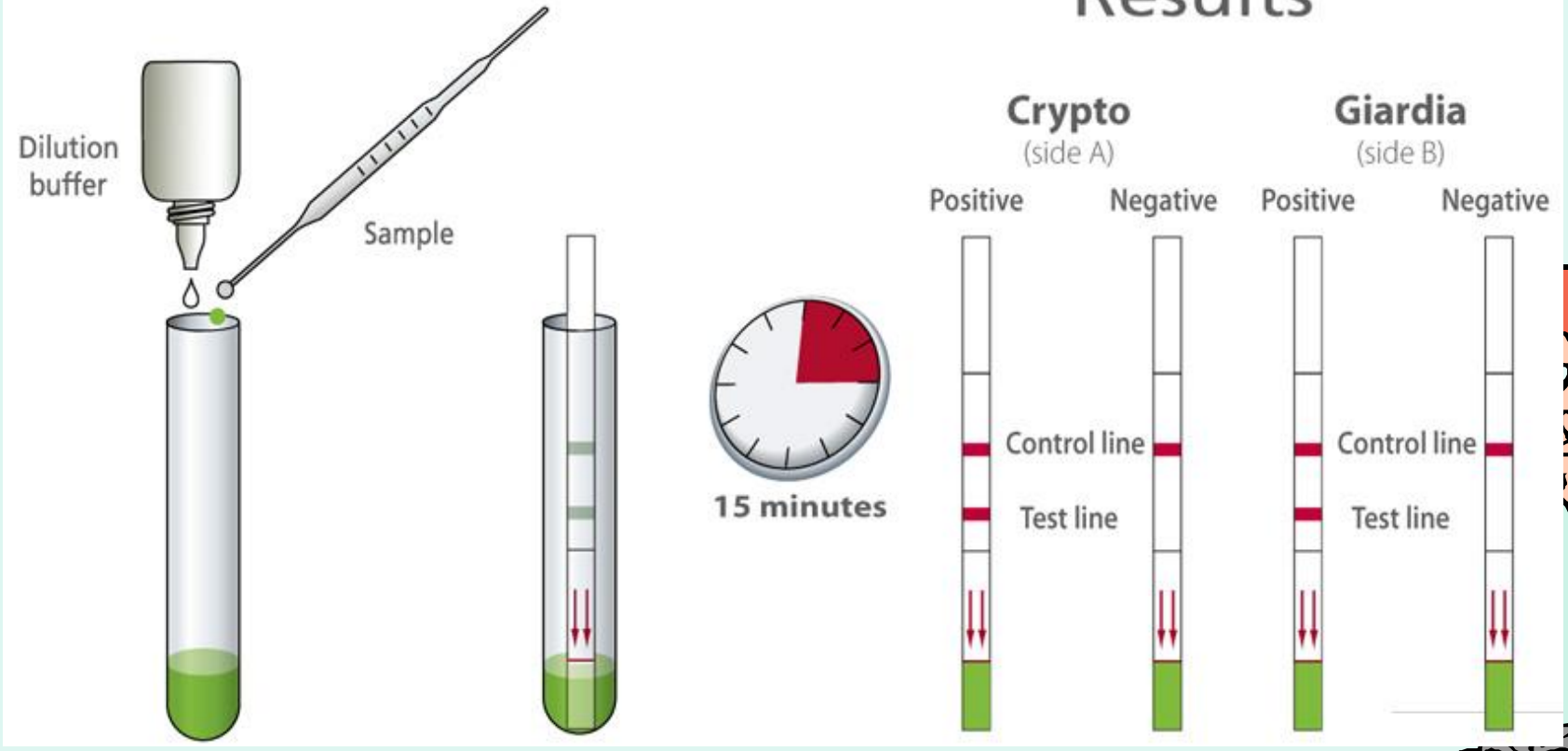
Applications

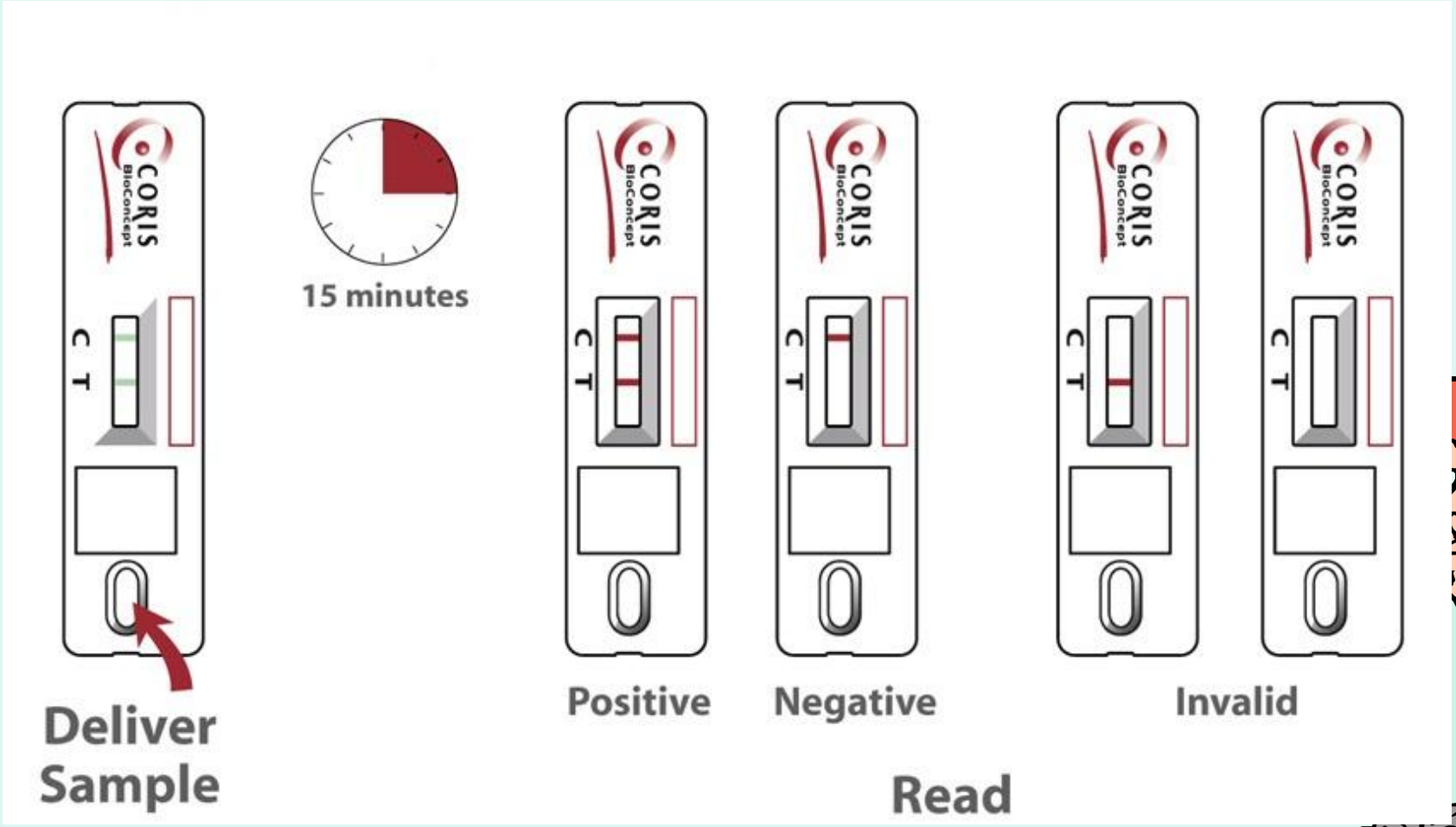
- -*Hymenolipes*
- *-Cryptosporidium and Girdia*
- *-E. hisolytica*
- -Urine-CCA (Circulating Cathodic Antigen) for *Schistosoma mansoni*.

Forms of immunchromatography tests



Crypto-Giardia Duo-Strip



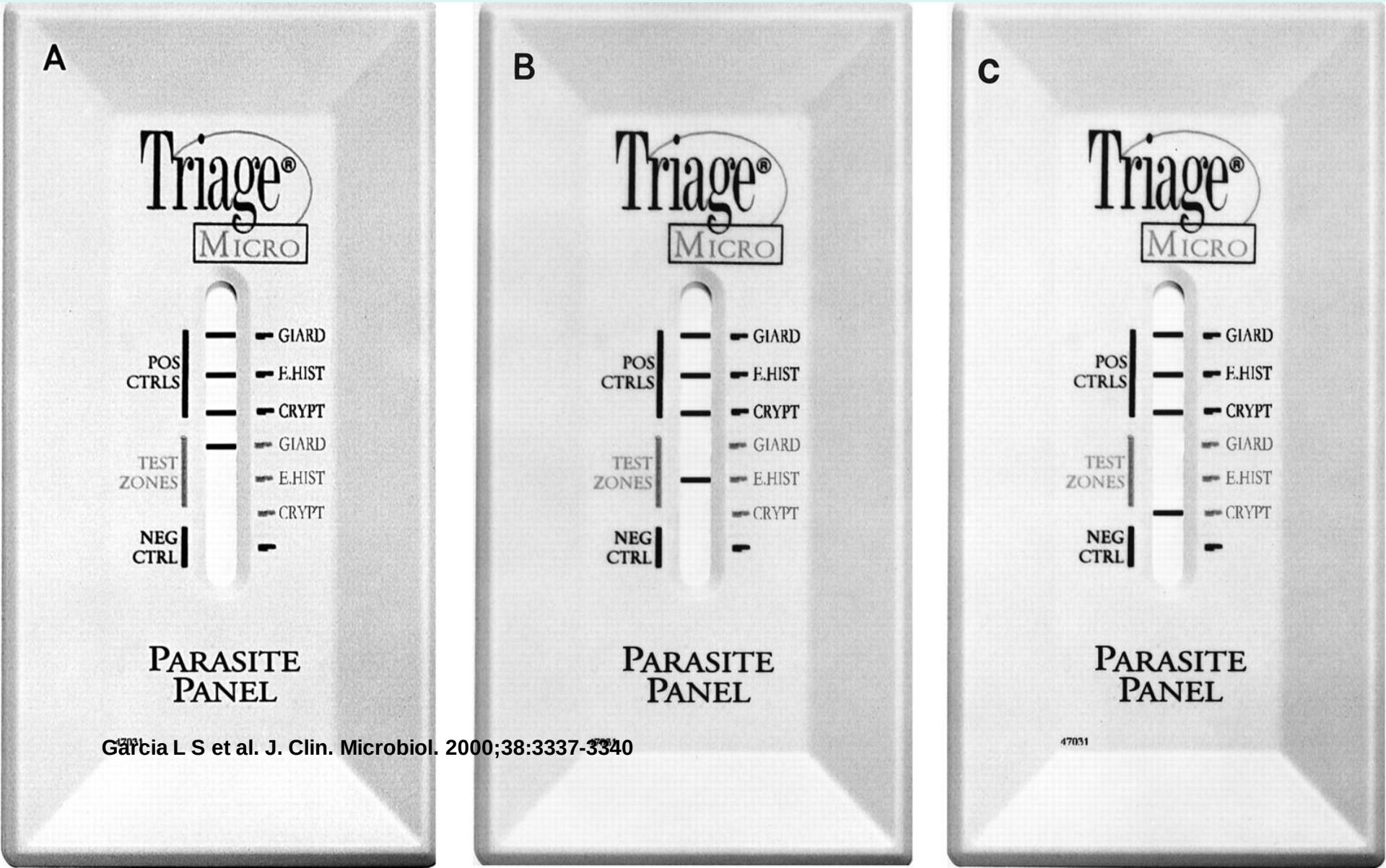

CORIS BioConcept
C T
15 minutes
Deliver Sample
Positive
Negative
Invalid
Read

ImmunoCard STAT! Cryptosporidium/Giardia Rapid Assay (Meridian Bioscience, Inc.).

Garcia L S et al. J. Clin. Microbiol. 2003;41:209-212

Journal of Clinical Microbiology

parasite panel demonstrating positive results.



García L S et al. J. Clin. Microbiol. 2000;38:3337-3340

Advantage

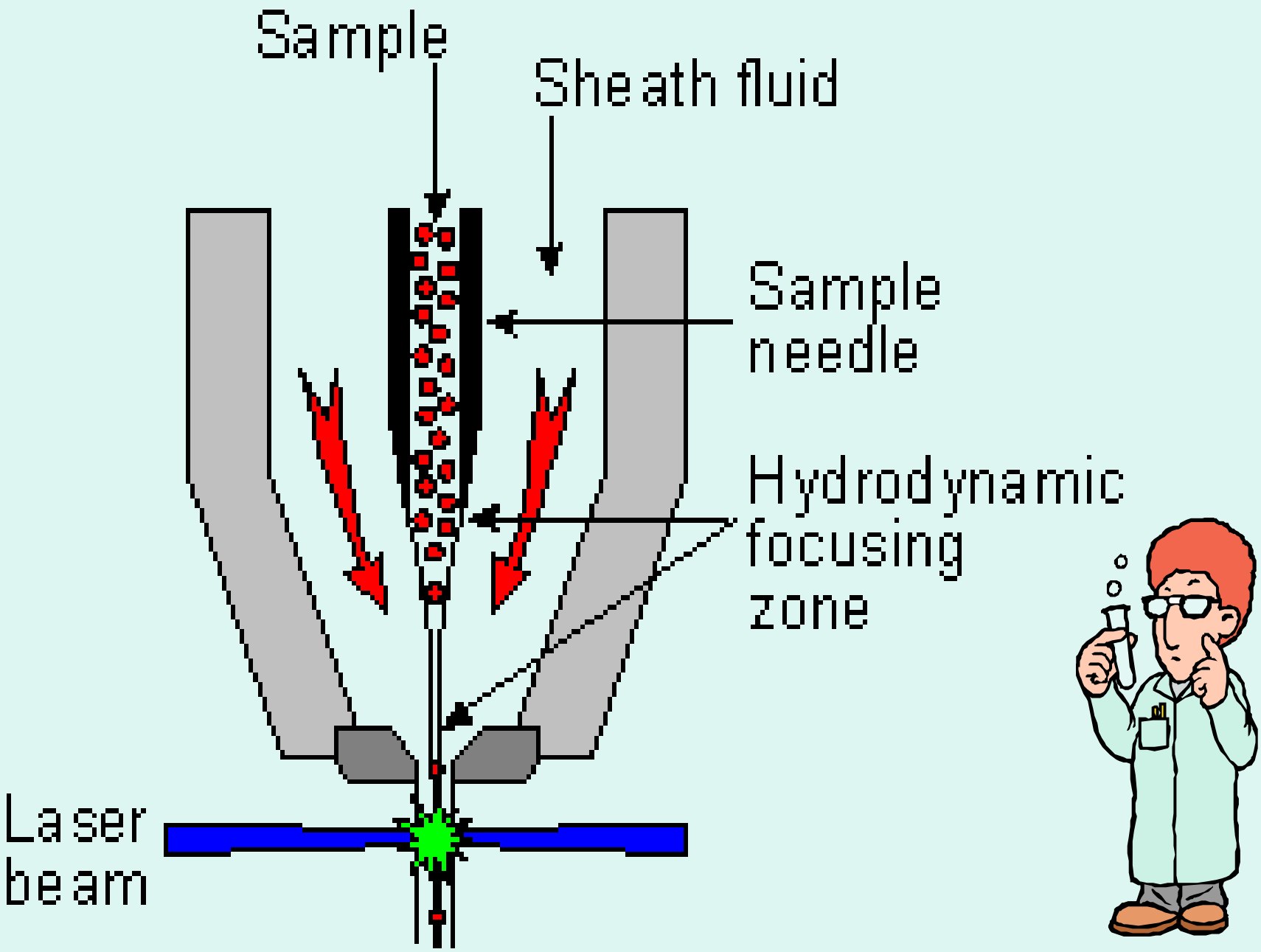
- Rapid
- Stable at temperatures up to 40°C,
- Easy to use,
- Cost-effective

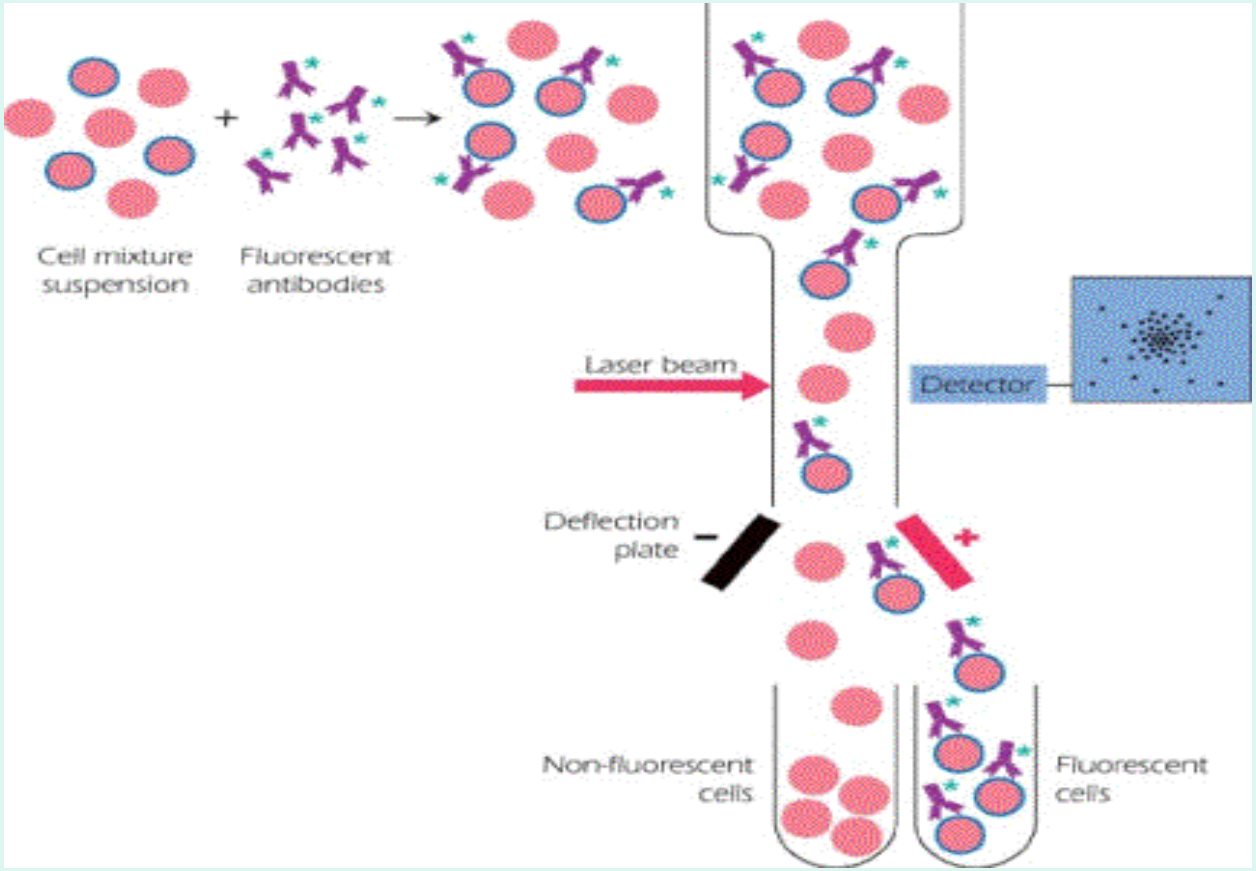
Disadvantage

- They are useless at detecting very low-density infections.

Hoping future techniques

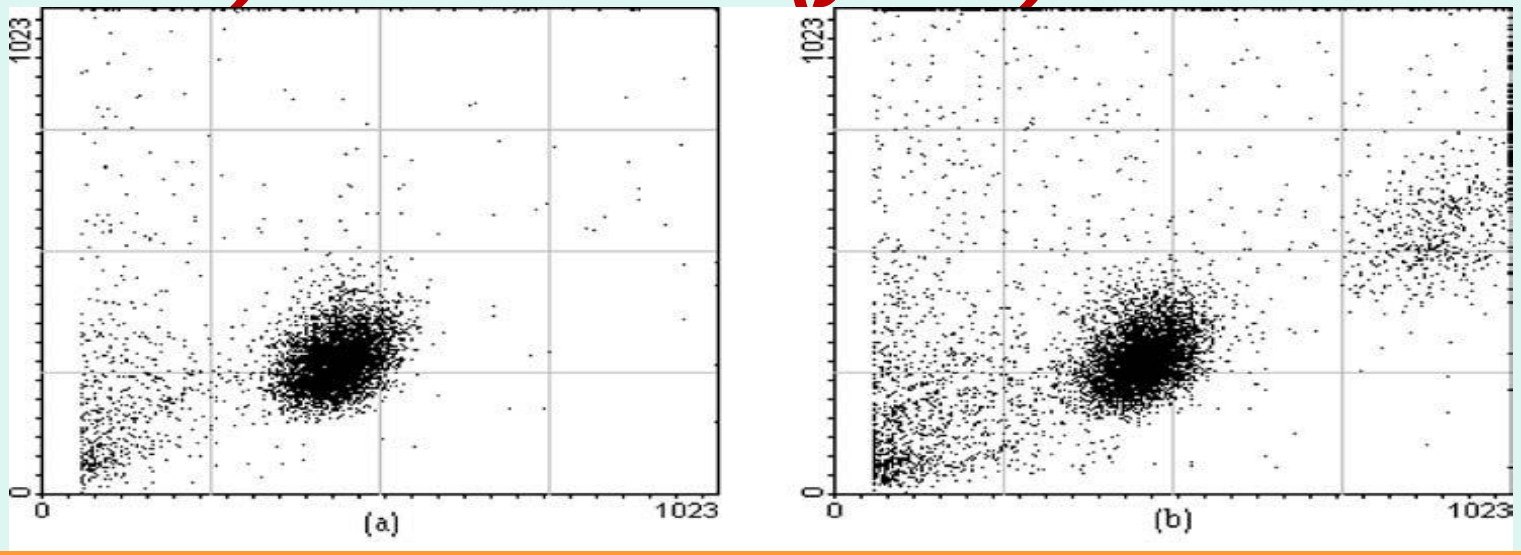
1-Flowcytometry


Sample
Sheath fluid
Sample needle
Hydrodynamic focusing zone
Laser beam


Cell mixture suspension
Fluorescent antibodies
Laser beam
Detector
Deflection plate
Non-fluorescent cells
Fluorescent cells

Cryptosporidium in stool

FSC (*x-axis) versus SSC (yaxis).*

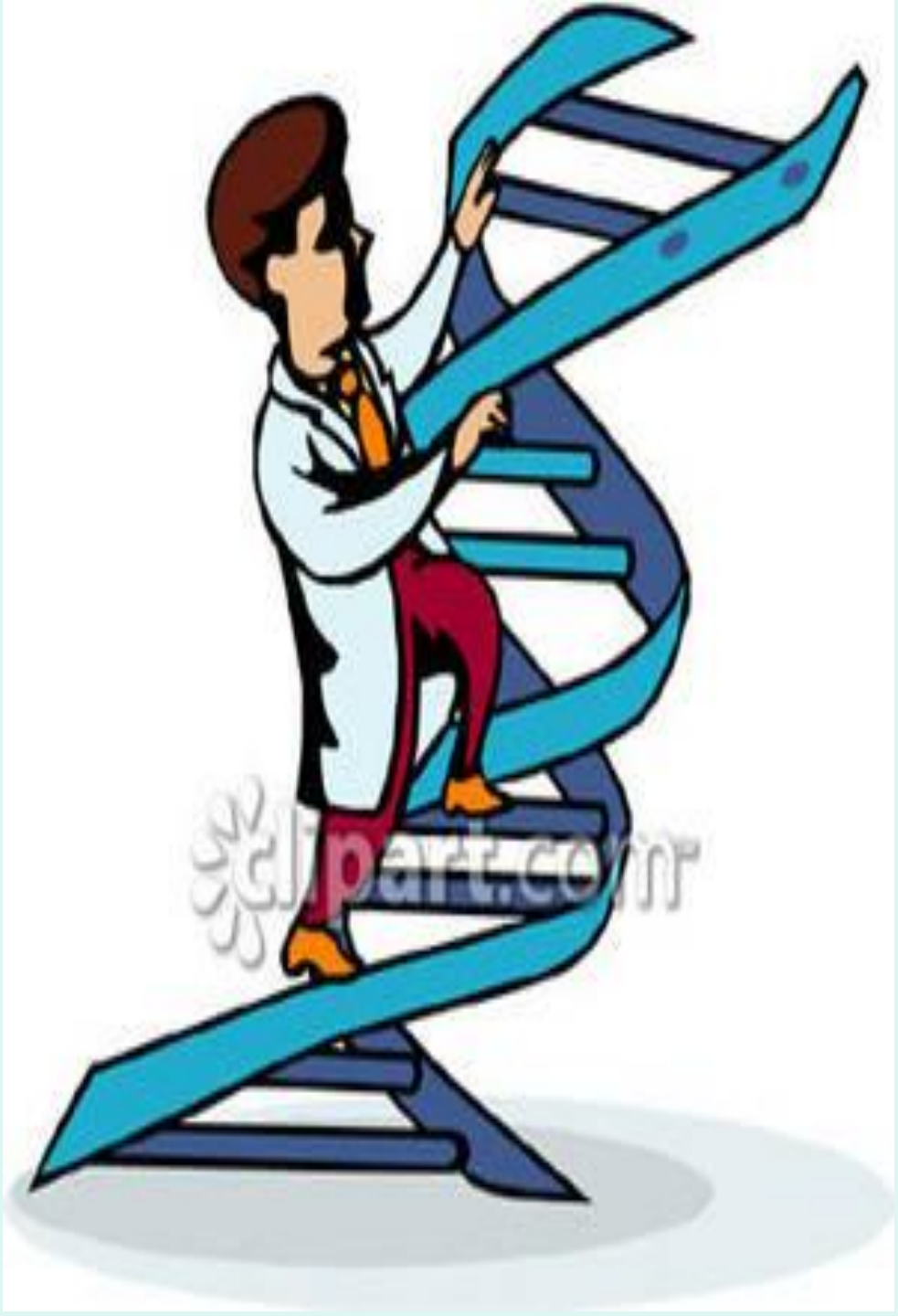


The scatter-plots for *Cryptosporidium oocysts representing FSC vs SSC*

(a): The scatter-plot for oocysts without staining.

(b): The scatter-plot for oocysts stained with direct immunofluorescent antibody

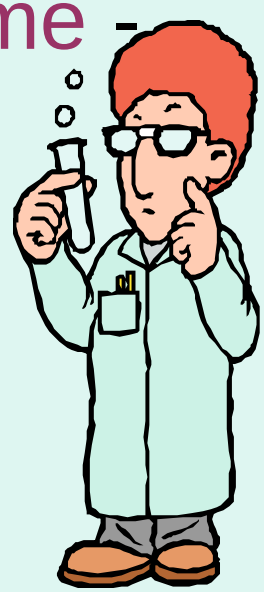
2- Molecular Based Approaches

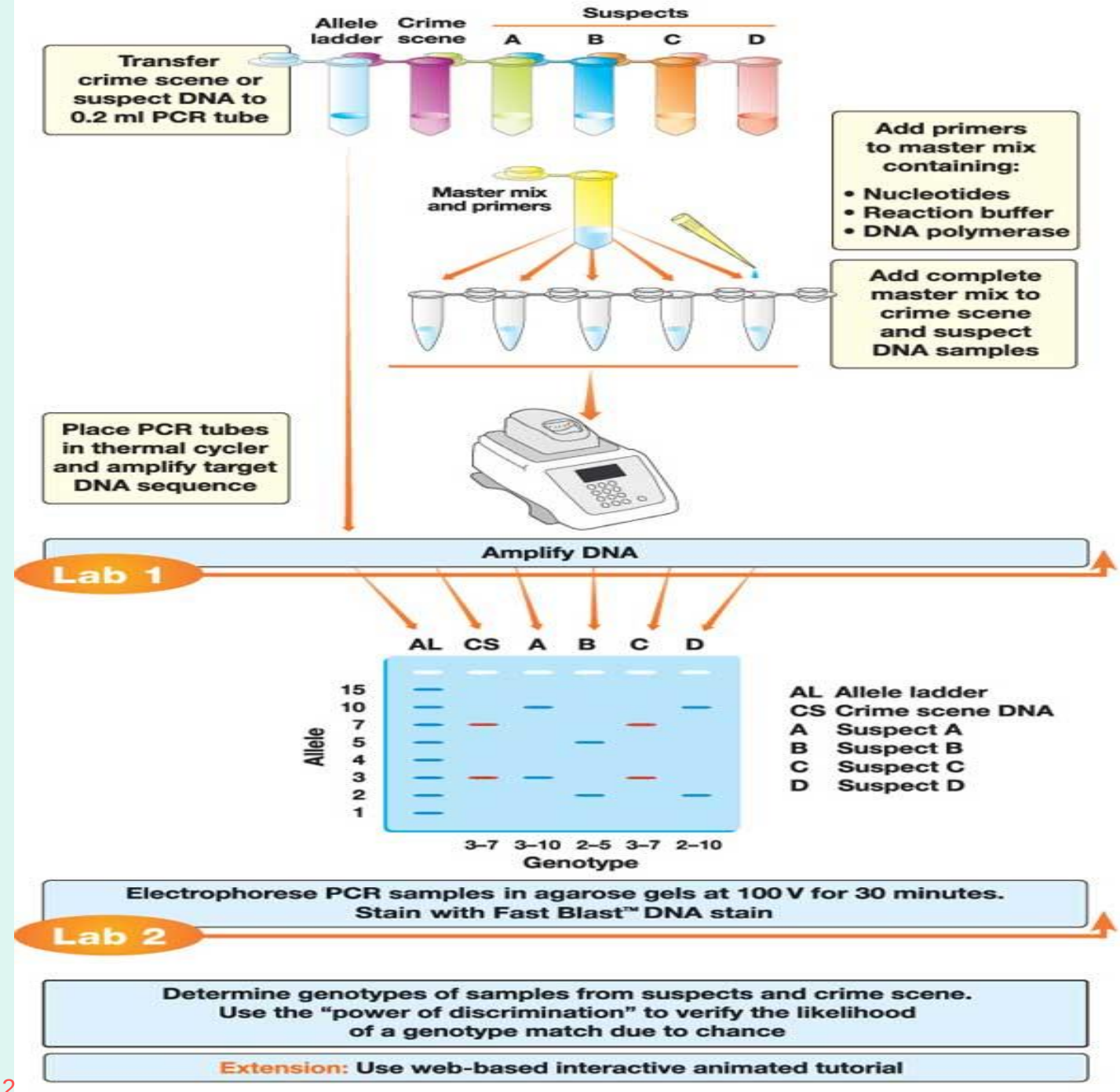


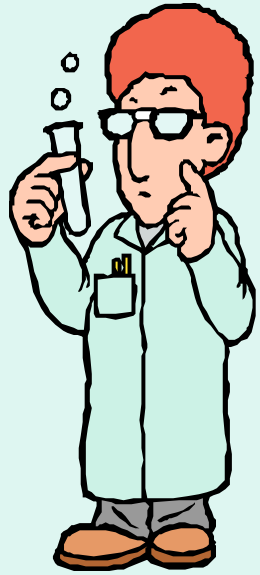
A-Conventional PCR

Four basic components:

- A DNA template .
- Primers
- A heat-stable DNA-polymerase enzyme -
- Free nucleotides

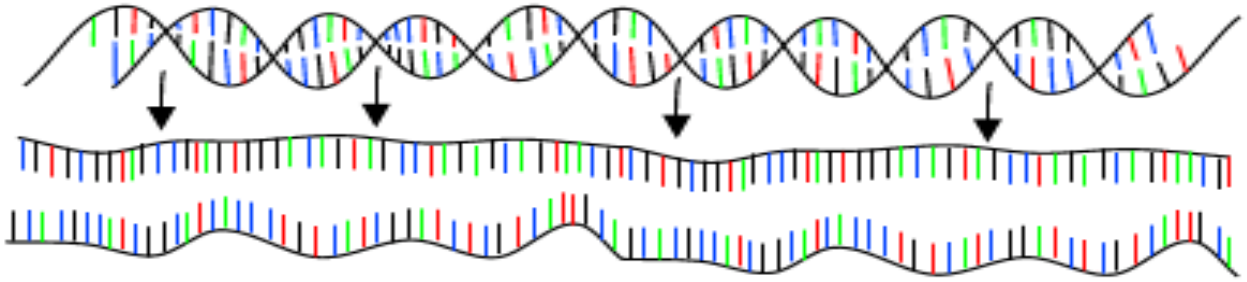



Allele ladder
Crime scene
Suspects
A
B
C
D
Transfer crime scene or suspect DNA to 0.2 ml PCR tube
Master mix and primers
Add primers to master mix containing:
• Nucleotides
• Reaction buffer
• DNA polymerase
Add complete master mix to crime scene and suspect DNA samples
Place PCR tubes in thermal cycler and amplify target DNA sequence
Lab 1
Amplify DNA
AL CS A B C D
Allele
15
10
7
5
4
3
2
1
3–7 3–10 2–5 3–7 2–10
Genotype
AL Allele ladder
CS Crime scene DNA
A Suspect A
B Suspect B
C Suspect C
D Suspect D
Electrophorese PCR samples in agarose gels at 100 V for 30 minutes.
Stain with Fast Blast™ DNA stain
Lab 2
Determine genotypes of samples from suspects and crime scene.
Use the "power of discrimination" to verify the likelihood of a genotype match due to chance
Extension: Use web-based interactive animated tutorial



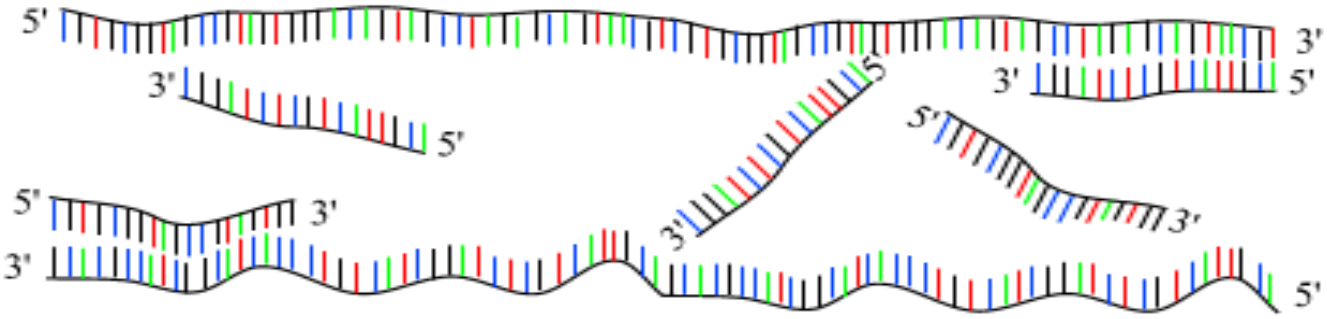
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

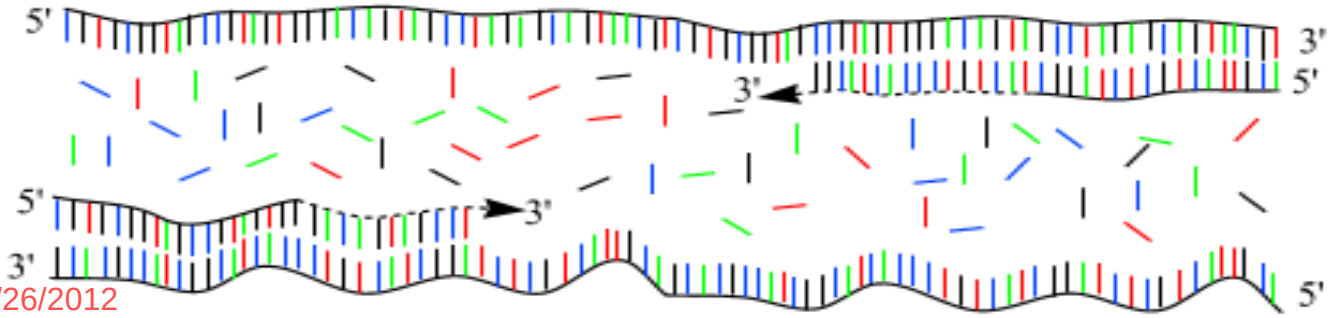
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)

Disadvantage of PCR

- The introduction of these methods in daily laboratory practice is still uncommon especially in rural endemic regions.

- PCR-based methods also suffer by the lack of standardization. DNA extraction, choice of primer sets, and use of various amplification protocols are all factors that may cause this diversification in results

Advantage

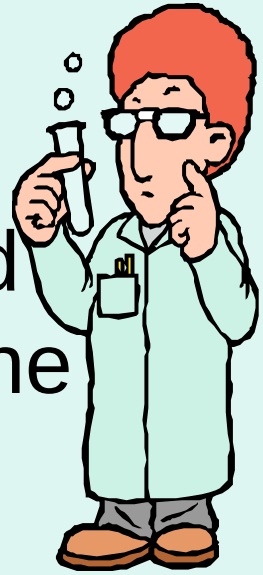
- DNA-based methods have shown excellent sensitivity and specificity

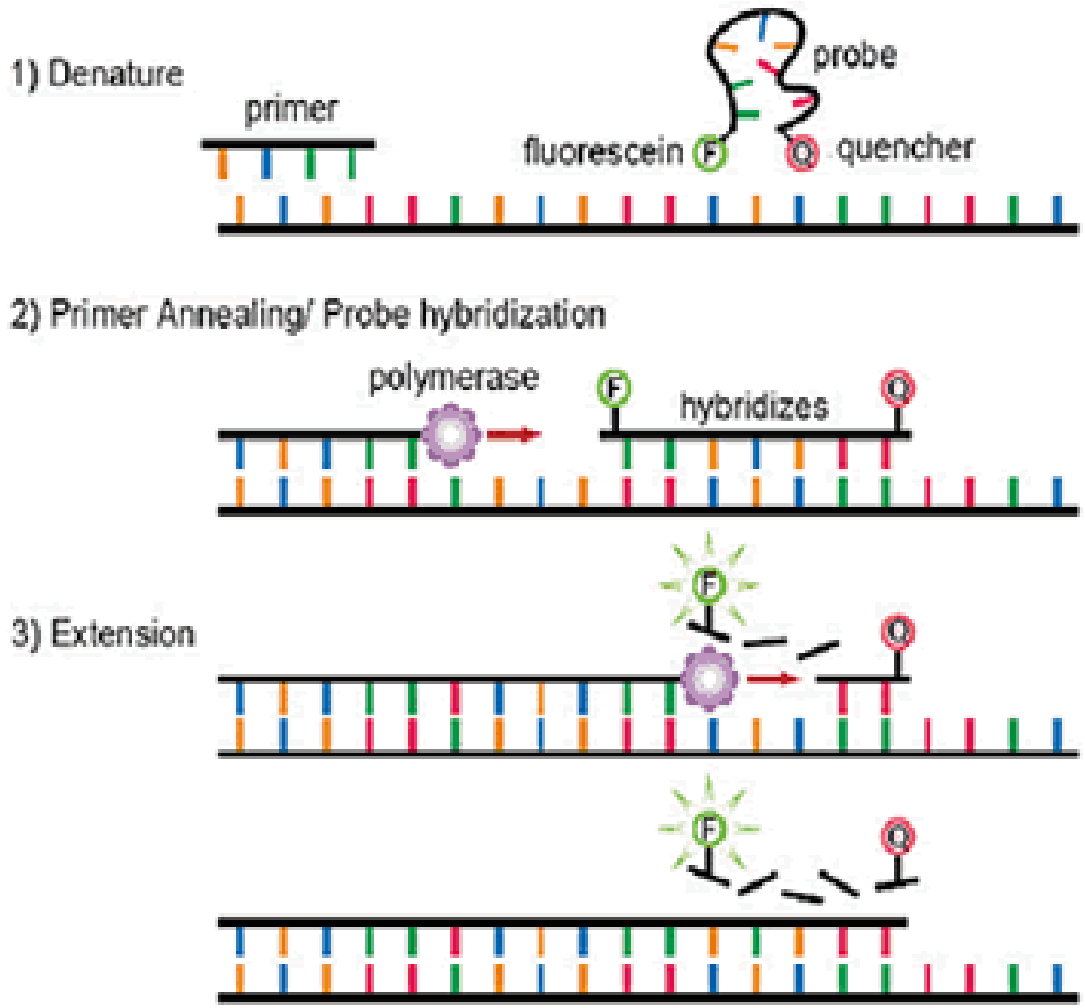
B-Real-time PCR (RT-PCR)

- **Principle**:

This method utilizes an additional primer, the probe, which also binds specifically to the target DNA sequence. Probes have a fluorescent 'reporter' dye at one end and a 'quencher' dye, which inhibits fluorescence at the other. During the extension stage the probe is broken apart by the DNA-polymerase and begins to fluoresce more strongly.

- The fluorescence can be measured at each cycle and increases in proportion to the number of target sequence copies produced. This means that the assay can be made quantitative by recording the cycle at which the fluorescence intensity rises above the background level for each test sample and for a set of standards run at the same time. A standard curve can then be drawn and the amount of target DNA present in the sample can be calculated from the standard curve.





TaqMan® Probe Method

Advantage

- Quantitation.
- This eliminates gel electrophoresis thereby greatly reducing the risk of contamination and the introduction of false-positives

Disadvantage

- It requires expensive equipment.
- Specialized personnel for analysis of the results

C-loop-mediated isothermal amplification (LAMP)

- Principle:

- The target sequence is amplified at a constant temperature of 60 - 65 °C using 4 sets of primers and a polymerase.

- Amplification results in visible turbidity due to precipitation, which allows easy visualization by the naked eye,

Advantage

- Results in higher yields
- Eliminates the need to buy a thermal cycler
- Shortens the reaction time by eliminating time lost during thermal changes.

The future adoption of LAMP as a diagnostic tool for parasite infections in rural endemic regions shows promise

Application :

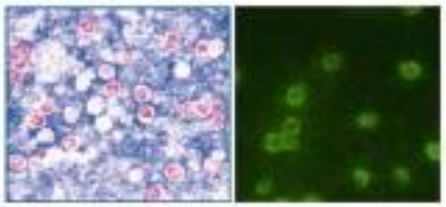
E. histolytica

G. lamblia

Cryptosporidium

Schistosoma mansoni

Summary



Staining method
-Ziehl Neelsen
-Malachite Green
-Immunofluorescence
-Immuno/DAPI

Microscopy

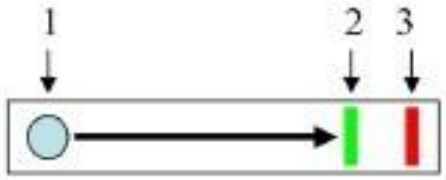
A

B

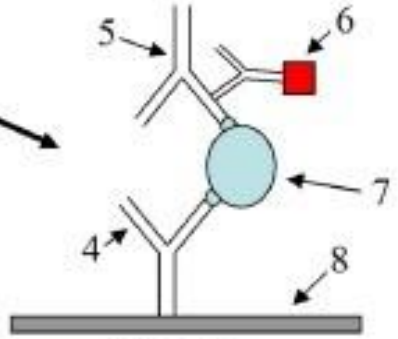
C

D

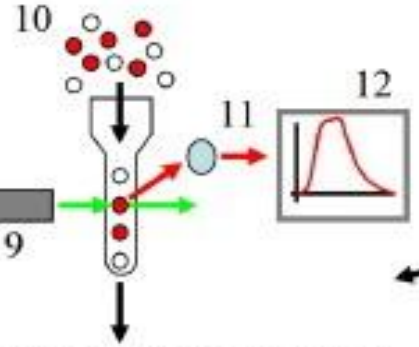
INTESTINAL PARASITES



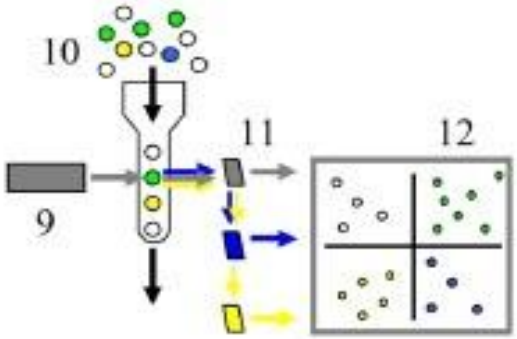
Immunochromatography dip-stick or cartridge



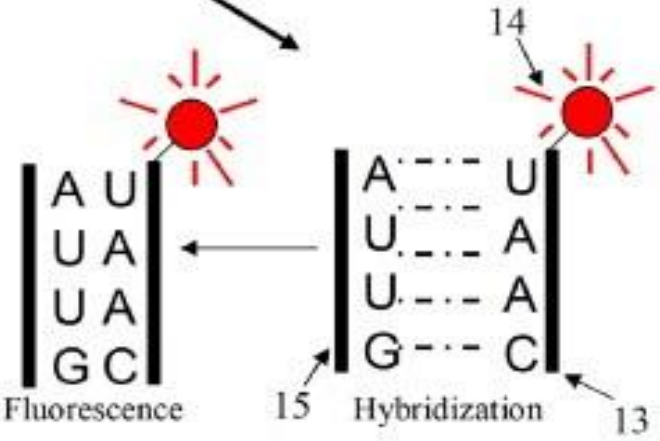
Enzyme linked Immunoassay



One colour" flow cytometry



"Two colour" flow cytometry



Fluorescence

Hybridization


Thank You
Терима касих

Any Question?