



Diagnostic Parasitology

It is important unit or divisions of
Clinical Laboratory Science

Clinical Laboratory Science

Clinical Laboratory Science, also called **Medical Technology**, is the health profession that provides laboratory information and services needed for the diagnosis and treatment of disease.

Clinical Laboratory Scientists

1-perform a variety of laboratory tests, **ensure the quality of the test results,**

2-explain the significance of laboratory tests,

3-evaluate new methods and study the

effectiveness of laboratory tests.

Diagnostic Parasitology Important ?

- The laboratory of diagnostic parasitology is **not authorized** to order tests; this function is the **responsibility of the examiner** **.and** depending on the **patient's history**,
- **Although more specific diagnostic tests are recommended but suggestion or designs depended on examiners experience**
- It is very important that **examiner** **become familiar with the test order** options available from the laboratory testing menu.
- They must also have an **understanding of the pros and cons of each test** when considered within the context of **the patient's symptoms and clinical** history.
- Without the appropriate test orders and collection procedures, test results may be **misleading or even incorrect**.

Laboratory Safety Rules

Laboratory should be designed in such a way that you can work safely:

1. it must be easily cleaned.
2. contains a sink for washing.
3. Illumination is adequate for all laboratory activities.
- 4-**Must be considered all body fluids as a potentially infectious materials.**
- 5-A lab coat and gloves should be worn during laboratory work.

Note to remove gloves before using telephone

Do not eat food, drink, or chew gum in the laboratory.

Do not use **laboratory glassware as containers for food or drinks.**

Work areas should be kept clean and tidy at all times.

Do not place **contaminated pipettes on the bench top.**

Keep hands away from face, eyes, mouth, and body while using chemicals or lab equipment. **Wash your hands with soap and water after performing all experiments.**

Never use mouth suction for chemicals or body fluids Keep fingers, pencils, loops etc. out of your mouth. Perform adequate sterilization before washing or disposing waste.

Summary: Collection of Fresh Stool Specimens

1. **Occupational Safety and Health Act regulations (Standard Precautions)** should be used **for handling all specimens.**

2. Interfering substances (e.g., mineral oil, or antibiotics) should be avoided when stool specimens are collected.

3. Contamination with urine or water should be avoided.

4. **Recommendation** for collection: two (minimum) or three specimens collected, one every other day or within a 10-day time frame; .

5. Liquid stool should be examined or preserved within 30 **min of passage (trophozoites).**

Soft stool should be examined or preserved within 1 h of passage (trophozoites and cysts*).

Formed stool should be examined or preserved within 24 h

6. Fresh or frozen fecal specimens are required for the following fecal **immunoassays (either as a single-organism test or combined with other organisms such as *G. lamblia* or *Cryptosporidium* spp.): *E. histolytica*/*E. dispar* group and *E. histolytica*).** *Dientamoeba fragilis* trophozoites can be found in

Parasites and parasitism

- **PARASITE** - live organism living in or on, and having some metabolic dependence on another organism known as a **host**
- **PARASITISM** - a relationship in which one of the participants, the parasite, either harms its host or in some sense lives at the expense of the host

- **Protozoa:** unicellular organisms, e.g. *Plasmodium* (malaria)
- **Metazoa:** multicellular organisms, e.g. helminths (worms) and arthropods (ticks, lice)
- **An endoparasite:** “a parasite that lives within another living organism” e.g. malaria, *Giardia*
- **An ectoparasite:** “a parasite that lives on the external surface of another living organism” e.g. lice, ticks

Parasites

→ according to which site they inhabit

- **Intestinal and urogenital parasites**
(protozoa and/or helminths)
- **Tissue and blood parasites**
(protozoa and/or helminths)

- **A-Intestinal & urogenital parasitic protozoa**
- 1-Ameboids (*Entamoeba histolytica* / *E. coli* / *endolimax nana* / *iodamoeba buetschlii* /
- 2- Flagellat *Giardia lamblia* .*Trichomonas* Spp.
- 3- ciliate / *Balantidium coli*.
- **B-Intestinal helminthes**
- 1- flukes (*Fasciolopsis Buski* / *Heterophyes heterophyes* (Intestinal *Schistosomes* spp.))
- 2- **Cestoda** (*Taenia* spp. / / *hymenolepis* spp. / *Dipylidium caninum* / *Diphyllobothrium*)
- 3- **Nematodes** (*Enterobius vermicularis* / *Trichuris trichiura* / *Ascaris lumbricoides* / *Necator americanus* *Ancylostoma duodenale* / *Strongyloides stercoralis* /

A- Blood and tissue protozoa

Free living ameba's *Negleria fawcarii* *Acanthamoeba* spp.

Flagellates *Trypanosoma* Spp., *Leishmania* SPP.,
sporozoa / *Plasmodium* spp. / *Toxoplasma* / *Cryptosporidium*
Spp

Blood and tissue helminthes

- blood flukes (*Schistosomes hematobium.*)

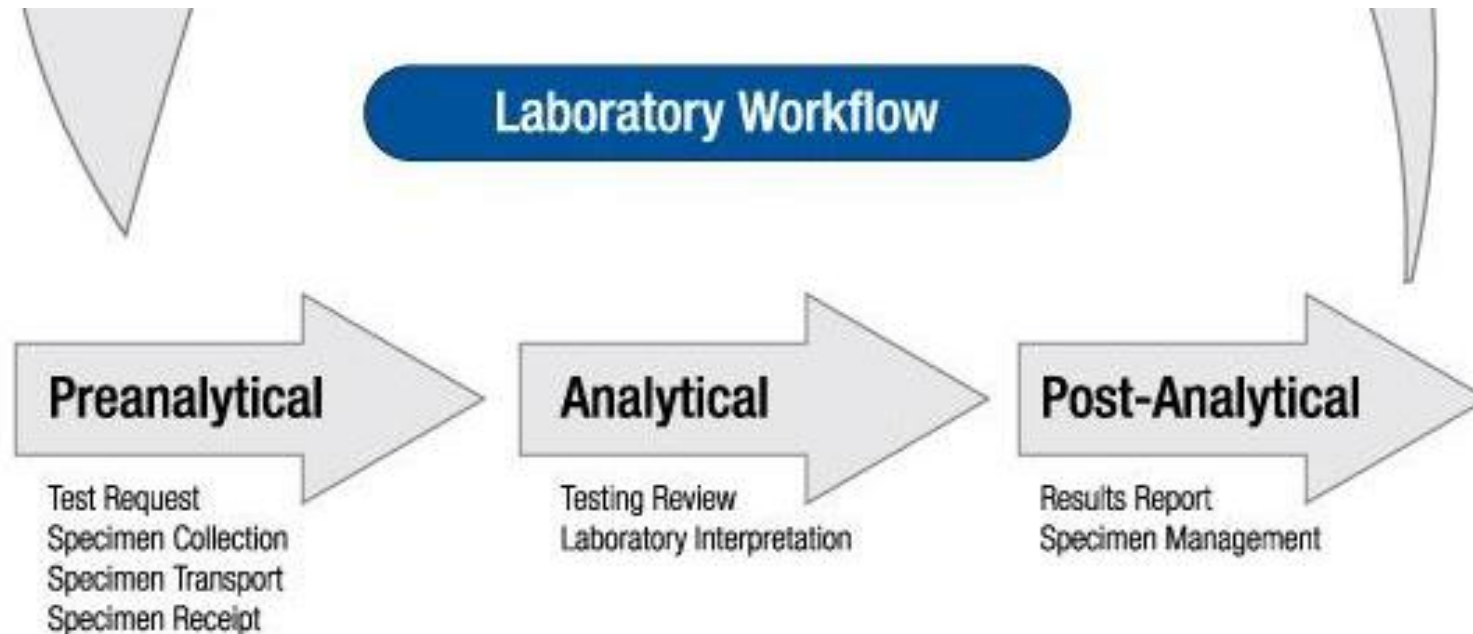
- liver flukes (*Fasciola* spp.) / *Clonorchis sinensis*

3- lung flukes (*Paragonimus*)

Cestodes echinococcus

Filarial worm , *Wuchereria bancrofti* / *Loa loa* , *Onchocerca*
volvulus , *Dracunculus medinensis*

Laboratory Workflow



Quality Systems

Organization
Personnel
Equipment
Purchasing/Inventory

Process Control
Documents/Records
Information Management
Laboratory Information System

Internal Assessment
Process Improvement
Service/Satisfaction
Facilities and Safety

Specimens

Stool

- ❖ **Blood (Serum and plasma)**
- ❖ **Others (anal swab, duodenal aspirate, sputum, urine, urogenital specimen , Skin test)**
- ❖ **Tissues and aspirates**

Examination of the stool sample:

Macro examination:

- **Mucoid blood stained** (acute amoebic dysentery or Giardiasis),
- Parasites can be detected (Adult stage of nematodes, cestodes)

Microscopic examination:

- Saline mount
- Iodine Mount
- Thick smears – not commonly used
- **Permanent stained smears**
 - Iron hematoxyline
 - Whearley's tri chrome stain
- Concentration methods
 - Floatation techniques
 - Sedimentation techniques
- Antigen detection
- Molecular diagnosis

Microscopic examination

Direct wet mount:

Thin emulsion of small amount of faeces

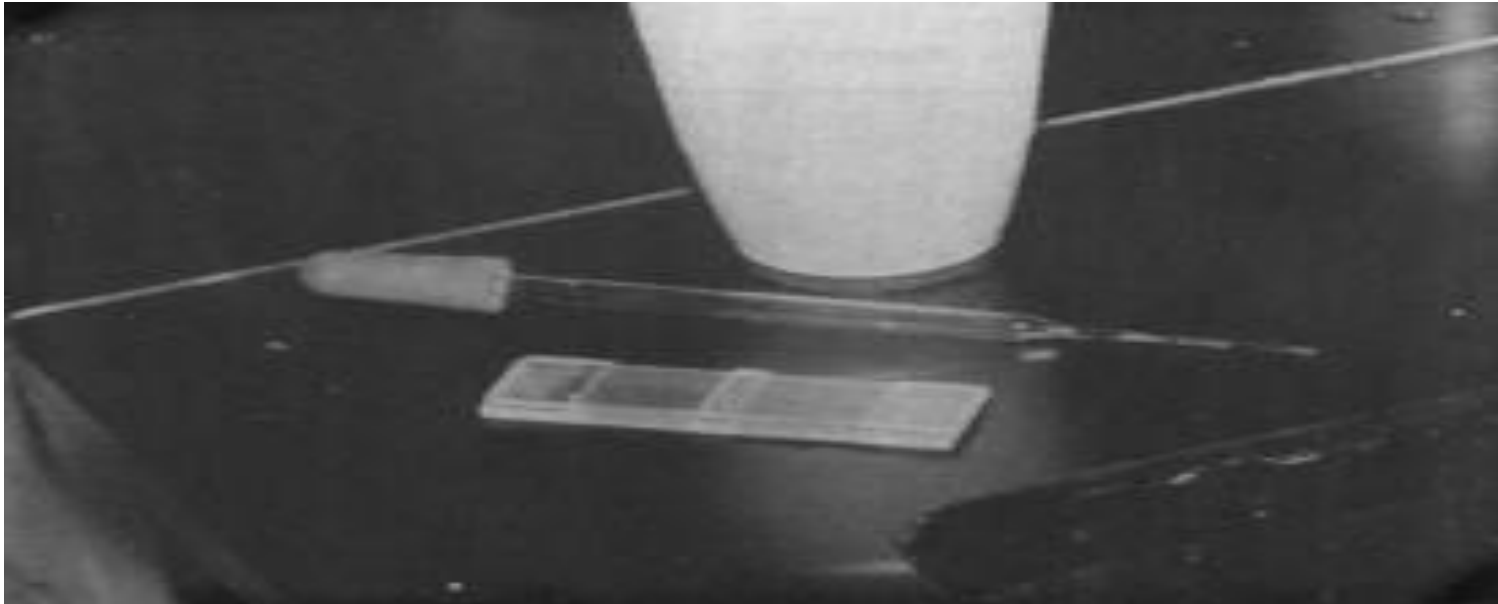
Few drops of saline

- Some times add lugol's iodine (nuclear details, glycogen vacuole in cyst)
- Protozoa (trophozoite), cyst, eggs and larva of helminths, and crystals
- Not, the major problem in stool examination summarized in Un known material like indigestive food particle ,fatty drops and fungi spatially that shape like some stage of parasite

Concentration methods

- What we are doing in **Scanty parasites in the sample**
- **Floatation** (eggs and cyst float , solution of high specific gravity)
 1. saturated sodium chloride (helminthic eggs ex.ascaris , hookworms)
 2. Zinc sulphate centrifugation floatation (cyst).
- **Sedimentation** (solution of low specific gravity):
 - formol ether
 - Egg count in 1 gram .

Stall's technique for counting helminth egg



3 gm stool and 42 ml water

0.15 ml on slid

Multiply result in 100

Number in 1 gm

Molecular diagnosis

(using stool sample)

If an unequivocal identification of the **parasite** can not be made, the stool specimen can be analyzed using molecular techniques such as **polymerase chain reaction (PCR)**. PCR amplified fragments can be analyzed by using **restriction fragment length polymorphisms (RFLP)** or **DNA sequencing** if further characterization is needed.

- *A- Blood and tissue protozoa*
- Free living ameba's *Negleria fawcarii* *Acanthamoeba* spp.
- Flagellates *Trypanosoma* Spp., *Leishmania* SPP. ,
- sporozoa / *Plasmodium* spp. / *Toxoplasma* / *Cryptosporidium* Spp
- *Blood and tissue helminthes*

1-blood flukes (*Schistosomes hematobium.*)

2- liver flukes (*Fasciola* spp.) / *Clonorchis sinensis*

- 3- lung flukes (*Paragonimus*)

- *Cestodes echinococcus*

- *Filarial worm* , *Wuchereria bancrofti* / *Loa loa* , *Onchocerca volvulus*

Dracunculus medinensis

Blood examination

- Fresh capillary blood of finger or ear lobe
- Venous blood collected in EDTA (anticoagulant)

Blood sample will be used for :

- **Microscopic examination(Thin Smear, Thick smear, Wet mount for microfilaria).**
- **Molecular diagnosis**
- **Detection of parasite antigen**
- **Isolation of organisms**
- **Special tests**

Stopper color	Additive	Uses
Red	None	Collecting serum sample
Purple	EDTA	Collecting plasma sample
Blue	Sodium citrate	PT & PTT tube
Black	Sodium citrate	ESR tube
Gray	Sodium fluoride	Blood sugar tube
Green	Lithium heparin	Collecting heparinated blood
Gold	None	Serum separator tube (SST) contains a gel at the bottom to separate blood from serum

Note Therapeutic drug monitoring specimens should not be collected in tubes that contain gel separators as some gels absorb certain drugs causing a falsely lowered result.



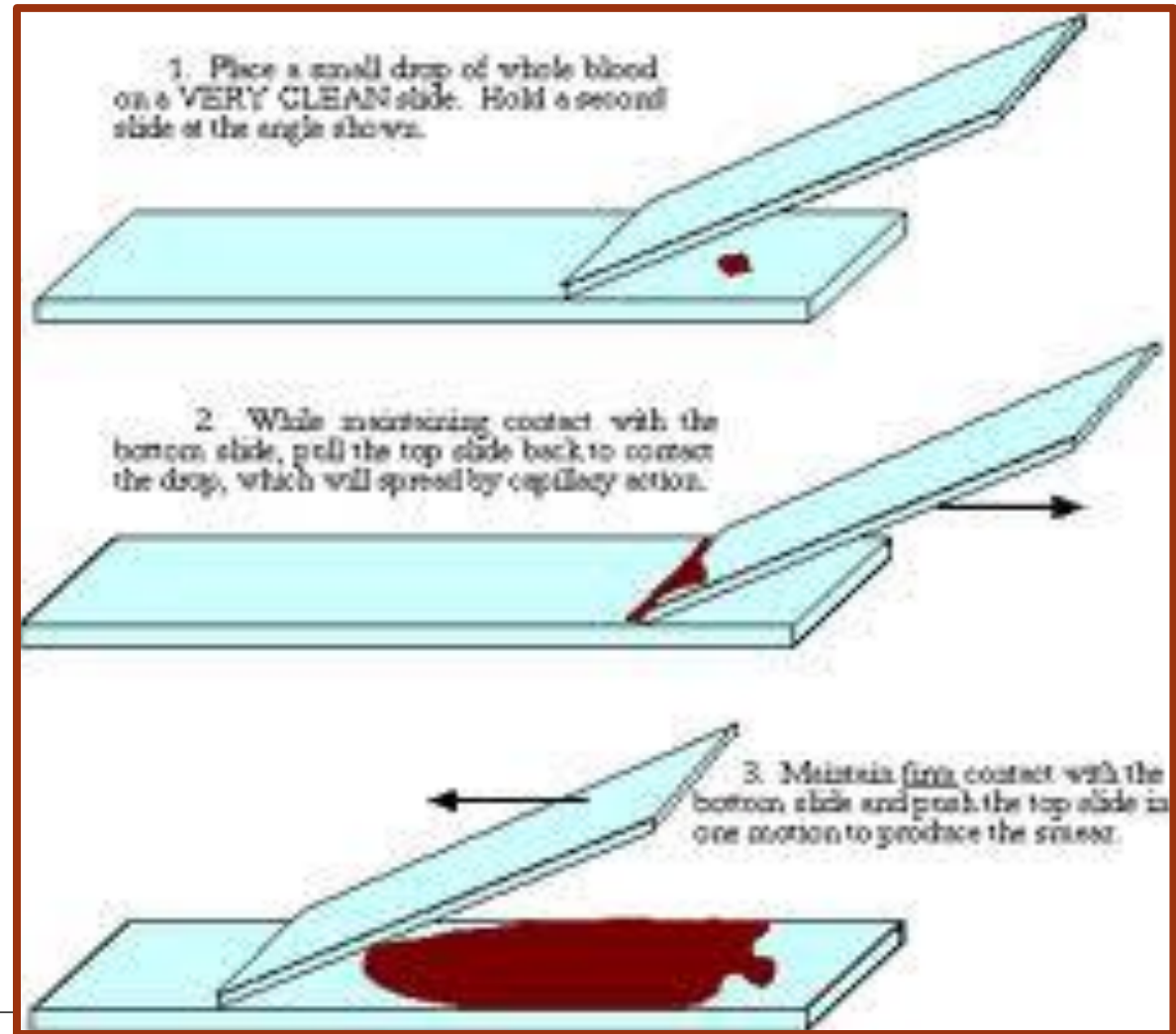
Thick blood film

- Screen large amount of blood (light infection)
- Can be stained latter



Thin blood film

In malaria Parasitized red blood cells and parasites
Definite species identification



Molecular diagnosis

(using blood sample)

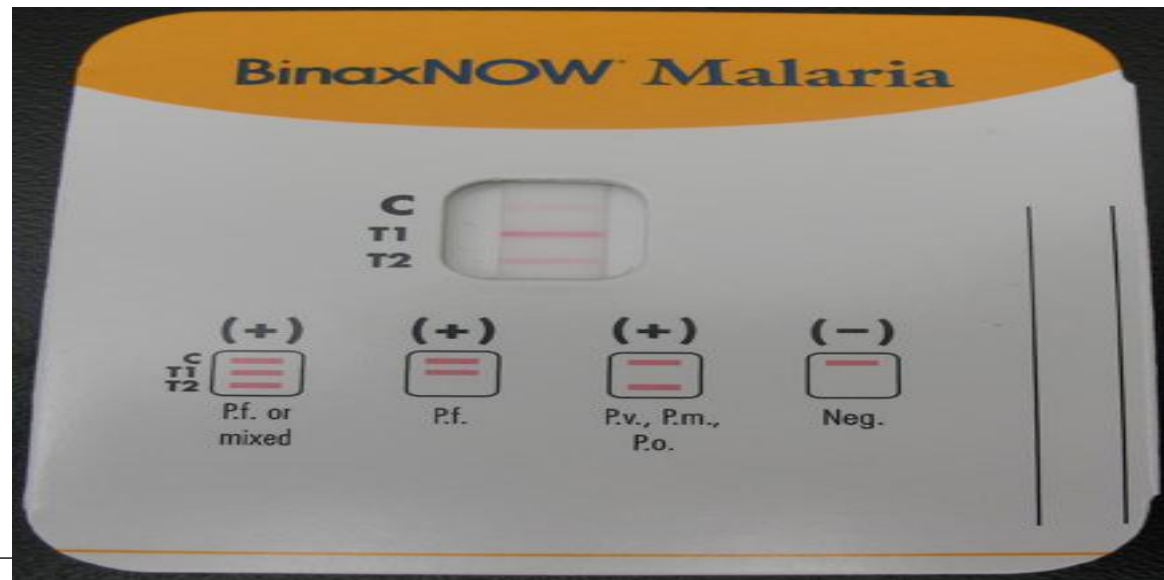
- Collect a 1-5 ml blood sample in tube with EDTA.
- Blood can be collected on filter papers (e.g Whatman)
- DNA is extracted using DNA extraction kits

Species-specific diagnosis of malaria

Detection and speciation of *Plasmodium* is done with a two step nested PCR using the primers of Snounou et al 1993.

Detection of parasite antigens (in blood sample)

- Rapid diagnostic tests for malaria employing **immunochromatographic** methods based on the detection of malarial antigens present in peripheral blood.
- only diagnose only *P. falciparum* malaria.
- Currently, the only available RDT for malaria in the United States is the **BinaxNOW® Malaria Test**.



Isolation of Organisms

(from blood)

- ❑ The diagnosis of *Leishmania* spp. is made by microscopic identification of the **nonmotile, intracellular form (amastigote)** in stained sections from lesions, and by **culture of the motile, extracellular form (promastigote)** on suitable media.
- ❑ Slides should be fixed and stained before they are sent unless reagents are not available.
- ❑ Serologic tests are also available to detect for **anti-leishmanial antibodies**; however, these tests are often **not sensitive**, particularly for diagnosing **cutaneous leishmaniasis**.

Special Tests: MQ Testing

- **Mefloquine** is recommended by CDC as a prophylactic against malaria.
- When individuals who are on mefloquine prophylaxis exhibit signs of malaria, blood samples are collected and analyzed for the presence of the drug.
- The drug is extracted from the blood and the concentration is determined by high-performance liquid chromatographic (HPLC) methods.
- Determining the level of mefloquine in the blood helps assess if the individual was adherent with his/her medication.
- This procedure is also useful to determine treatment failure due to a mefloquine resistant form of malaria.

Serum, plasma and others Specimen Requirements

○Serum/plasma is required for all parasitic disease **immunodiagnostic** tests.

○CSF and eye fluids (vitreous or aqueous) are acceptable for selected diseases Exp **Amoebic meningitis & toxocariasis**

- **Serology – All tests available**

- IHA
- ELISA
- CIEP counterimmunoelectrophoresis (CIEP)
- IF

- **More useful in**

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

Skin Tests Casoni's test (Leishmanin test)

Cultivation of parasites

Culture methods are used for :

Amoeba

- **Leishmania**
- **Trypanosoma**
- **Malarial parasite**

Animal inoculation

- **Leishmania** (young hamster)
- **Trypanosomes** (rat, mouse)
- **Toxoplasma** (all lab animals)

Xenodiagnosis: In chagas' disease and malarialiasis

(Vector infected experimentally)

Sputum examination

Microscopic examination of sputum can identify:

- *Paragonimus westermani* eggs
- *Strongyloides stercoralis* larva
- *Ascaris lumbricoides* larvae
- hookworm larvae, and rarely *Entamoeba histolytica*.
- ❑ Sputum should be obtained from the lower respiratory passages not saliva.
- ❑ Sputum specimens should be collected first thing in the morning.

Vaginal swabs

▪ Demonstration of *Trichomonas vaginalis* trophozoites is usually done by preparing wet mounts made from vaginal swabs or scrapings.



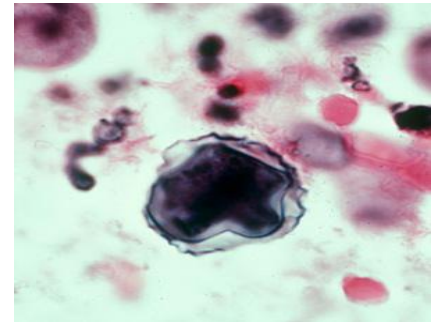
▪ If the specimen cannot be examined immediately, it should be preserved in PVA and stained smears examined later.

Commercially available tests for detection of *Trichomonas*

Organism	Kit name	Manufacturer - distributor ^a	Type of Test ^b
<i>Trichomonas vaginalis</i>	T. vag	Chemicon	DFA
	Quik-Trich	PanBio	LA

Tissue Specimens for Free-living Amebae (FLAs)

Tissue specimens, including biopsy, surgical or necropsy specimens, may be collected for the detection of free-living amebae (*Naegleria*, *Balamuthia*, and *Acanthamoeba*).



The desired specimens include:

Tissue slides stained with hematoxylin and eosin (H&E).

- **Unstained slides (for indirect Immunofluorescence, or IIF).**
- **Unfixed brain tissue or CSF for PCR.**
- **Unfixed corneal scrapings (for *Acanthamoeba*).**
- **Paraffin-embedded tissue block.**

Cellulose Tape or Swube Tube Procedure for Demonstration of Pinworm Eggs

The most reliable and widely used technique for demonstrating pinworm eggs (*Enterobius vermicularis*) is the cellulose tape or swube tube procedure.

- The adhesive part of the swube tube or tape is applied to the perianal area first thing in the morning.
- Specimens should be collected on three consecutive mornings prior to bathing.
- If an infection is present, eggs and sometimes adult worms of *Enterobius vermicularis* will be present on the tape and can be seen under the microscope



E. Vermicula

Urine Specimens



Urinary schistosomiasis

- Presence of *S. haematobium* eggs in urine is diagnostic for
- Eggs usually shed in the urine around midday, so an optimum urine specimen for diagnosis should be collected at noon.
- The specimen should be immediately centrifuged at $400 \times g$ and the sediment examined by wet mount.

Trichomonas vaginalis

- Motile trophozoites may also be found in the urine, especially in infected male patients.
- Urine specimen should be centrifuged at $400 \times g$, the sediment mixed with a drop or two of saline, and examined by wet mount.





**THANK YOU FOR LISTEN,
ARE YOU WISH TO VISIT THIS LAKE**