

**Tishik University- Nursing**

**Medical Parasitology**

**lec.11-12**

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**parasite** is an organism that lives in or on another organism, called *the host*, and often harms it.

Parasitism: is a non-mutual relationship between species, where one species, the parasite, benefits at the expense of the other, the host.



## Parasite occur as

- **Protozoa:** unicellular organisms, e.g. Plasmodium (malaria)
- **Metazoa:** multicellular organisms, e.g. helminths (worms)
- **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

# Examples of important intestinal protozoa

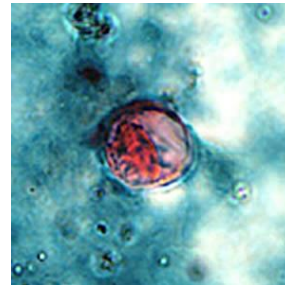
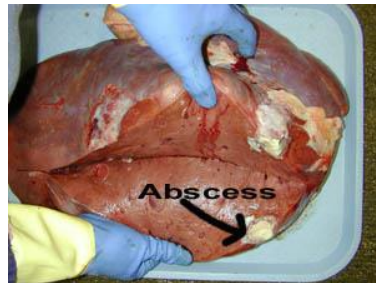
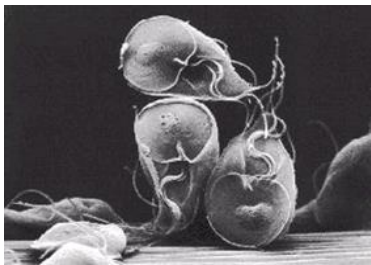
Transmitted by the faecal-oral route and cause diarrhoea

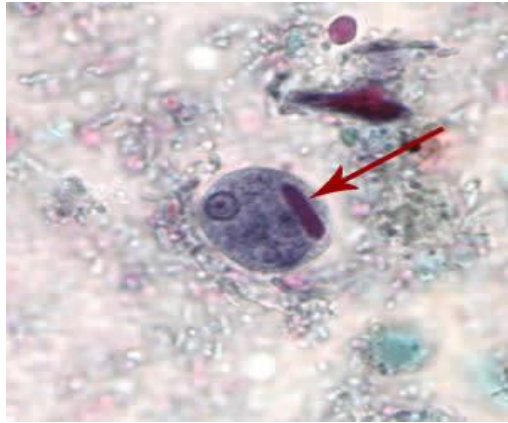
*Giardia lamblia*: world-wide distribution, lives in the small intestine and results in malabsorption

*Entamoeba histolytica*: may invade the colon and cause bloody diarrhoea – amoebic dysentery. Also causes amoebic liver abscess.

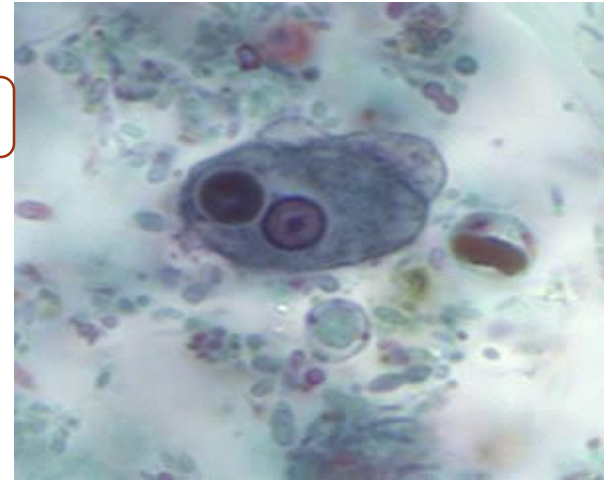
*Cyclospora* : parasitizes the small intestinal mucosa and may cause diarrhoea for several weeks

*Balantidium coli*: a large motile ciliated parasite that lives in the colon of pigs, humans and rodents and can lead to colonic ulceration



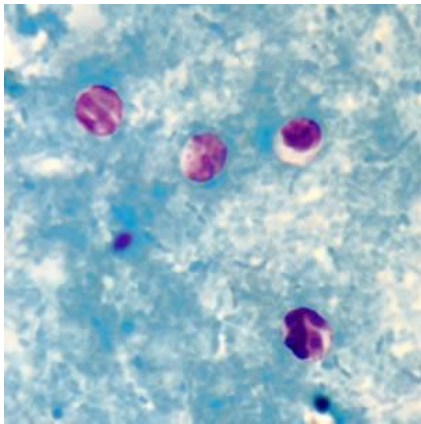


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Cyst of *E. histolytica* stained with trichrome. Note the chromatoid body with blunt ends (red arrow)

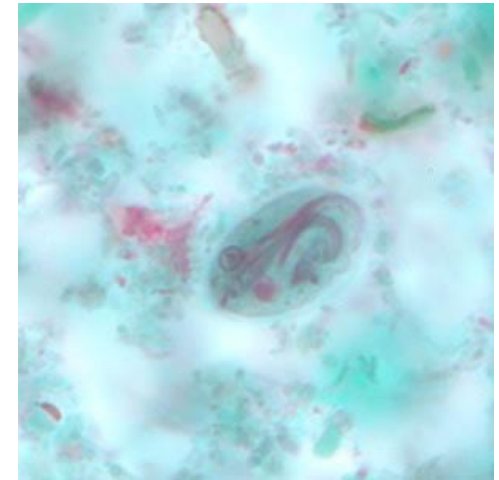
Trophozoites of *E. histolytica* with ingested erythrocytes stained with trichrome.



*Cryptosporidium* sp. oocysts stained with modified acid-fast.



*G. duodenalis* trophozoite stained with trichrome

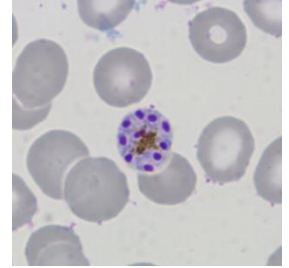


*G. duodenalis* cyst stained with trichrome.

# Examples of important systemic protozoa

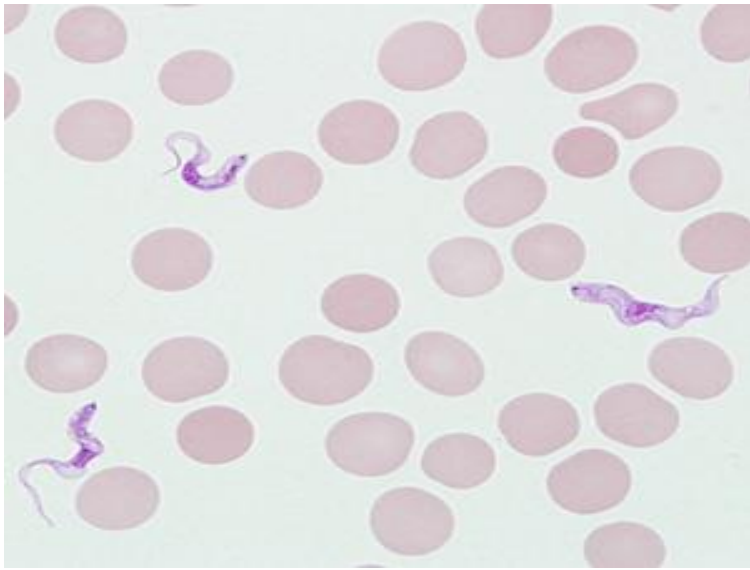
## Detected in the blood

- *Plasmodium*: the cause of malaria *P. malariae*
- *Toxoplasma gondi*: transmitted by the ingestion of oocysts from cat faeces. Infection can lead to ocular problems and is also a cause of neonatal toxoplasmosis

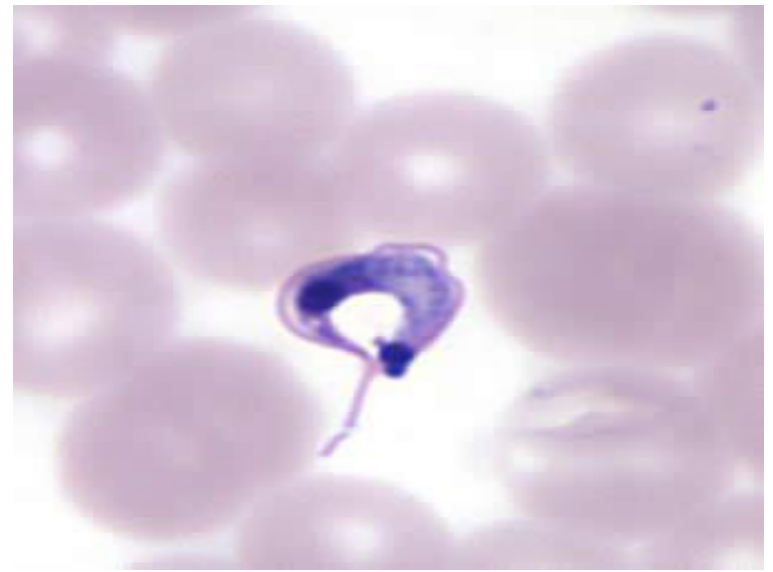


- *Trypanosoma* cause
  - In Africa - sleeping sickness (transmitted by the Tsetse fly)
  - In South America - Chagas disease (transmitted by the Reduviid bug)

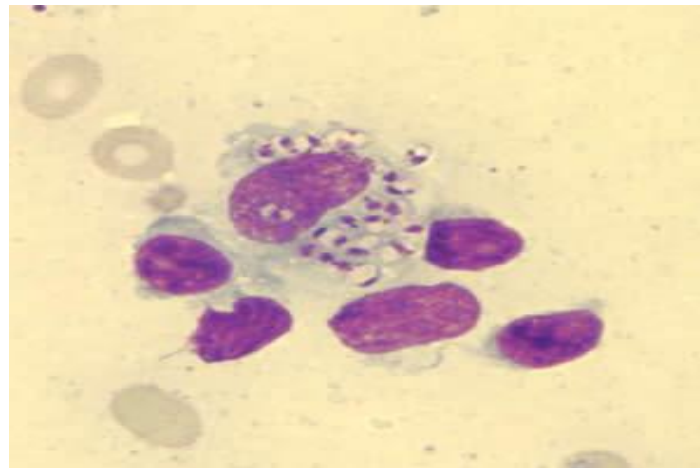




*Trypanosoma brucei* sp. in a thin blood smear stained with Giemsa.



*T. cruzi* trypomastigote in a thin blood smear stained with Giemsa.



*Leishmania* sp. amastigotes in a Giemsa-stained tissue scraping



# Examples of important intestinal nematodes

## *Trichuris* (whipworm)

- A soil transmitted helminth
- prevalent in warm, humid conditions
- Can cause diarrhoea and anaemia in heavily-infected people



## *Enterobius* (pinworm)

prevalent in cold and temperate climates  
but rare in the tropics  
found mainly in children

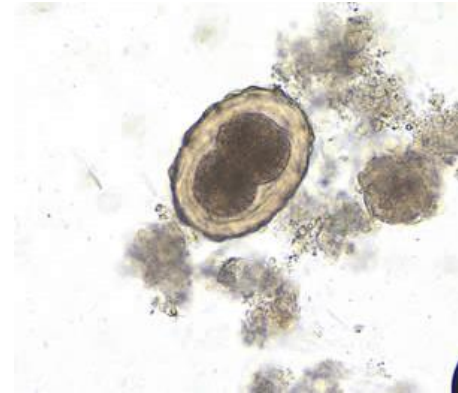


## *Ascaris* (roundworm)

- Found world-wide in conditions of poor hygiene, transmitted by the faecal-oral route
- Adult worms live in the small intestine
- Causes eosinophilia

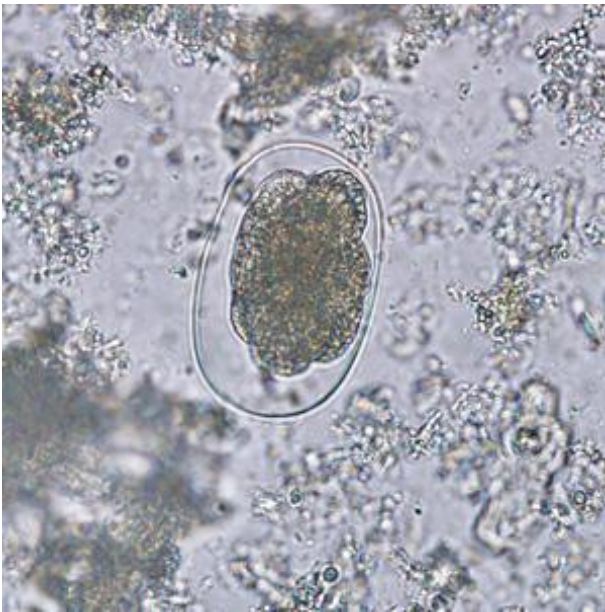






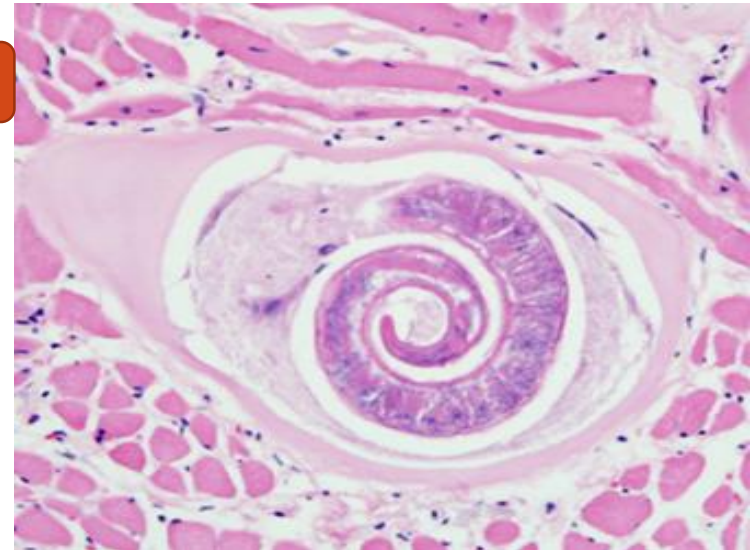
Adult female *A. lumbricoides*. Unfertilized egg of *A. lumbricoides*

Fertilized egg



Hookworm egg in an unstained wet mount

information



*Trichinella* larva in tongue muscle of a rat, stained with hematoxylin and eosin

# Examples of important systemic nematodes

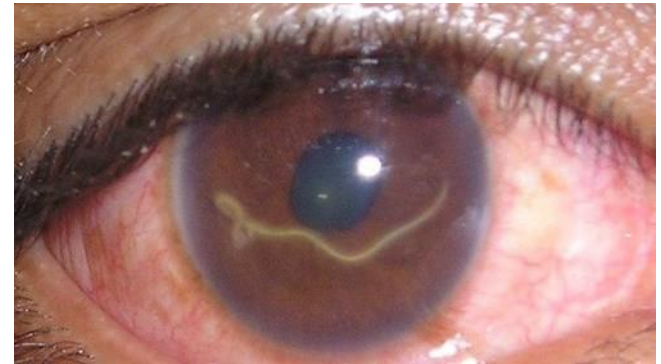
## Filaria worms including:

- *Onchocerca*
- Transmitted by the simulium black fly, this microfilarial parasite can cause visual impairment, blindness and severe itching of the skin in those infected

*Onchocerca volvulus*



- *Toxocara*
- A world-wide infection of dogs and cats
- Human infection occurs when embryonated eggs are ingested from dog or cat faeces
- It is common in children and can cause visceral larva migrans (VLM)



# Examples of important flatworms : cestodes

## Intestinal : (“tapeworms”)

### *Taenia saginata*

- worldwide
- acquired by ingestion of contaminated, uncooked beef
- a common infection but causes minimal symptoms

### *Taenia solium*

- worldwide
- acquired by ingestion of contaminated, uncooked pork that contains cysticerci
- Less common, but causes cysticercosis – a systemic disease where cysticerci encyst in muscles and in the brain – may lead to epilepsy

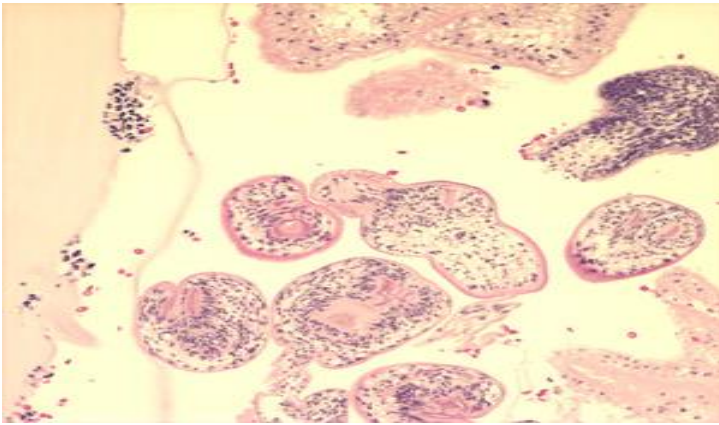
*Taenia saginata*



*Taenia solium*







Protoscolexes in a hydatid cyst removed from lung tissue, stained with hematoxylin and eosin (H&E).



*Taenia* sp. egg in unstained wet mounts

information



Proglottid of *T. saginata* injected with India Ink

# Diagnosis of Parasitic Infections

1. Clinical
2. Laboratory

## **Purpose of laboratory diagnosis :**

- Confirmation of clinical suspicion
- Identification of unsuspected infection

# Specimens

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

# Stool examination

## Sample collection:

- Sample is collected in clean, dry container
- Handled carefully
- Sometimes use preservative (10% formalin)
- Samples in some cases fresh (amoeba, ciliates)
- Liquid and soft stool examined within 15 min
- **Not mixed with urine or disinfectant (as they will kill trophozoites)**
- Specimens obtained by enema or laxatives are often positive for worm eggs or adult worm.



# Examination of the stool sample:

## Gross examination:

- Mucoid blood stained (acute amoebic dysentery), Parasites can be detected (nematodes, cestodes)

## Microscopic examination:

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

# Microscopic examination

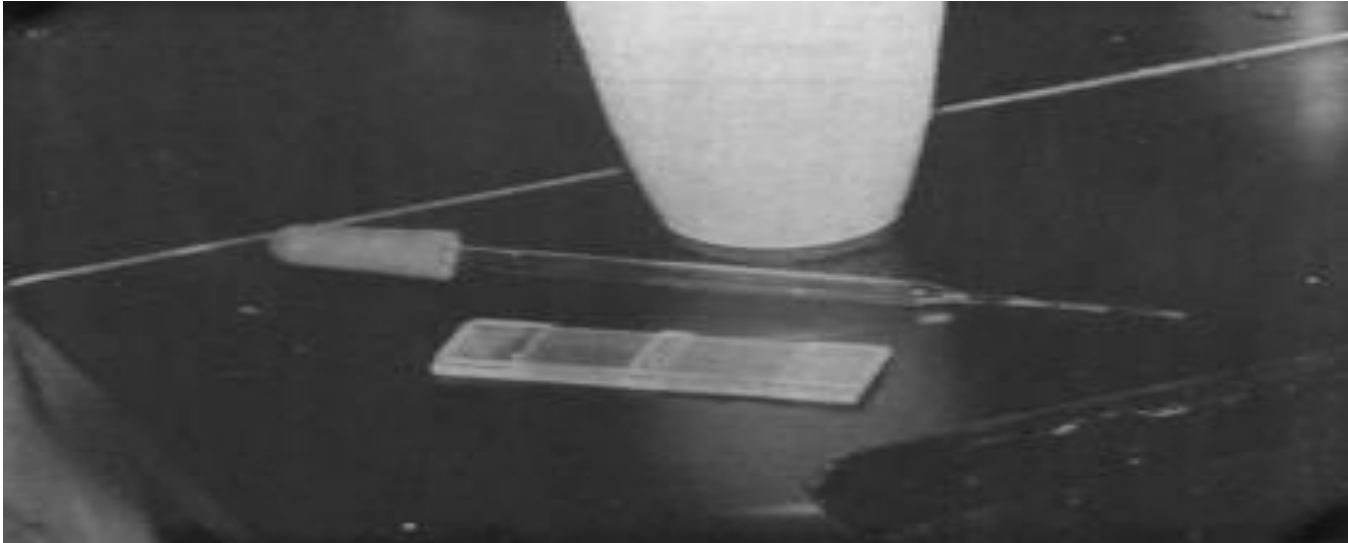
## Direct wet mount:

- Thin emulsion of small amount of faeces
- Few drops of saline
- Sometimes add lugol's iodine (nuclear details, glycogen vacuole in cyst)
- Protozoa (trophozoite), cyst, eggs and larva of helminths, crystals (charcot leyden)

# Concentration methods

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

# Stoll'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

# Immunodiagnostic (antigen detection)

- Fresh or preserved stool samples are the appropriate specimens

## Amebiasis

- **EIA kits** are commercially available for detection of fecal antigens for the diagnosis of intestinal amebiasis.
- These assays use **monoclonal antibodies** that detect the galactose-inhibitable adherence protein in the pathogenic *E. histolytica*.
- Several EIA kits for antigen detection of the *E. histolytica*/*E. Dispar* (*non-pathogenic amoeba*) group are available , but only the **TechLab** kit is specific for *E. histolytica*.

# Cryptosporidiasis

Several kits are combined tests for *Cryptosporidium*, *Giardia*, and *E. Histolytica*.

DFA test identifies oocysts in concentrated or unconcentrated fecal samples by using a fluorescein isothiocyanate (FITC)-labeled monoclonal antibody is the most sensitive.

# Giardiasis

DFA assays may be purchased that employ FITC-labeled monoclonal antibody for detection of *Giardia* cysts.

Organism	Kit name	Manufacturer - distributor <sup>a</sup>	Type of Test <sup>b</sup>
<i>Cryptosporidium</i> spp.	Crypto CELISA	Cellabs	EIA
	PARA-TECT™ Cryptosporidium Antigen 96	Medical Chemical Corporation	EIA
	ProSpecT Rapid	Remel	EIA
	ProSpecT	Remel	EIA
	Cryptosporidium	TechLab	EIA
	Cryptosporidium	Wampole	EIA
	Crypto CEL	Cellabs	IFA
	XPect Crypto	Remel	Rapid
<i>Cryptosporidium</i> spp./ <i>Giardia duodenalis</i>	PARA-TECT™ Cryptosporidium/Giardia DFA 75	Medical Chemical Corporation	DFA
	Merifluor	Meridian	DFA
	ProSpecT	Remel	EIA
	Crypto/Giardia CEL	Cellabs	IFA
	ColorPAC*	Becton Dickinson	Rapid
	ImmunoCard STAT!*	Meridian	Rapid
	XPect	Remel	Rapid
<i>Cryptosporidium</i> spp./ <i>Giardia duodenalis</i> / <i>Entamoeba histolytica</i> / <i>dispar</i>	Triage	BioSite	Rapid
<i>Entamoeba histolytica</i>	Entamoeba CELISA	Cellabs	EIA
	E. histolytica	Wampole	EIA
	E. histolytica II	TechLab	EIA
<i>Entamoeba histolytica</i> / <i>E. dispar</i>	ProSpecT	Remel	EIA
<i>Giardia duodenalis</i>	Giardia CELISA	Cellabs	EIA
	PARA-TECT™ Giardia Antigen 96	Medical Chemical Corporation	EIA
	ProSpecT	Remel	EIA
	Giardia II	TechLab	EIA
	Giardia	Wampole	EIA
	GiardiaEIA	Antibodies, Inc.	EIA
	Giardia CEL	Cellabs	IFA
	ProSpecT	Remel	Rapid
	Simple-Read Giardia	Medical Chemical Corporation	Rapid
	<i>Wuchereria bancrofti</i>	Filariasis CELISA	Cellabs



# 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.

- **Sample :**

Fresh stool should be kept cold or frozen till DNA extraction.

Samples collected in a preservative should be compatible with molecular detection (TotalFix, Unifix, modified PVA (Zn- or Cu-based), and Ecofix)

- **DNA Extraction** better using commercially available kits (Qiagen)

- **PCR analysis:**

### **Conventional PCR:**

DNA is tested by PCR with diagnostic primers. Amplified DNA fragments are electrophoretically resolved on an agarose gel for analysis of results.

### **Real-Time PCR**

The DNA amplification is monitored by measuring the fluorescence signal generated in the reaction vessel. The fluorescence signal is measured every cycle and is proportional to the amount of accumulated PCR product.

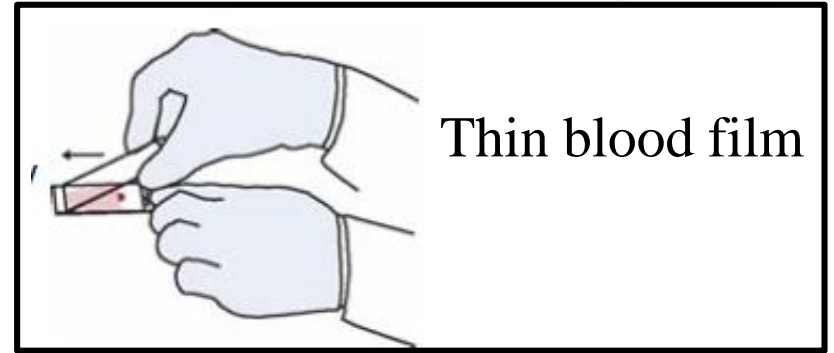
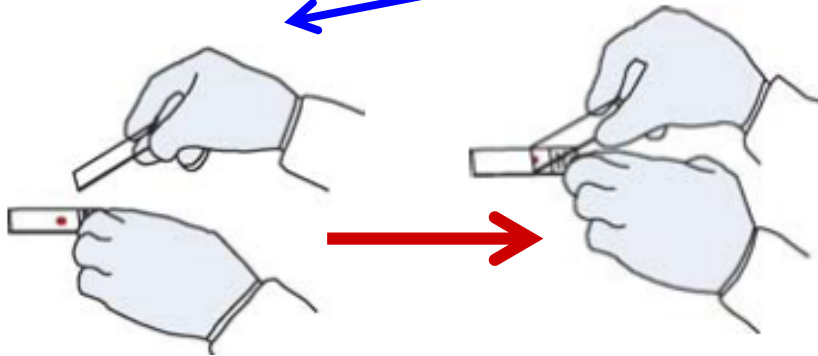
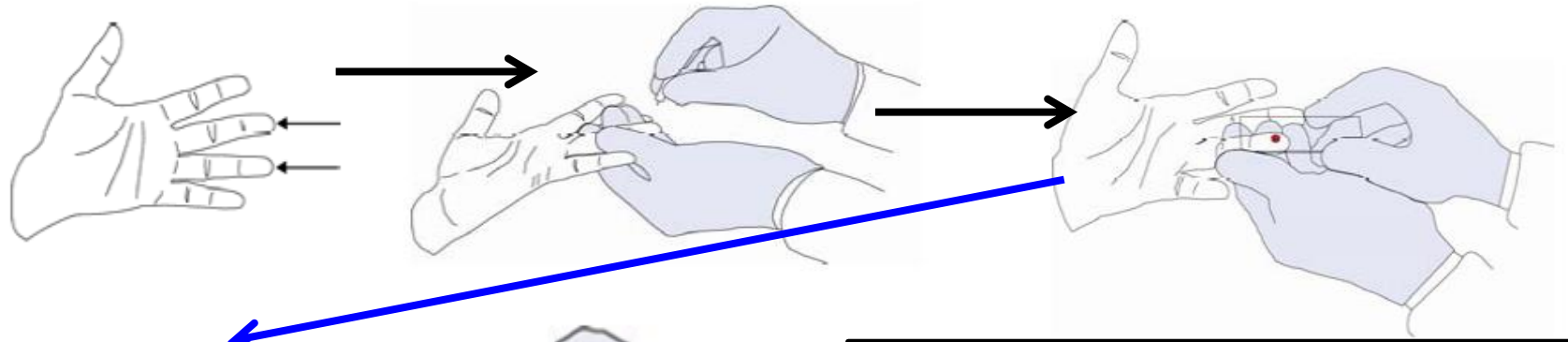
# Blood examination

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

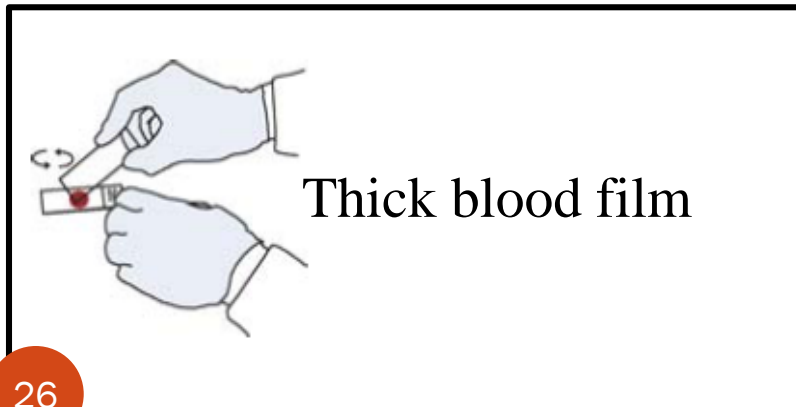
## **Blood sample will be used for :**

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

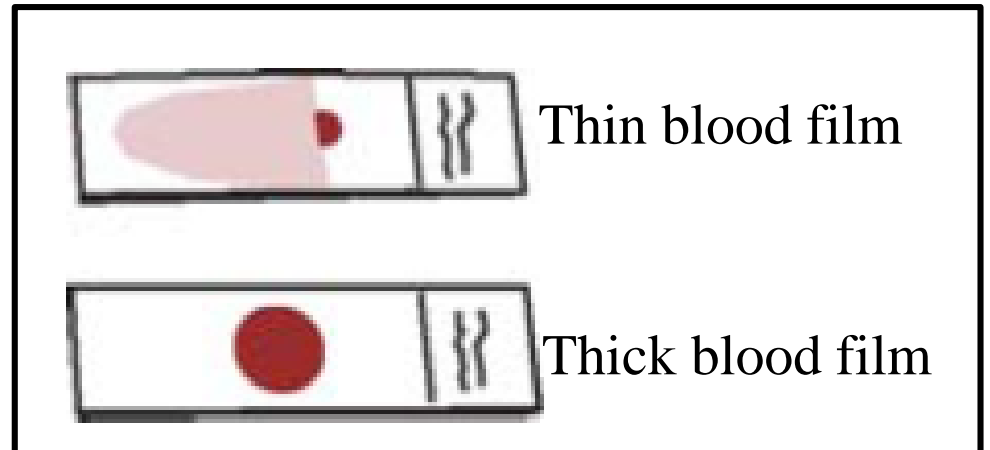
# Blood sample preparation for microscopic examination



Thin blood film



Thick blood film



Thin blood film

Thick blood film

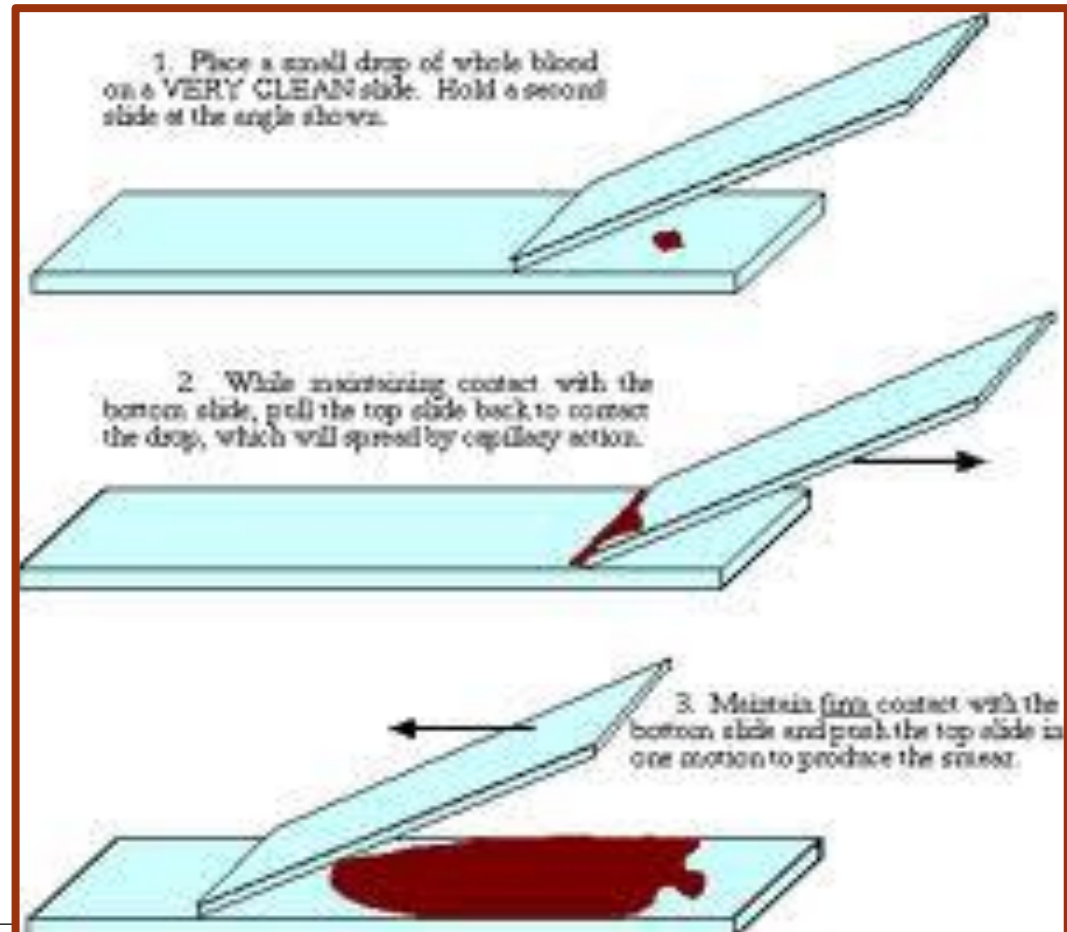
# Thick blood film

- 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



# Microfilaria

- Sample collection according to periodicity of microfilaria
- Concentration by sedimentation or membrane filtration (examine the filter)
- DEC provacation method



Microfilaria of *Wuchereria bancrofti*

# 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

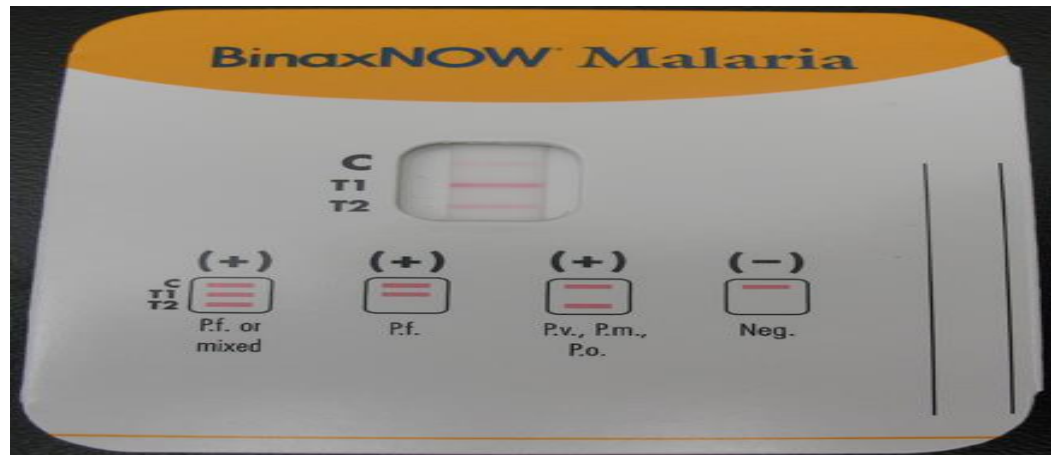
Detection and speciation of *Plasmodium* is done with a two step nested PCR using the primers of Snounou et al 1993.



# 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**.



Organism	Kit name	Manufacturer - distributor <sup>a</sup>	Type of Test <sup>b</sup>
<i>Plasmodium</i>	BinaxNOW® Malaria Test	Inverness Medical	Rapid (HRP2 and aldolase)
	Malaria-Ag	Cellabs	EIA
	OptiMal	Flow	Rapid (LDH)
	MAKROmed malaria test	MAKROmed Manufacturing, LTD	Rapid (HRP2)
	Paracheck Pf	Orchid	Rapid (HRP2)
	Visitect Malaria Pf	Omega Diagnostics LTD	Rapid (HRP2)
<i>Wuchereria bancrofti</i>	ICT Filariasis	Binax	Rapid
	Filariasis Ag-CELISA	Cellabs	EIA

# 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.

## Giardiasis

Giardiasis is caused by the parasite known as *Giardia intestinalis*. This parasite is found in fecal matter, so it can be contracted through oral contact with feces. This can occur if proper hand washing is not done after using the bathroom, changing a baby's diaper or cleaning bathroom fixtures. Drinking water from contaminated sources and accidentally swallowing contaminated swimming water can also cause giardiasis infection. The symptoms of giardiasis include greasy stools, upset stomach, diarrhea, nausea, flatulence and abdominal cramps.

## African Sleeping Sickness

African sleeping sickness is caused by protozoa in the *Trypanosoma* genus. These organisms are carried by the tsetse fly and transmitted to humans via tsetse fly bites. According to the World Health Organization, *Trypanosoma brucei gambiense* causes more than 90 percent of African sleeping sickness cases. *Trypanosoma brucei rhodesiense* is responsible for less than 10 percent of reported cases of the disease. Signs and symptoms of African sleeping sickness can include insomnia, progressive confusion, seizures, irritability, personality changes, weight loss, loss of concentration, slurred speech and difficulty talking and walking.

## Leishmaniasis



Leishmaniasis is caused by the Leishmania parasite, which is found in southern Europe, the subtropics and the tropics. This disease is spread by the bites of sand flies that are infected with the parasite. Cutaneous leishmaniasis affects the skin, while visceral leishmaniasis affects the spleen, liver and other organs. Cutaneous leishmaniasis causes skin sores that can change in size and shape as the disease progresses. Visceral leishmaniasis symptoms include weight loss, fever, liver enlargement, spleen enlargement and abnormal blood counts.

### Toxoplasmosis

Toxoplasmosis is caused by an organism known as Toxoplasma gondii, which the Mayo Clinic cites as one of the most common parasites in the world. While most people with this disease do not have any symptoms, serious complications can occur when toxoplasmosis develops in people with compromised immune systems. Infants born to mothers infected with the disease can also experience serious complications. Toxoplasmosis signs and symptoms are flu-like in nature and include body aches, headache, fatigue, fever, swollen lymph nodes and sore throat.

## Malaria

Malaria is spread by mosquitoes that are infected by the parasites *Plasmodium falciparum*, *P. ovale*, *P. vivax* and *P. malariae*. According to the Centers for Disease Control and Prevention, the United States has approximately 1,300 cases of malaria each year, most of which are related to travel to or immigration from South Asia and sub-Saharan Africa. Malaria signs and symptoms include shaking, headache, chills, fatigue and muscle aches.

## Babesiosis

Babesiosis is caused by the *Babesia* parasite, which is carried by ticks. This disease can also be spread through blood transfusions if a donor has a *Babesia* infection that has not been diagnosed. Babesiosis caused by tick bites usually occurs in New England, New Jersey, New York, Minnesota and Wisconsin. This condition may not cause any signs or symptoms, but some common symptoms of babesiosis include fatigue, nausea, body aches, headache, loss of appetite, chills, fever and sweats. This disease can be life-threatening in people who have weakened immune systems, serious health problems or spleens that do not function properly.