DIAGNOSTIC MICROBIOLOGY

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Which factors should precipitate testing?

- CLINICAL SYMPTOMS
- CONTACT WITH INFECTED INDIVIDUALS
- TRAVEL HISTORY
- IMMUNE STATUS OF THE PATIENT (e.g. compromised patient -increase in the number of patients whose immune systems are compromised through underlying illness, chemotherapy, transplantation)
- DOCUMENTED PREVIOUS INFECTION
- SCREENING (e.g., outbreak situation)

How determine causative agent of the disease?

- **DIRECT OR INDIRECT METHODS**

- **Direct methods** (e.g, microscopy, cultivation of specific nucleid acids, detection of specific antigens) = highly specific and unambigously recommendable, however, in some cases: either low sensitivity (microscopy) or expensive, but **important - the possibility of testing the sensitivity to ATB.**

- **Indirect methods** (e.g. serological methods = sometimes can be of low sensitivity and specificity)

How determine causative agent of the disease?

- Examination of exact sample (dependence on clinical symptoms and signs!!!) isolated:

  - from exact site;
  - at the exact time interval;
  - transport to laboratory examination under adequate conditions (standards)
  - examined by adequate methods (standards)

MATERIAL

Clinical symptoms = specific material in which the causative agent can be detected

= isolation at exact time

e.g.,

- stool
- urine
- blood
- cerebrospinal fluid
- sputum
- organ biopsies, aspirates
- smears, etc.

Body site	Specimen (examples)	Test options (examples)
Blood	**whole blood, serum, anticoagulated blood, etc.**	culture, QBC microhematocrit centruifugation, Buffy coat films, Knott concentration, membrane filtr. techniq. immunoassays, animal inoculation
Bone narrow	**aspirate**	culture, histopathology, thick and thin smears, PCR
CNS	**spinal fluid, brain biopsy specimen**	culture, wet examination, stained smears, immunoassays, PCR
Eye	**apirates from below surface, biopsy specimen**	culture, wet preparation, stained smears,
Skin	**smears, scrapings, apirates from below surface, biopsy specimen**	culture, histopathologic testing, squash preps (stained smears),
Intestinal tract	**fresh stool**	culture,direct wet smear, concentr., permanent stained smear, ag. det.
	anal smear	culture, direct wet smear
	preserved stool	concentration, permanent stained smear
	sigmoidoscopy material	direct wet smear, stained smear
	duodenal contents	
	anal impression smear	exam. of tapes for pinworm eggs
Liver and spleen	**sputum, induced sputum, broncholaveolar lavage fluid, transbronchial aspirate, brush biopsy specimen, aspirate, open-lung biopsy specimen**	wet preparation, stained smear, immunoassays, histopathologic testing, PCR
Lymph node	**biopsy specimen**	culture, stained smear, histopathol. test., PCR
Muscle	**biopsy specimen**	histopathologic testing, PCR
Urogenital system	**vaginal discharge, urethral discharge, prostatic secretions, urine, biopsy specimen**	culture, wet preparation, stain.smears, histopathol.test.

DETECTION OF THE AGENT

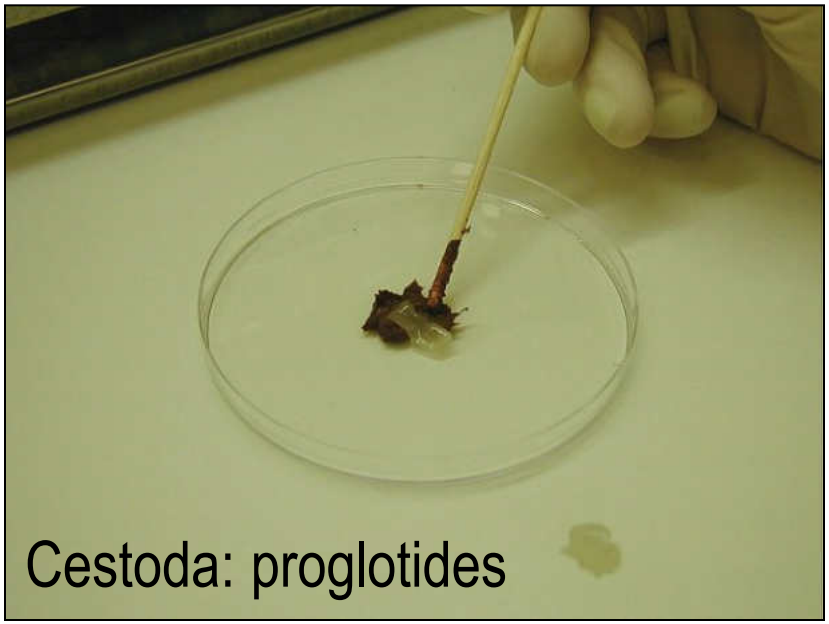
1) DIRECT – macroscopically or microscopically

- **culture**: predermined culture media or tussue cultures under controlled laboratory conditions
- **non-concentration methods**: nativ fresh mounts
  stained smears
- **concentration methods**: flotation
  sedimentation
  filtration
- **specific methods**: detection of DNA, circulating antigens

Detection of the parasite DNA: limited use

Material: e.g. incondesable blood, stool, urine (fresh, frozen, fixed in pure **100% alcohol**)

MACROSCOPICAL examination of samples



Cestoda: proglotides

Ascaris lumbricoides

CULTURE

BLOOD CULTURE

Very important - e.g., due to sepsis, pneumonia, fever of unknown origin, puerperal sepsis, pelvic inflammation, neonatal epiglottitis…

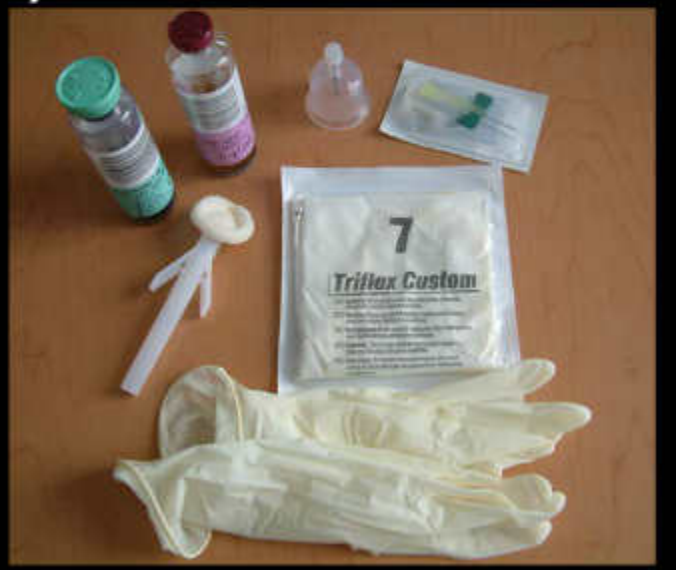
Source: Wikimedia Commons

Principles for Collection

- Gloves will be worn in accordance with standard precautions.
- A physician's order must be obtained for specimen collection.
- Appropriate verification of the patient's identity, by means of an armband or area specific procedure, will occur before the specimen collection.
- Cultures should be drawn before administration of antibiotics, if possible.
- If at all possible, blood cultures should not be drawn from lines, but should be drawn via venipuncture.

Materials

- Chlorhexidine swabs (1-2 packages)
- Alcohol swabs
- Blood culture bottles (2 bottles per set)
- 2 syringes (adult: 20 cc, pediatric: 5 cc)
- 2 needles (adult: 22 gauge or preferably larger butterfly or standard needle; pediatric: 25 or 23 gauge butterfly or standard needle)
- Gloves (sterile & nonsterile)
- Tourniquet
- Sterile gauze pad
- Adhesive strip or tape
- Self-sticking patient labels
- Plastic zip lock specimen bags



Step 7 – Draw Blood

7. Draw blood. Note the appropriate volume to obtain:

	Syringe needed	Aerobic bottle	Anaerobic bottle
Adult	20 ml	10 ml	10 ml
Pediatric	20 ml	2.5 - 10 ml	2.5 - 10 ml
Infant	3 ml	0.5 -1 ml	0.5-1 ml
Adult (low volume)*		All	None

Do not overfill bottles (do not add more than 10 ml of blood to each bottle)

*In some cases, it may not be possible to obtain 20 ml blood from an adult. If 10 ml or less is obtained, place all of the blood in the aerobic bottle.

Quality Problem

Contamination of blood cultures produces false positive test results for blood stream infections leading to adverse patient outcomes and wasted healthcare resources.

Preventability/ Improvement

Blood culture contamination rates
Range: 0.6% - 12.5%
Target: ≤ 3% (ASM, CLSI)

Practices/ Interventions

- Venipuncture (versus IV catheter) collection
- Phlebotomy team (versus other staff)
- Prepackaged prep kit (versus no prep kit)

Intermediate Outcomes

- Blood culture contamination rates
- Additional testing/follow-up associated with re-evaluation
- Costs associated with above

Healthcare/Health Outcomes

- Diagnostic and treatment delays
- Diagnostic and treatment errors
- Unnecessary and increased hospital stays
- Unnecessary antibiotic use
- Hospital acquired infection
- Costs associated with above

- Morbidity
- Mortality

Harms

- Needle-stick injury risk
- Patient collection site infection

CEREBROSPINAL FLUID CULTURE

+ other normally sterile fluids – e.g., peritoneal, pleural, synovial

1-3 ml of fluid transported to the laboratory

as soon soon as possible

Source: Wikimedia Commons

DEFINITION OF SIGNIFICANT BACTERIURIA IN PREGNANCY

- in an asymptomatic pregnant woman, bacteriuria is considered significant if two consecutive voided urine specimens grow > 10^5 cfu/mL of the same bacterial species on quantitative culture; or a single catheterised specimen grows > 10^5 cfu/mL of a uropathogen

- in a pregnant woman with symptoms compatible with UTI, bacteriuria is considered significant if a voided or catheterised urine specimen grows > 10^3 cfu/mL of a uropathogen

- > 10^3 cfu/mL of uropathogens in a mid-stream sample of urine (MSU) in acute uncomplicated cystitis in women

- > 10^4 cfu/mL of uropathogens in an MSU in acute uncomplicated pyelonephritis in women.

- > 10^5 cfu/mL of uropathogens in an MSU in women, or > 10^4 cfu/mL uropathogens in an MSU in men,
- or in straight catheter urine in women, in a complicated UTI.

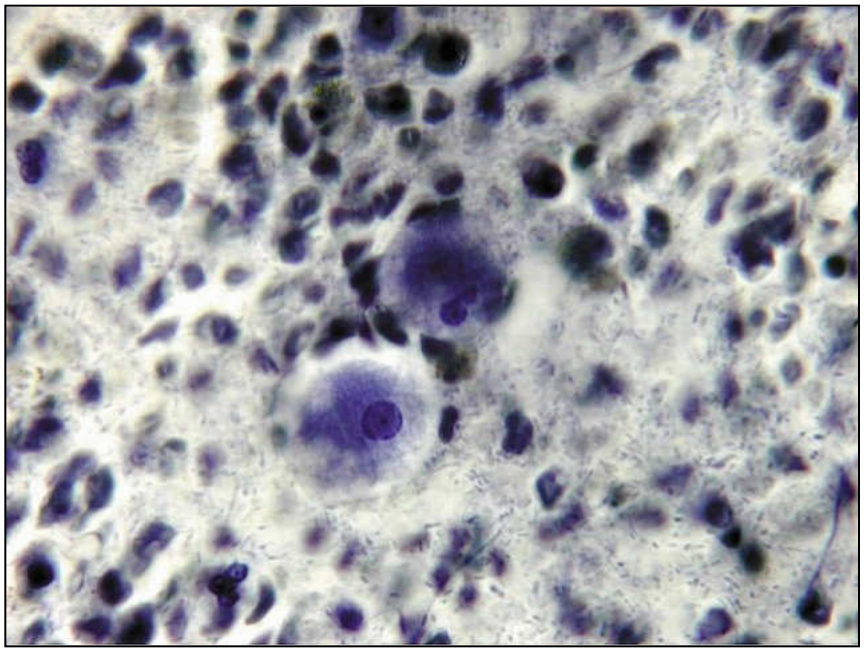
In a suprapubic bladder puncture specimen, any count of bacteria is relevant.

Escherichia coli – 10^8 / ml

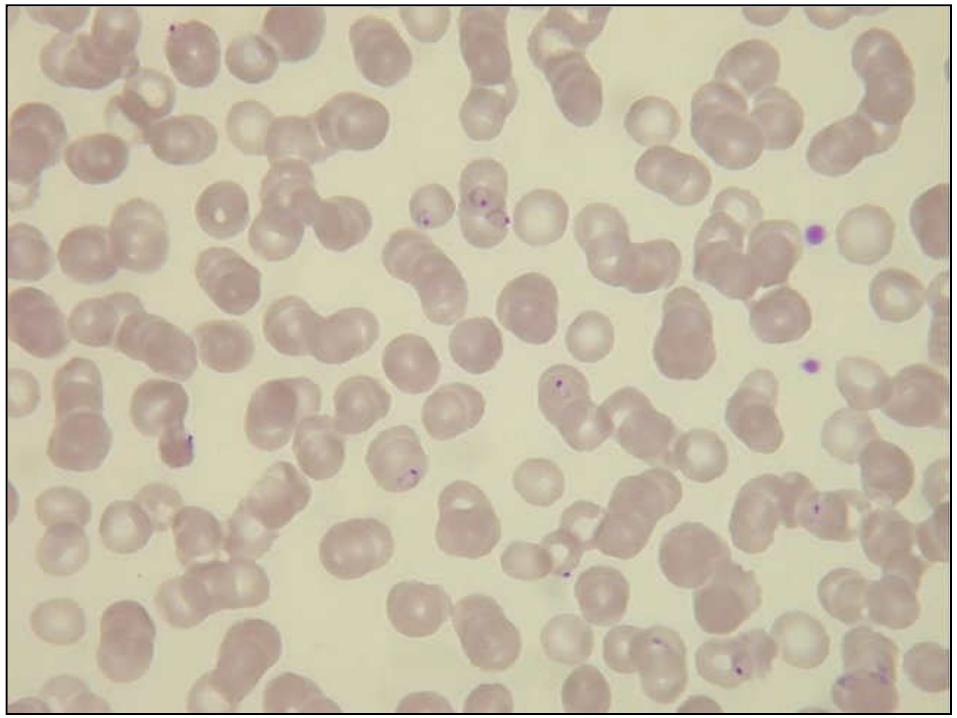
ASYMPTOMATIC BACTERIURIA

- diagnosed if two cultures of the same bacterial strain (in most cases the species only is available), taken > 24 h apart, show bacteriuria of > 10^5 cfu/mL of uropathogens

MICROSCOPICAL examination of samples



Entamoeba histolytica cysts

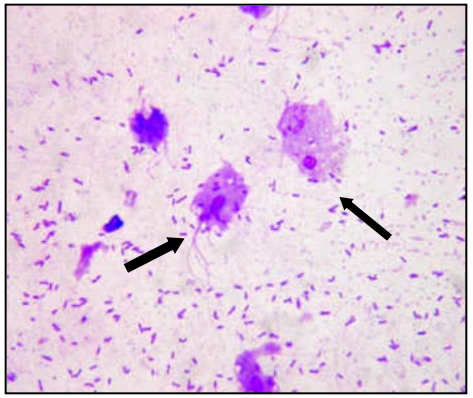


Plasmodium falciparum

CULTURE & MICROSCOPY

Material: **Smears**

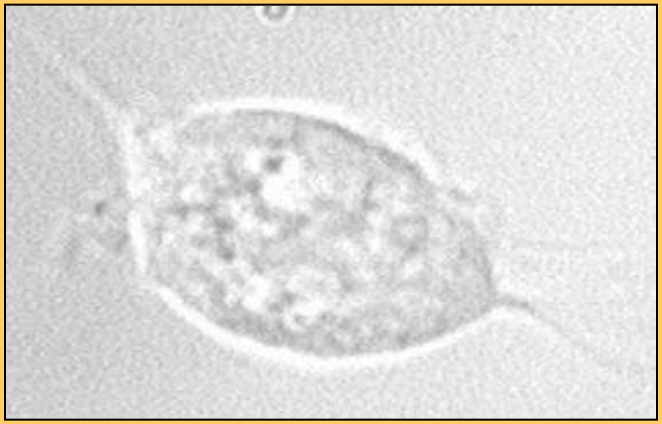
e.g., of vaginal mucosa *Trichomonas vaginalis*



1. MICROSCOPY

EXAMINATION of smear - *in vivo*, staining by Giemsa - MOP)

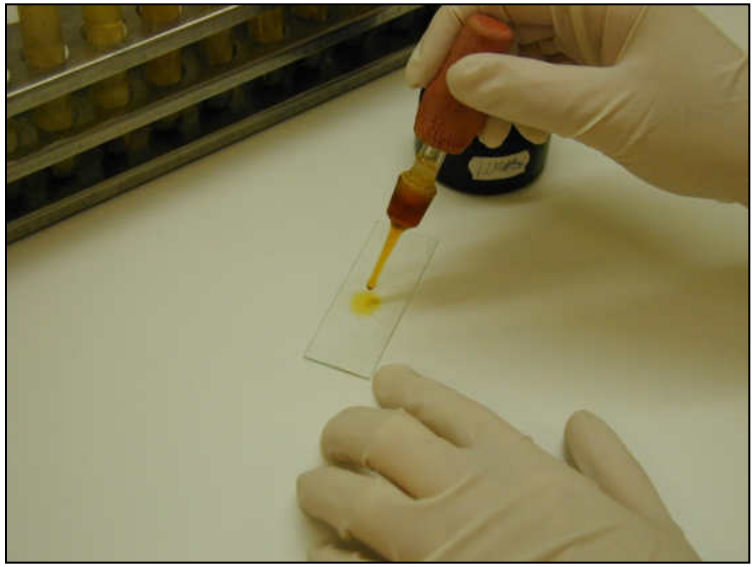
2. CULTURE → MIKROSCOPY

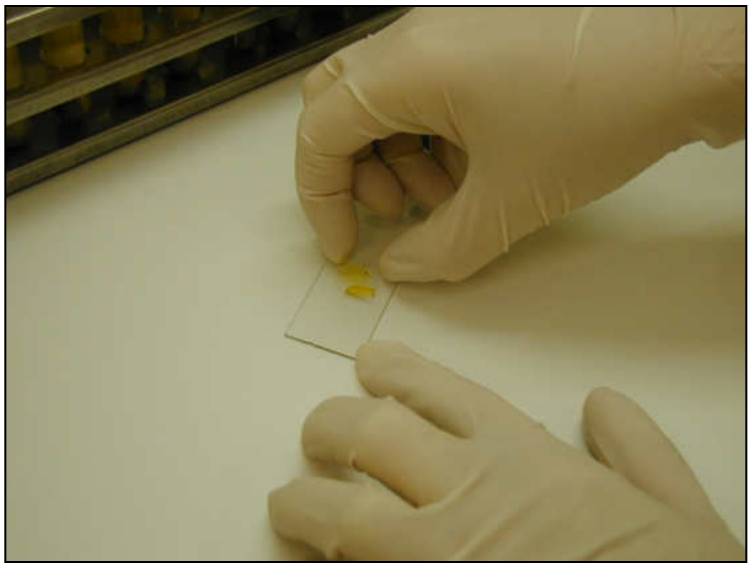


MICROSCOPY
NATIVE WET MOUNTS

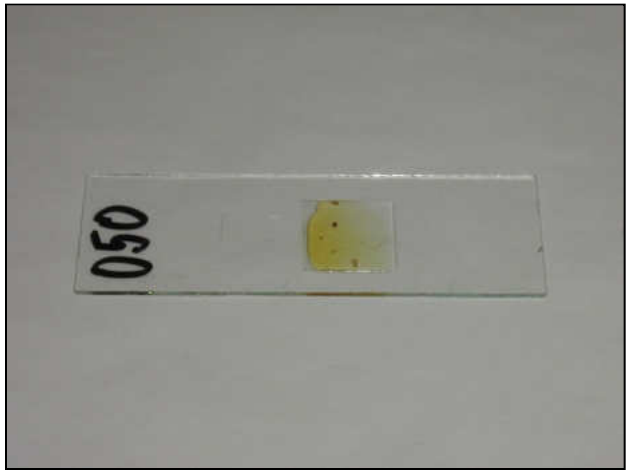
Enterobius vermicularis

MICROSCOPY
WET FRESH STAINED MOUNT





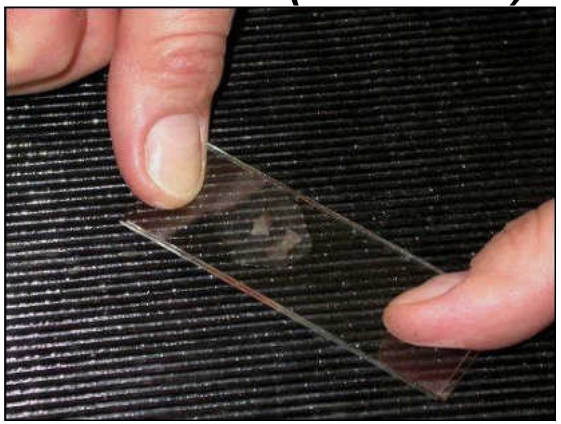
Staining e.g. by **Lugol's iodine** (e.g.,amoebae)



MICROSCOPY

WET FRESH MOUNTS OF ORGAN BIOPSIES

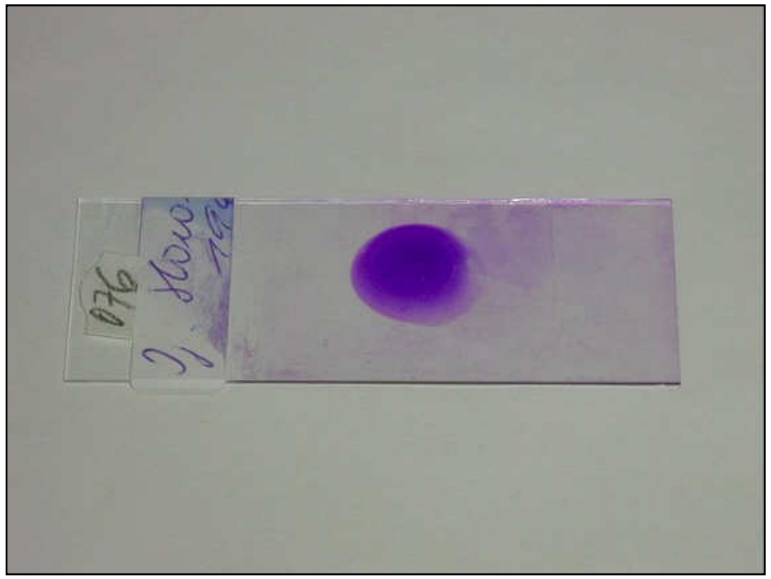
Quantitative compressed biopsy technique (QCTB)



e.g. *Schistosoma* eggs

MICROSCOPY

STAINED DRY SMEARS



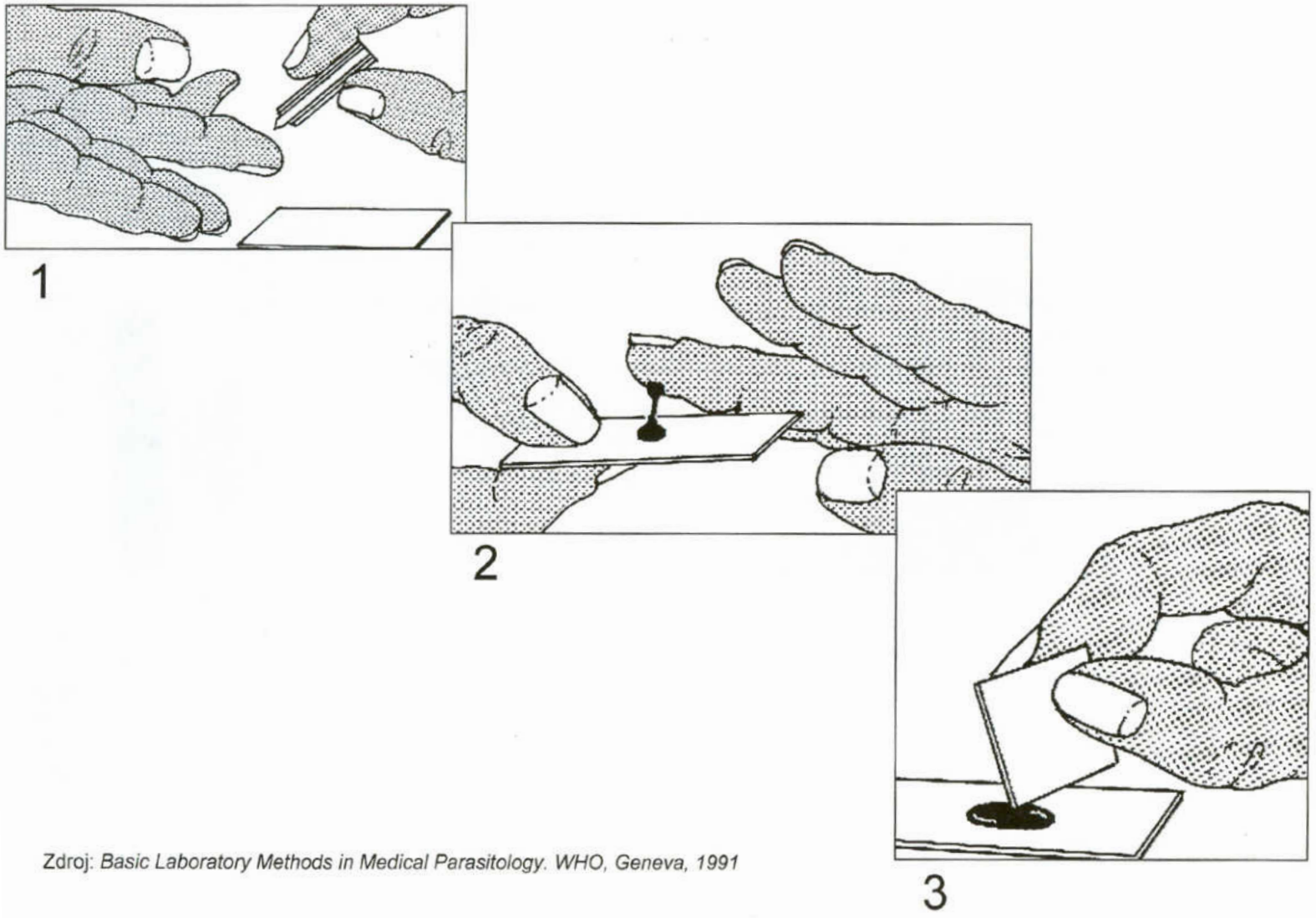
Thick smear stained by Giemsa (**no fixation** by methanol)

e.g. blood: malaria, filariases

material: peripheral blood

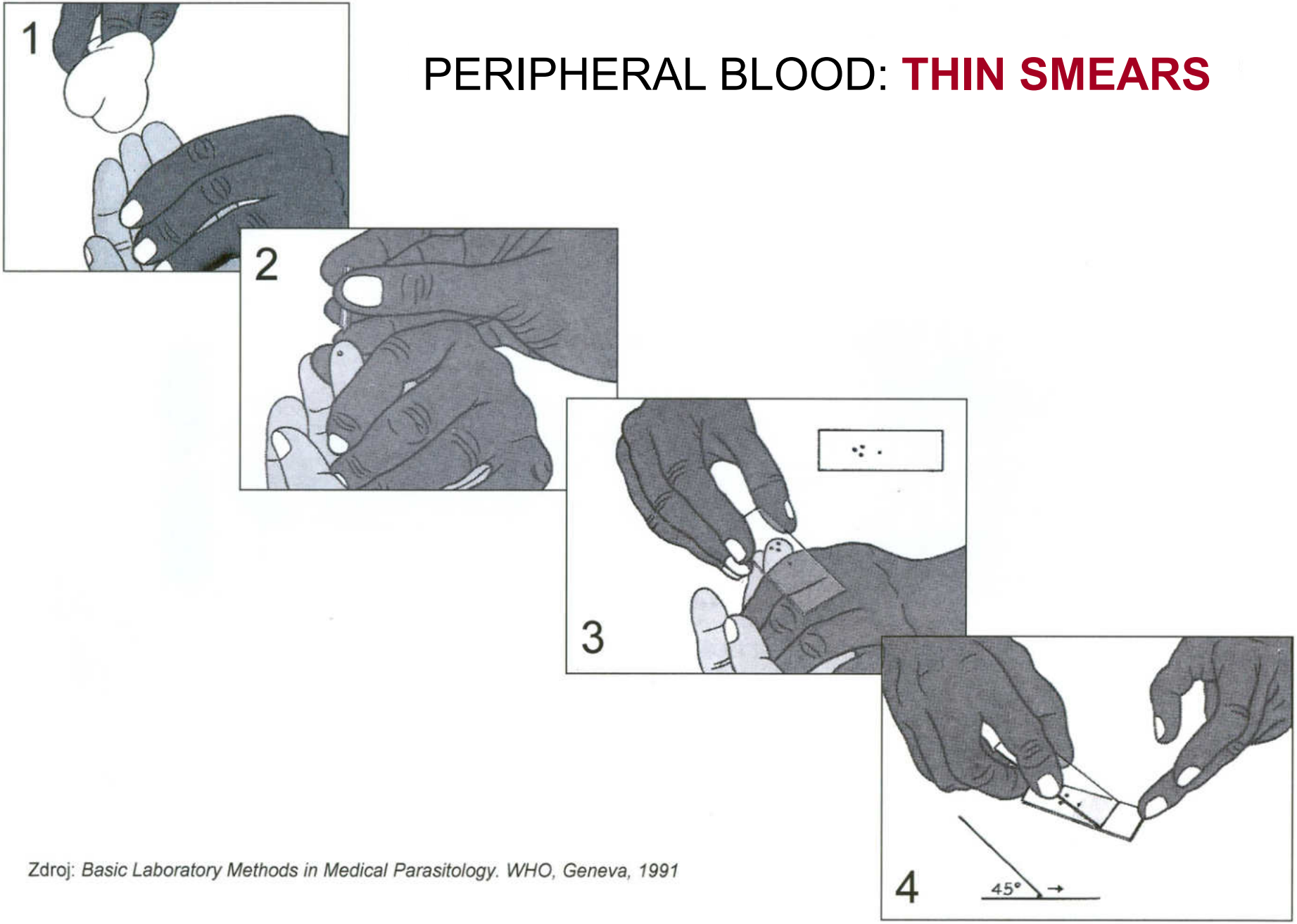
Thin smear: following fixation by methanol, staining by Giemsa

PERIPHERAL BLOOD: **THICK SMEARS**



Zdroj: *Basic Laboratory Methods in Medical Parasitology. WHO, Geneva, 1991*

PERIPHERAL BLOOD: **THIN SMEARS**



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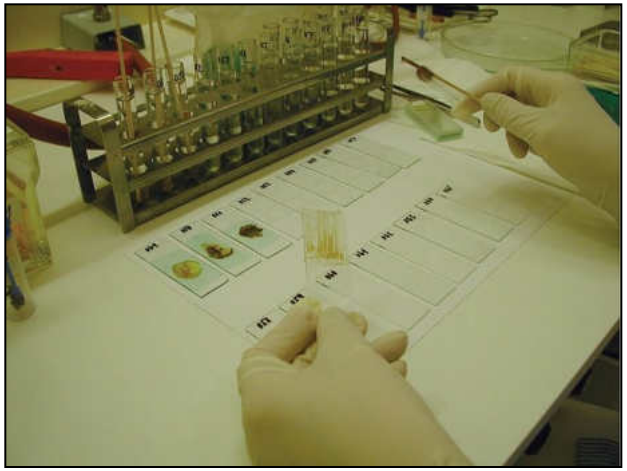
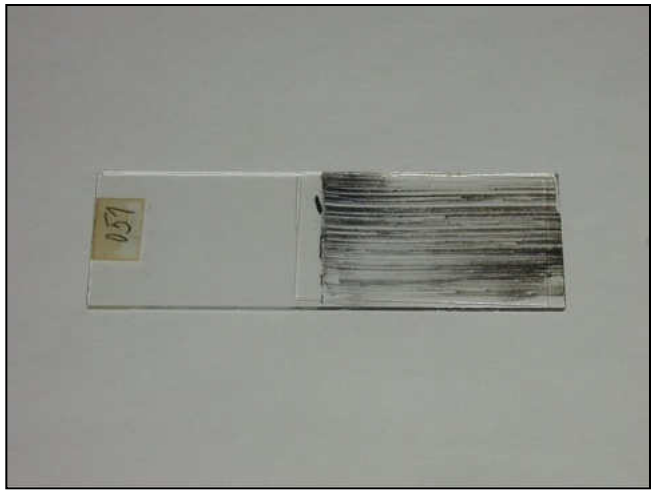
MICROSCOPY

EXAMINATION OF FAECAL SMEARS



smear → fixation → staining

e.g. eggs of intestinal protozoa



MICROSCOPY

EXAMINATION OF FAECAL THICK SMEARS

Kato-Katz Technique – celophane faecal thick smears

(glycerol-malachite green or glycerol-methylene blue solution; solutions are poured into the cellophane strips and soaked in this solution in a jar)

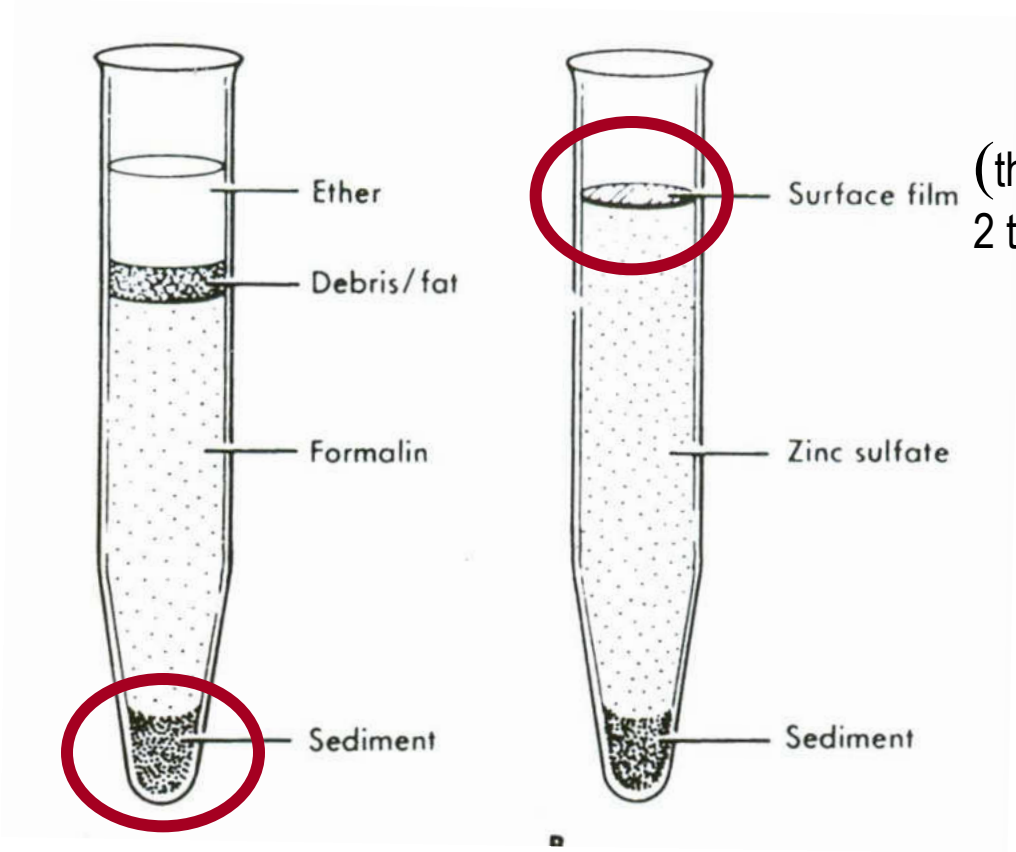
e.g. eggs of intestinal helmints

MICROSCOPY

EXAMINATION OF CONCENTRATED MOUNTS

FECAL CONCENTRATION PROCEDURES

various layers seen in the tubes after centrufugation



(the surface film should be within 2 to 3 mm of the tube rim)

A) Formalin-ether

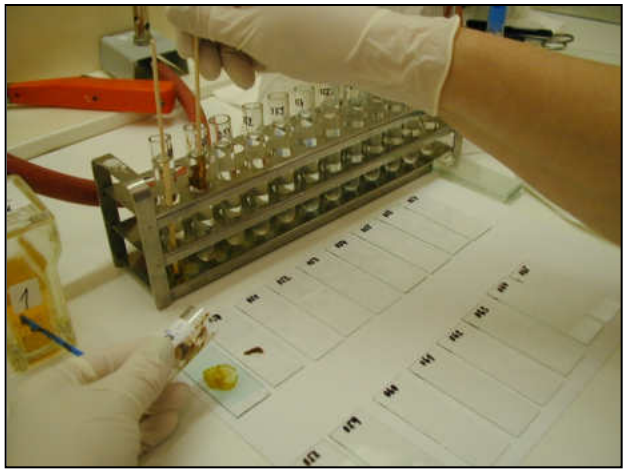
B) Zinc sulfate

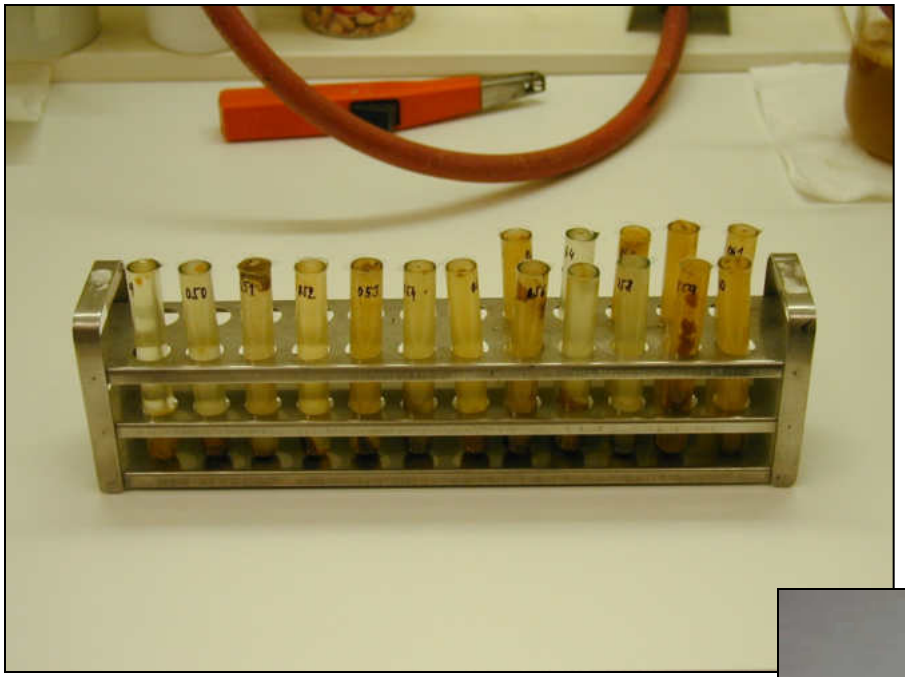
MICROSCOPY

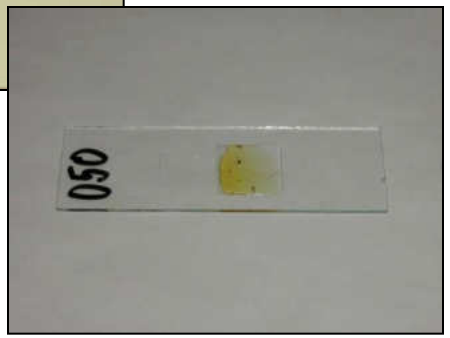
EXAMINATION OF CONCENTRATED MOUNTS

FLOTATION

Zinc Sulfate (33% aqueous solution)





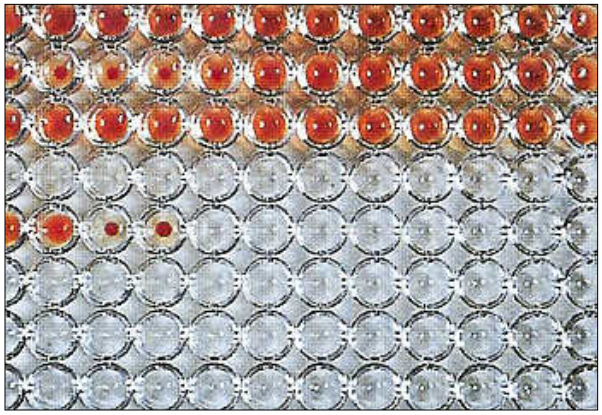


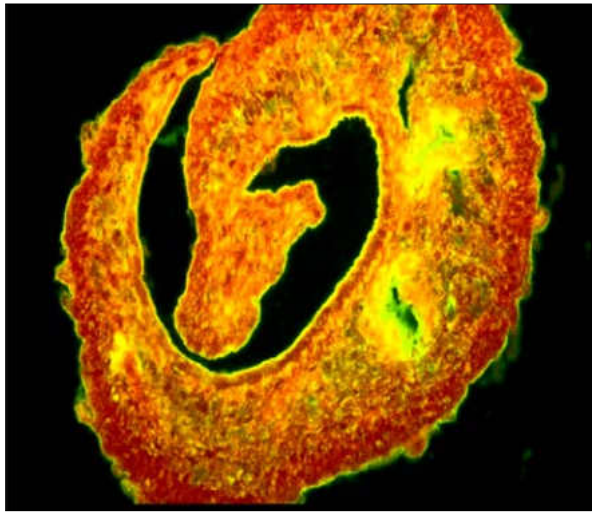
e.g. protozoan cysts, helminthic eggs

DETECTION OF THE AGENT

2) INDIRECT – using specifical methods, detection of specific **antibodies in the serum, vitreous humour, CTF**(when the agent is losalised in the organ/tissue)

methods: e.g., ELISA, IHA, IFAT, WB
material: condensable blood

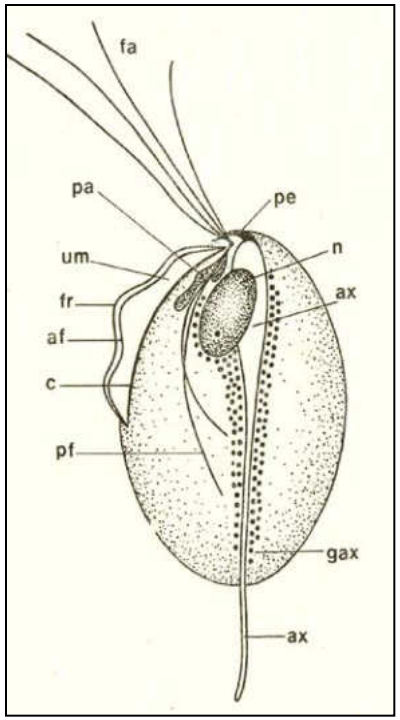




Examples

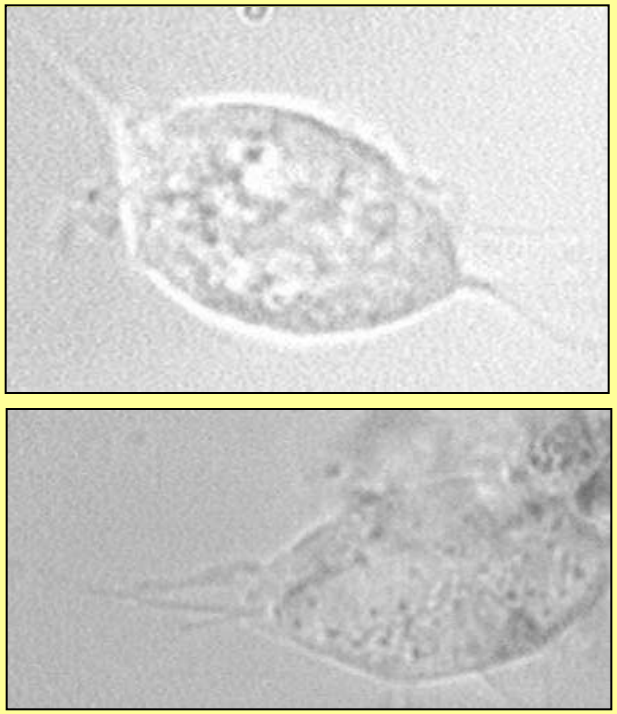
Trichomonas vaginalis

Trophozites: vaginal cavity and urethra
Transmission: veneral contacts
Diagnose: **examination of discharge,vaginal smears (staining mounts, culturing)**



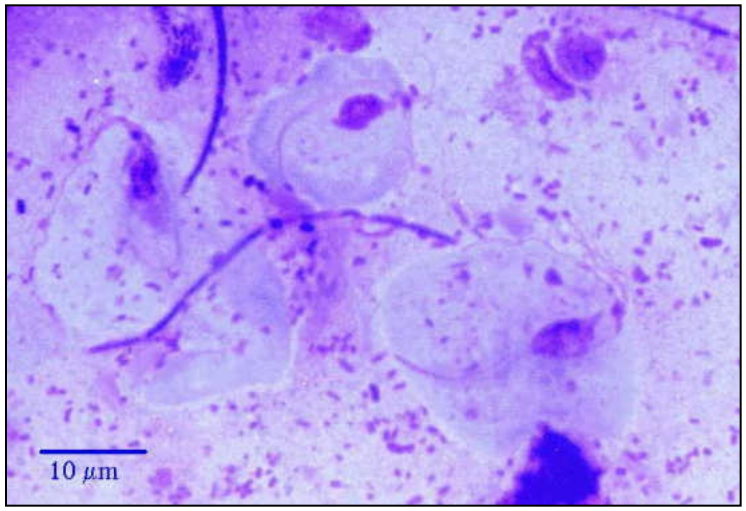
fa: anterior flagella, **fr:** posterior flagella, **n:** nucleus, **ax:** axostyle, **um:** undulating membrane

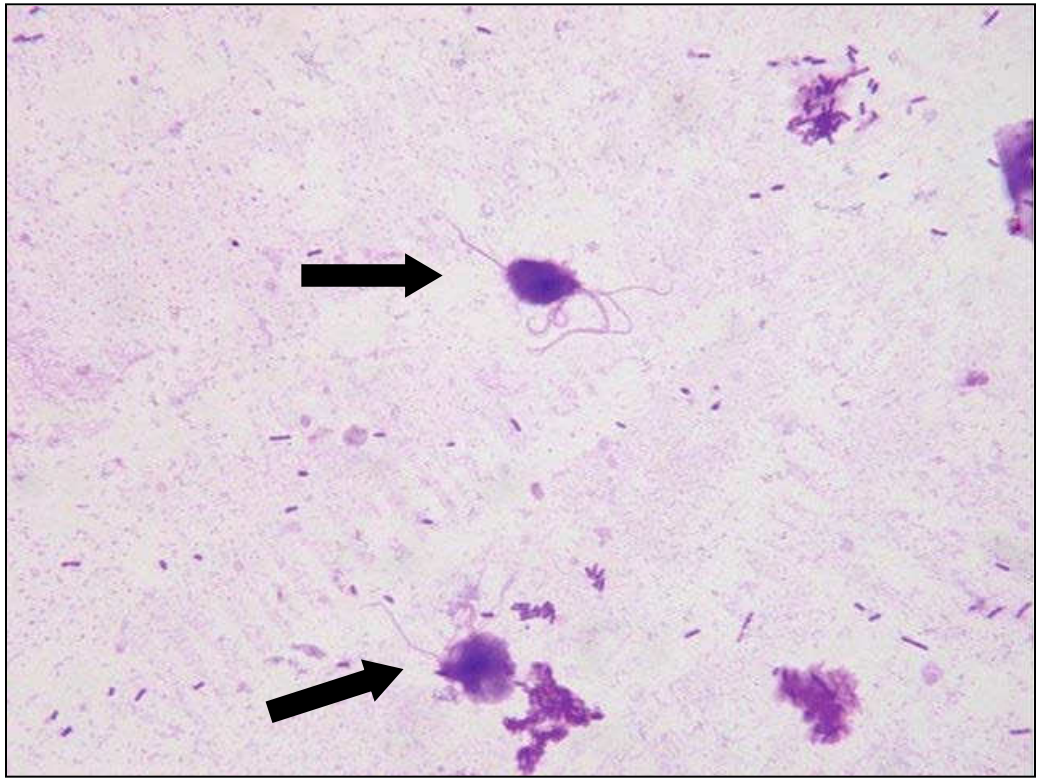
1) FRESH MOUNTS (culture)

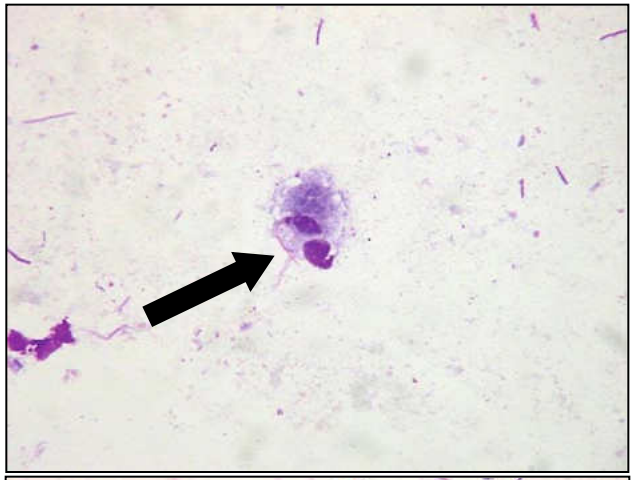


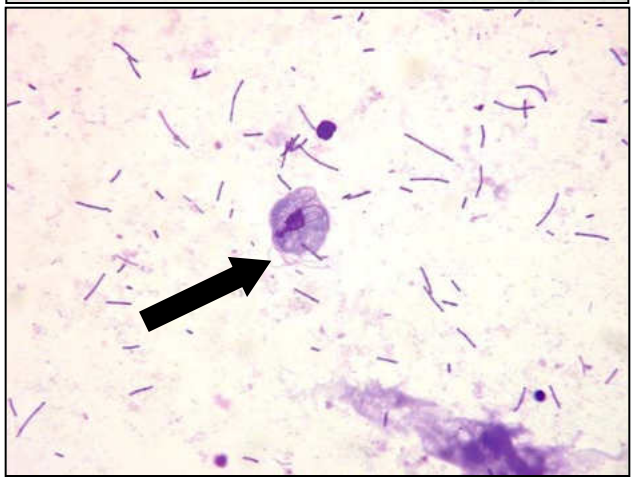
size: 10-30 μm x 6-20 μm

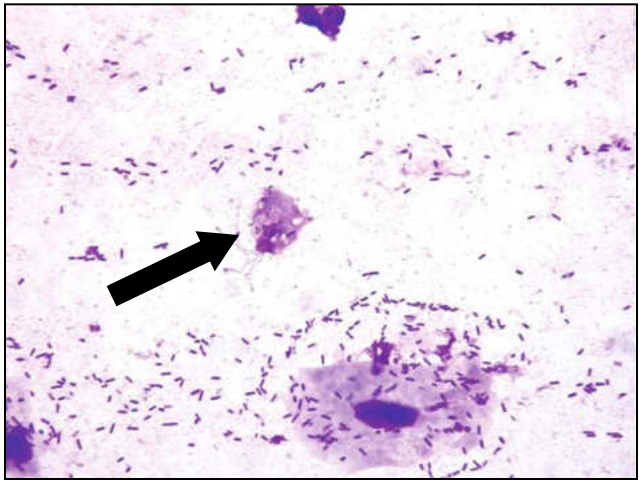
STAINED DRY SMEARS



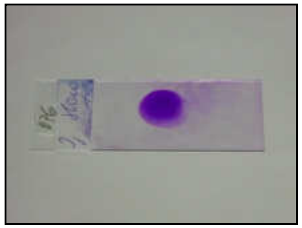


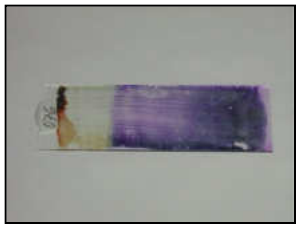






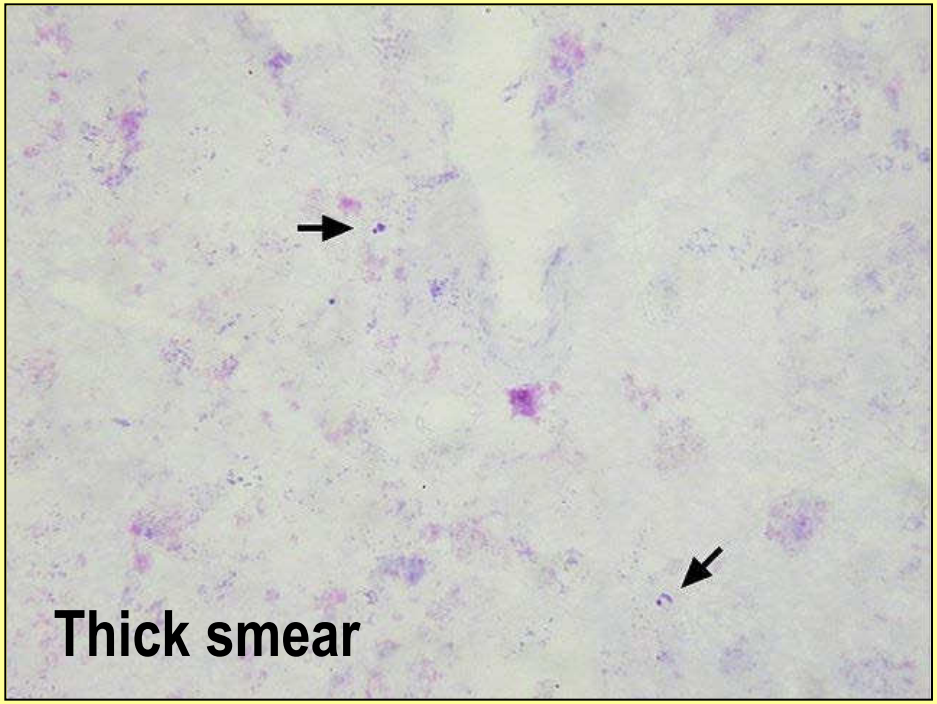
Plasmodium

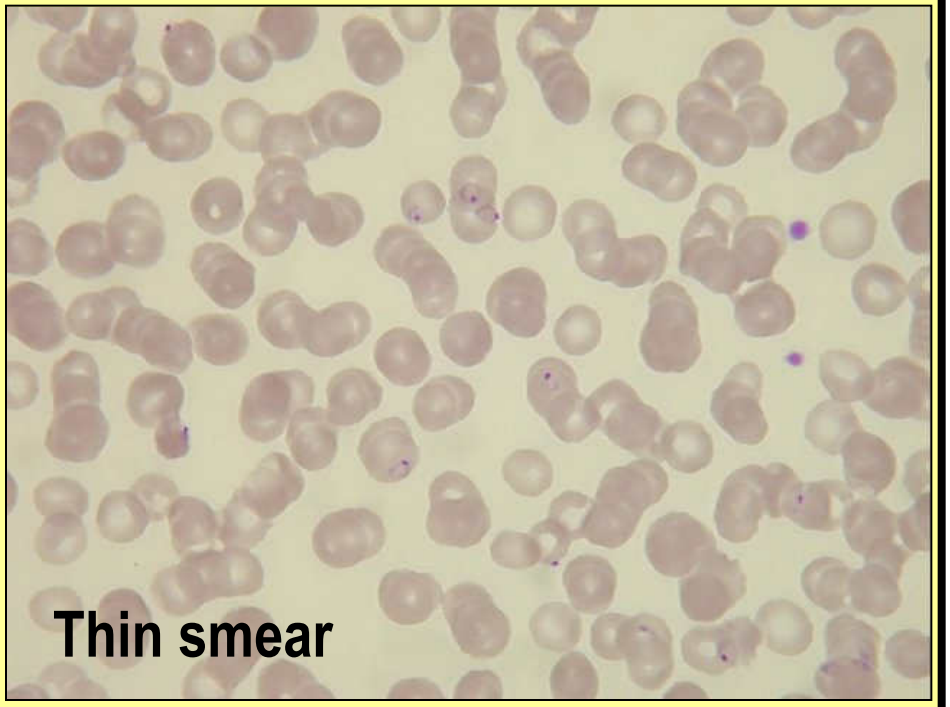




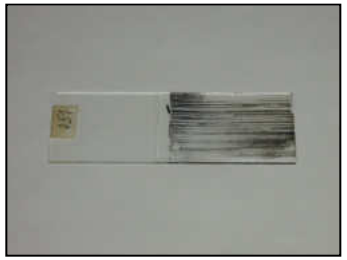
Disease: malaria
Transmission: vector
Diagnose: examination of peripheral blood smears and other techniques such as PCR

3) STAINED BLOOD SMEARS



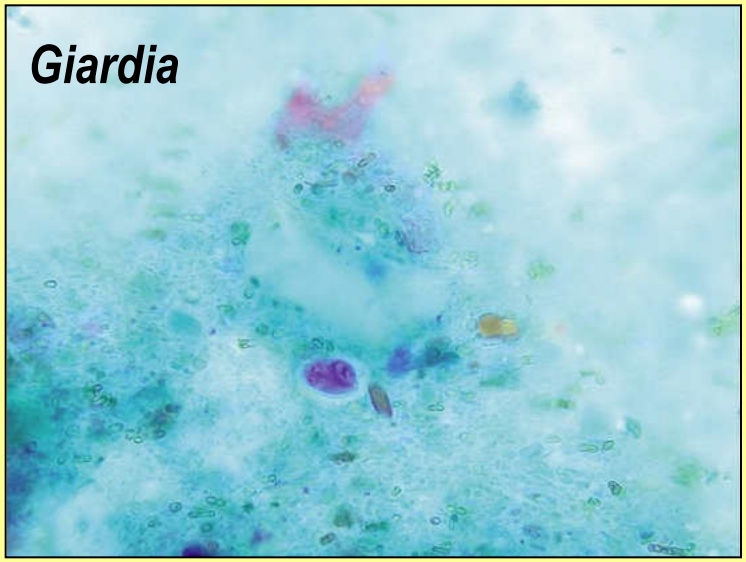


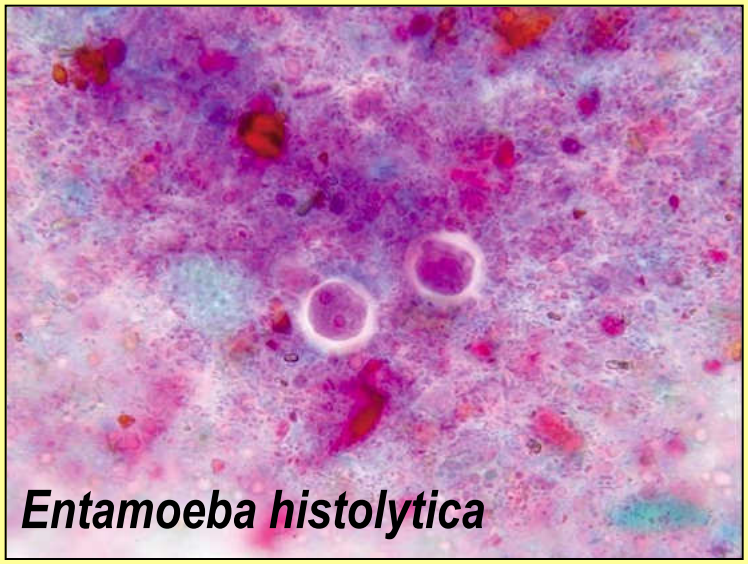
a) *Entamoeba histolytica*
b) *Giardia intestinalis*



Disease: **a),b)** intestinal and **a)** extraintestinal infections
Transmission: per os (food born infection)
Diagnose: intestinal: examination of stool,
extraintestinal: detection of antibodies, immaging

4,5) STAINED FAECAL SMEARS

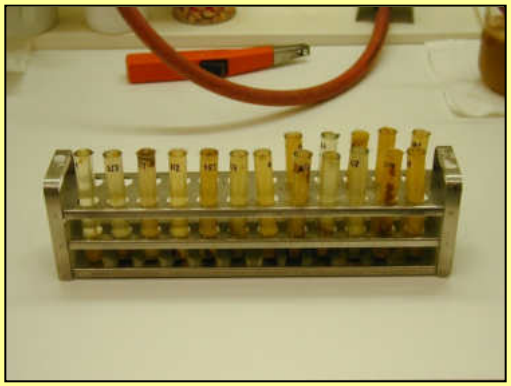


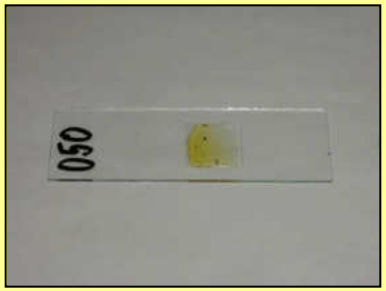


Ascaris lumbricoides

Disease: mainly intestinal infection
Transmission: per os (food born infection)
Diagnose: examination of stool

6) MOUNTS PREPARED BY FLOTATION METHOD





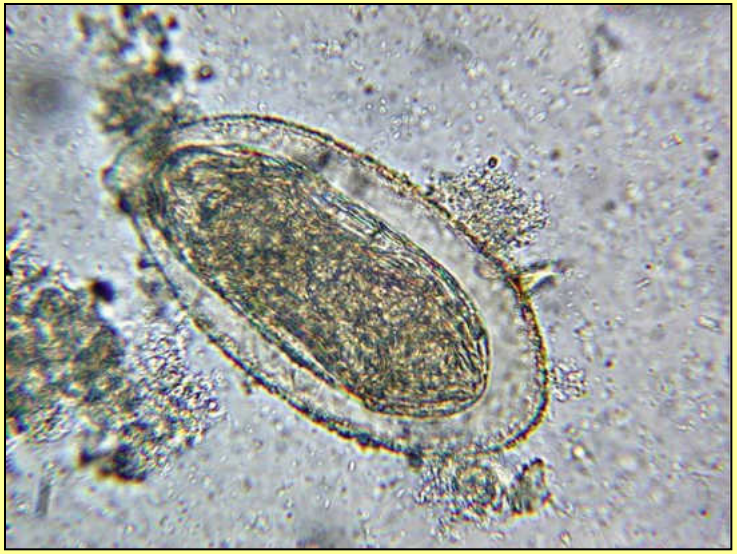
Size: 60 x 45 μm

Size: 80 x 45 μm

Schistosoma mansoni

Disease: intestinal and organ infection
Transmission: by cercariae (water-born infection)
Laboratory dg.: **examination of stool, detection of antibodies, immaging**

7) Quantitative compressed biopsy technique



Size:130-180 x 60-76 μm

Thank you for attention