Department of Parasitology

بسم الله الرحمن الرحيم

السلام عليكم ورحمة الله وبركاته

MISCELLANEOUS TECHNIQUES

PART I

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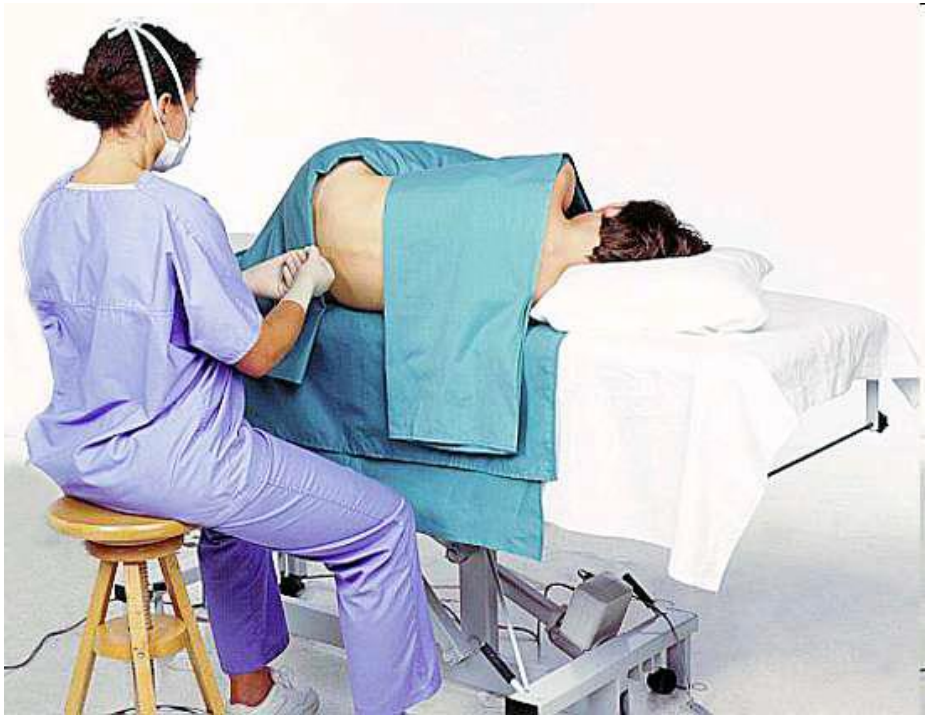
CEREBROSPINAL FLUID



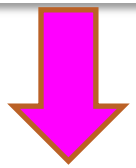
CSF analysis may be ordered if you've had CNS trauma. It may also be used if you have cancer and your doctor wants to see if the cancer has spread to the CNS.

In addition, CSF analysis may be ordered if you have one or more of the following symptoms:

- Severe, unremitting headache
- Stiff neck
- Hallucinations, confusion, or dementia
- Seizures
- Flu-like symptoms that persist or intensify
- Fatigue, lethargy, or muscle weakness
- Fever of unknown cause or rash
- Light sensitivity
- Speaking difficulties
- Trouble walking or poor coordination

If meningitis or encephalitis is suspected, tests may be performed to detect microorganisms.

❑ **Parasites found in CSF sample**



Trophozoite of *Naegleria fowleri*

Trypanosoma species

Trichinella spirallis larvae

Cestode

Taenia solium cysticercus larvae

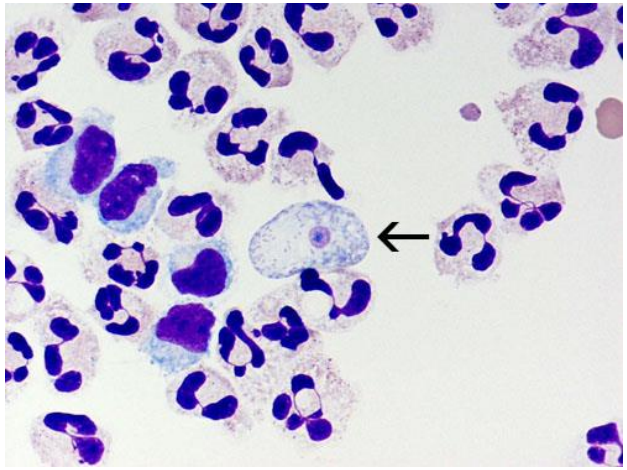
Echinococcus spp.

Toxoplasma gondii

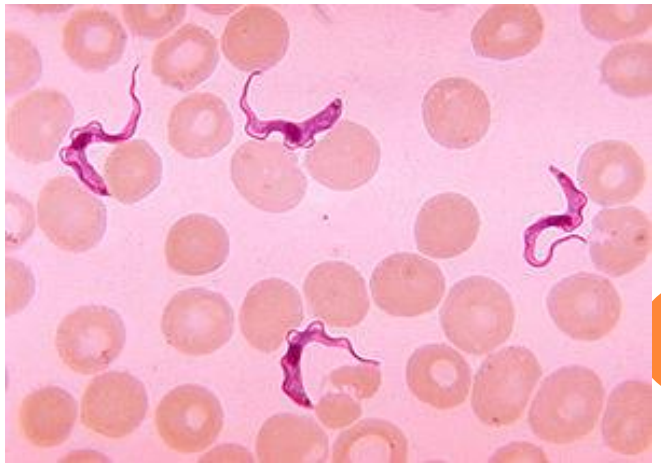
Microsporidia

Trichinella spirallis larvae

Naegleria floweri



Trypanosoma gambiense



Container:

- Sterile screw-cap sterile tube

Patient preparation:

- Disinfect skin before aspirating specimen

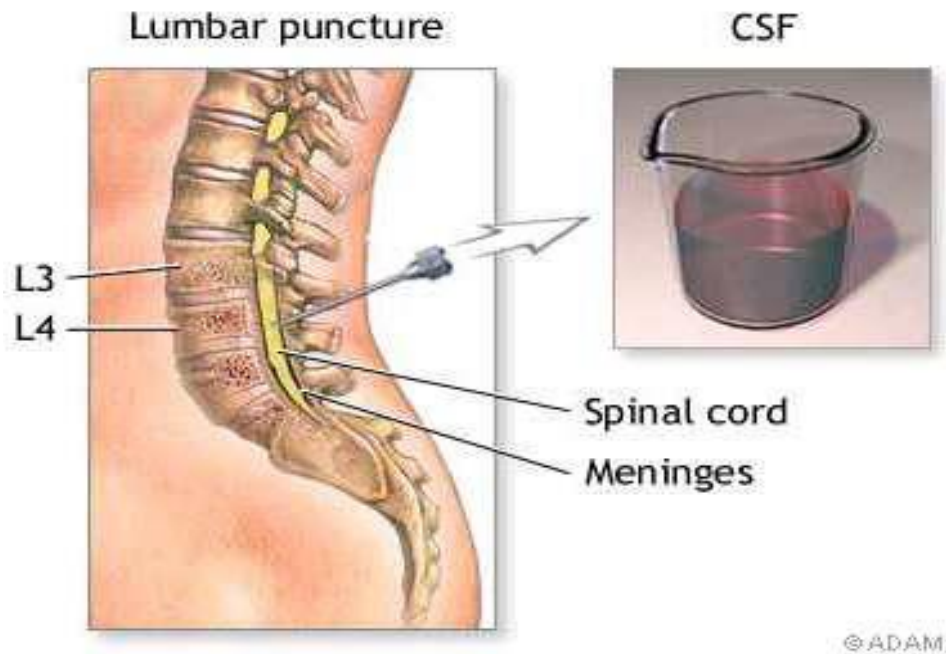
☞ **Collection:**

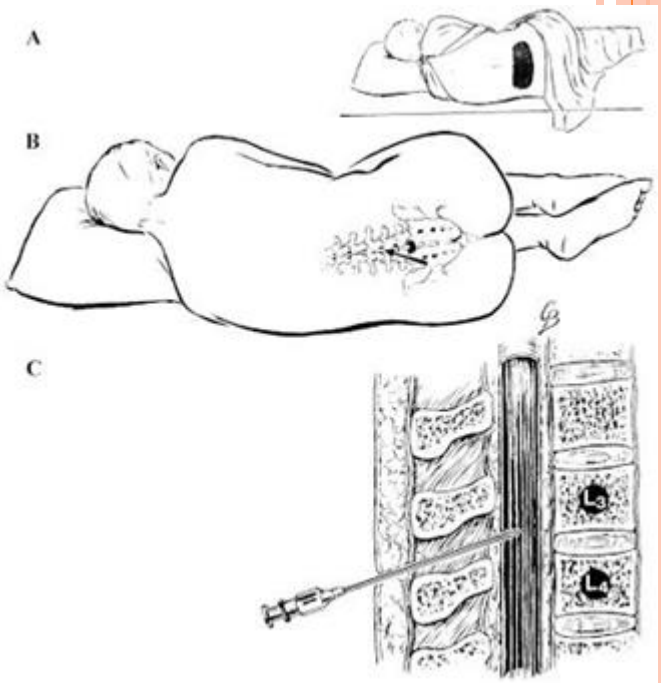
- Lumbar puncture (a spinal tap) is used to collect the CSF for examination. It should be collected by Physician trained in procedure with aseptic precautions to prevent introduction of Infection.

🕯 **Amount**

3-5 ml

- The fluid to be collected at the rate of 4-5 drops per second


Lumbar puncture
CSF
L3
L4
Spinal cord
Meninges
©ADAM, Inc.


A
B
C

TRANSPORTATION TO LABORATORY

The collected specimen of CSF should be dispatched promptly to Laboratory within 2 hours, any delay may cause disintegration of the cells and death of delicate pathogens, reducing the chance of isolation of pathogen.

PRESERVATION OF CSF

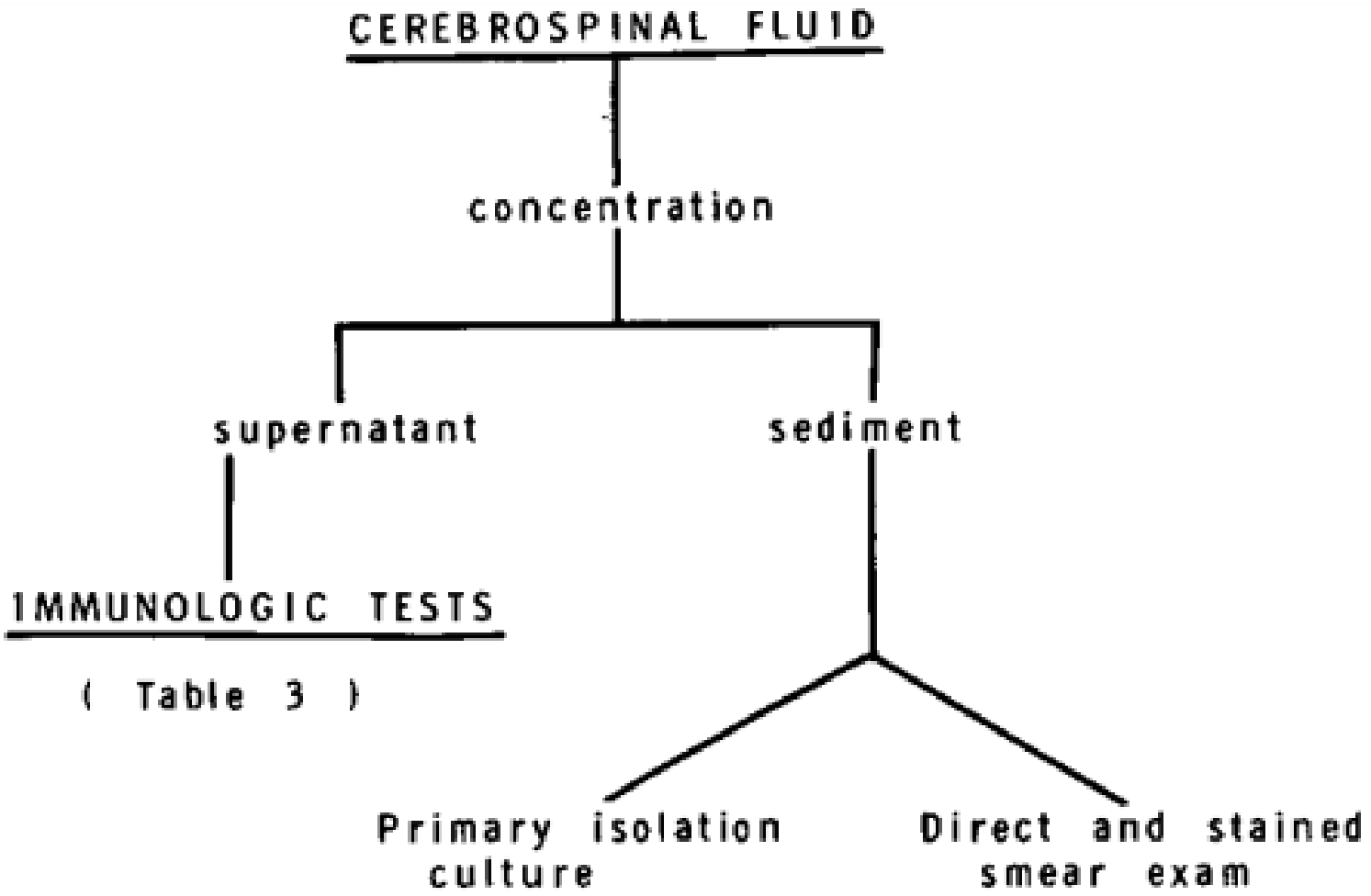
- It is important when there is a delay in transportation of specimens, the sample should not kept in the Refrigerator.
- If delay is anticipated leave the sample at Room Temperature (20-25°C).

a) The C.S.F must be examined as soon as possible, because the trypanosomes can survive only about 20 minutes and Naegleria round up and become non-motile (**Through 1 hour**).

b) The C.S.F should be centrifuged at 3000r.p.m for 10 minutes

c) The supernatant should be removed for immunologic testing and the sediment examined under the microscope using low magnification power.

EXAMINATION FOR PARASITES



ANIMAL INOCULATION

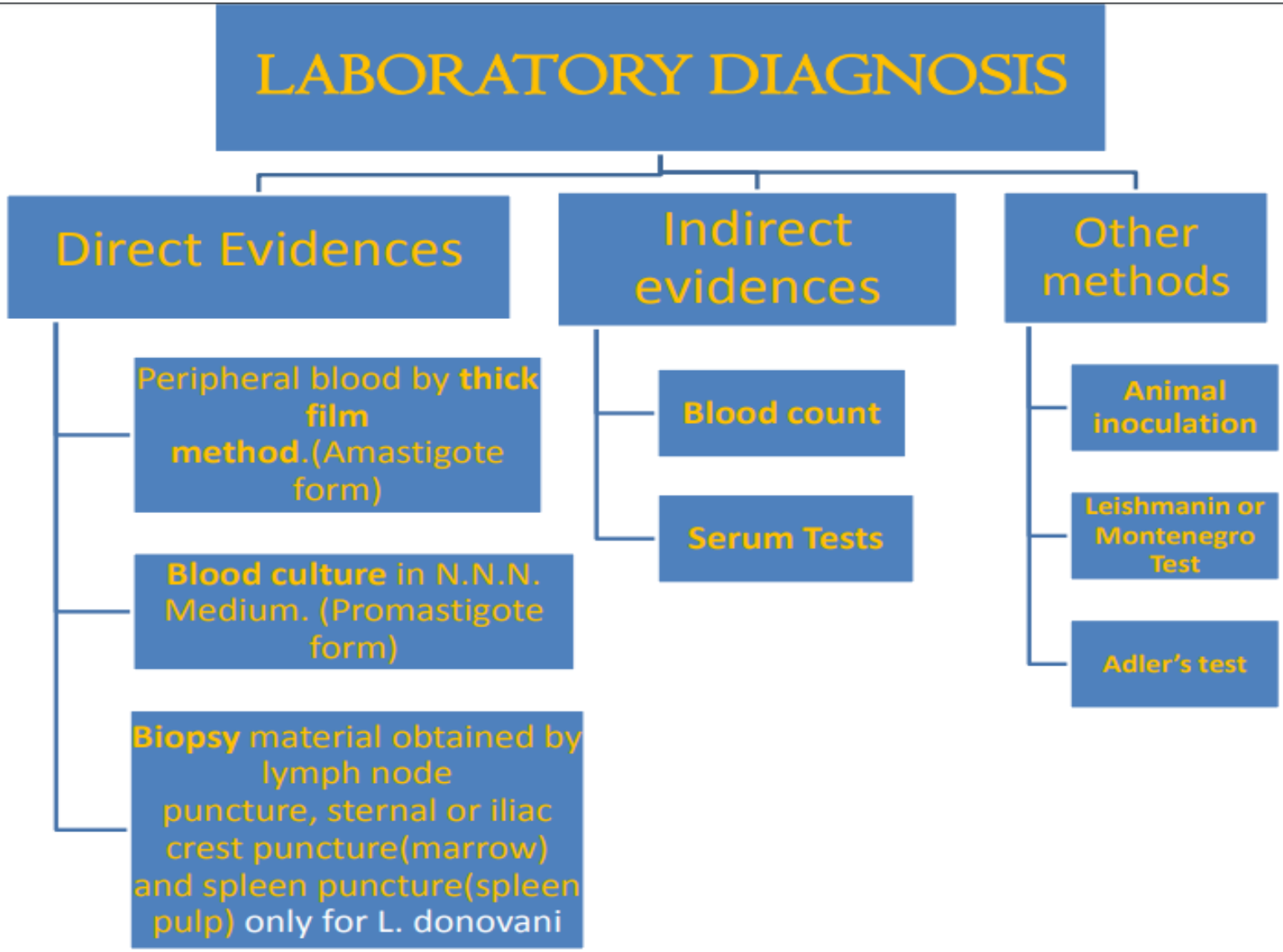
- Certain parasites have host specificity and require particular animals.
- Mice, guinea pigs, and hamsters are used.
- Suitable specimens for animal inoculation vary depending on the parasite suspected; these include blood, lymph node aspirates, CSF, and bone marrow.
- The specimens should be collected using aseptic technique.
- Very sensitive method but takes long time.

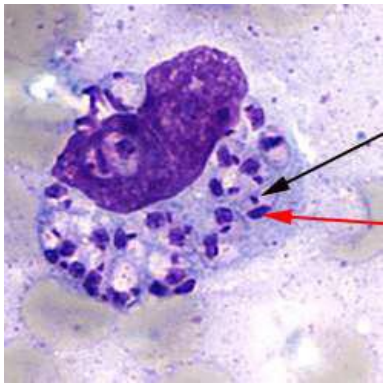
- Leishmania → (young hamster)
- Trypanosomes → (rat, mouse)
- Toxoplasma → (all lab animals)

TOXOPLASMA

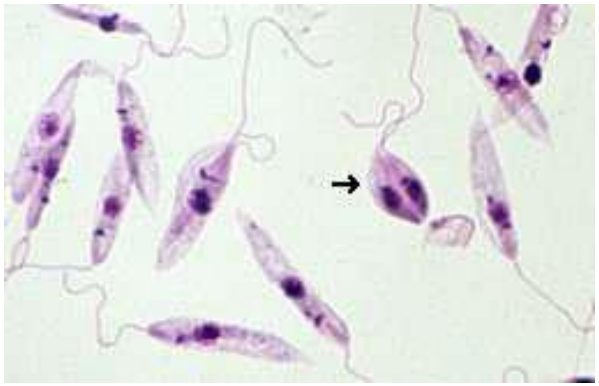
- Intraperitoneal inoculation of the body fluid, suspicious blood samples, cerebrospinal fluid, lymph node or ground tissue in laboratory bred mice free from infection
- Suspected material show trophozoites after 7 to 10 days post inoculation.
- The parasite is observed in its characteristic half-moon fixing the specimen with methanol and stain with Wright, or Giemsa stain and observed by a microscope.
- Confirmed by demonstration of tissue cyst in brain of inoculated animal.

LEISHMANIASIS

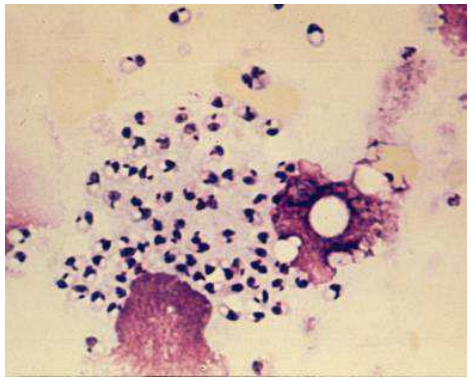


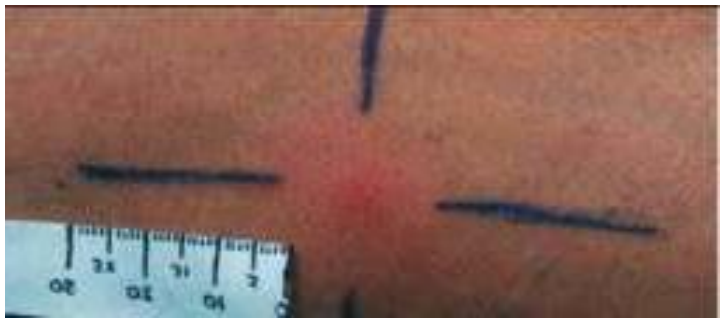


Amastigotes in a macrophage



Promastigote from culture in NNN medium





Montenegro Test

Biopsy material obtained by

- lymph node puncture,
- sternal or iliac crest puncture(marrow)

and

- spleen puncture(spleen pulp)

Amastigote form in a stained smear
Promastigote in culture in NNN medium

INDIRECT EVIDENCES

1. Blood count:-

- Leucopenia (progressive)
- Anaemia (raised ESR)

2. Serum Tests

- Aldehyde test- positive after 3 months.
- Antimony test- less reliable. Not used now.

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- Demonstration of antibodies (ELISA, DAT, IHA, IFA with specific antigen etc.)

3- **Molecular diagnosis**:- DNA Probes, PCR, etc.

OTHER METHODS

• **Animal inoculation** Wherever in vitro facilities are not there, the material from patients can be injected **intraperitoneally** in hamster or mice and the parasite is recovered from the animal. In positive cases, the amastigotes can be demonstrated in the stained impression smears of spleen from animals.

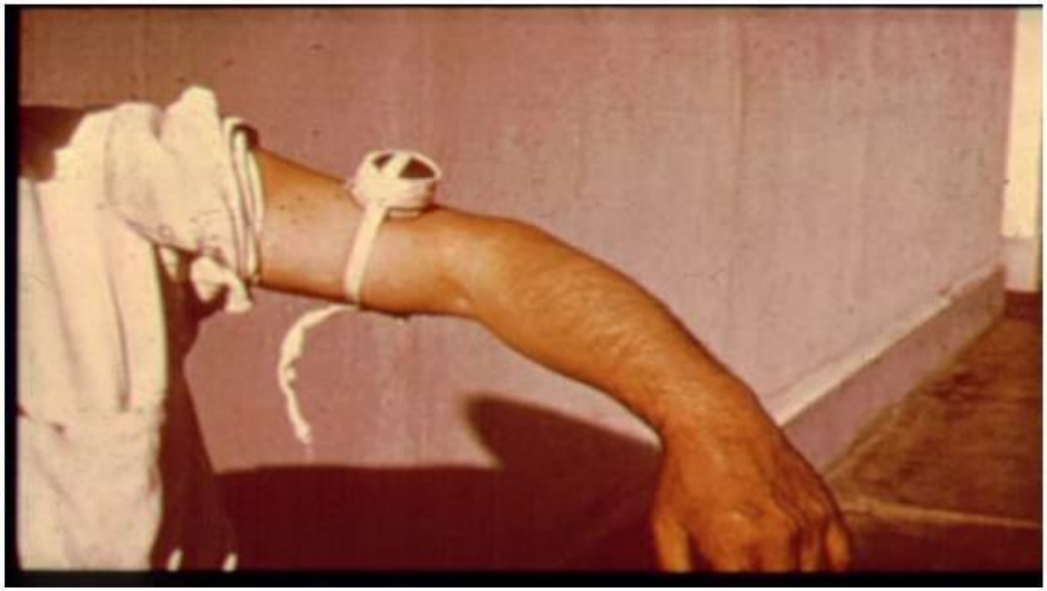
• **Leishmanin or Montenegro Test**

It is a delayed hypersensitivity test. 0.2 ml of Leishmania antigen is injected **intradermally**. The test is read after 48-72 hrs. Positive result is indicated by an induration of 5mm or more. In kala-azar (visceral leishmaniasis), this test is negative

XENODIAGNOSIS

- It is a technique used for the diagnosis of Chagas' disease. An uninfected reduviid bug is allowed to take a blood meal from the patient and the bug's feces is then examined to observe for the presence of *cruzi*.
- This procedure is primarily used in South America and Mexico.
- In a typical xenodiagnostic procedure, boxes containing 10 laboratory-reared, uninfected, and unfed (i.e., hungry) bugs were applied to a patient's arms and legs for 30 minutes, allowing the insects to take a blood meal. The bugs were kept in the box for around 30 days, at which point their feces were harvested, pooled, and examined microscopically for evidence of trypanosomes. If results at this point were negative, fecal inspection could be repeated after another 30 days. Other procedures describe "squashing" or homogenizing entire bugs prior to microscopic examination.

Diagnosis (Xenodiagnosis)



Highly efficient – demonstrate low level of parasite in blood

Method:

A Laboratory bred winged bug is starved for 2 weeks then fed on suspected patient's blood – 30 days later, it faeces & gut examined for trypanosomes.

TRICHINOSIS

- Trichinella larvae travel from the small intestine through the arteries to bury themselves inside muscle tissue, so stool sample tests don't often show evidence of the parasite. Your doctor can diagnose Trichinella infection by performing a physical exam. and discussing your signs and symptoms such as swelling around the eyes, muscle inflammation and fever.

To confirm the diagnosis, your doctor might use these tests:

- **Blood tests**

Your doctor may take a blood sample and test it for signs suggesting trichinosis — an increase in the number of a certain type of white blood cell (eosinophil) or the formation of antibodies against the parasite after several weeks.

- **Muscle biopsy**

While a blood test typically is enough to establish a diagnosis, your doctor might also recommend a muscle biopsy. A small piece of muscle is removed and examined under a microscope to look for Trichinella larvae.

SQUASH PREPARATION (TRICHINOSCOPY)

☞ It is used to identify *Trichinella* spp. cysts within muscle

1. Collect a small amount of fresh muscle.
2. Choose tissue from either the masseter or diaphragm as these two sites are most likely to yield positive results.
3. Place a small amount of tissue on a glass slide.
4. Cover with a second glass slide.
5. Press the two slides together using thumb and index finger, while still holding slides together, tape both ends of the slides together (scotch tape works well for this).
7. Trim away tissue not contained by the two slides.
8. Examine with a microscope using low power to identify larval cysts within the muscle.

☞ This technique is expensive and quite painful.

This specialized microscope is for identifying the Trichinae parasite in meat samples.

Trichinella worms cause Trichinosis in humans, hogs, rats, and other mammals.

Base allows microscope to swivel 45 degrees in one direction only.

A simple iris for light control, no condenser.

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SEROLOGIC DIAGNOSIS

- The detection of specific anti-*Trichinella* antibodies in human serum is vital for the diagnosis. An early upsurge in the IgE class of antibodies (which is observed in most cases) suggests that the immune system responds to Trichinella infection by an allergic reaction in the form of edema or a cutaneous rash.
- The demonstration of IgG antibodies is taken as a proof of infection. These may be detected from 12 to 60 days following primary infection, depending on the parasite species involved, the immune response and the larval infective dose.
- Enzyme-linked immunosorbant assay (ELISA) still represents the most commonly employed diagnostic technique, although the indirect fluorescent antibody test is sometimes used as well. The latex agglutination test is used when rapid diagnosis is required.


THANK YOU