Tetanus
Botulism
Leprosy
Rabies
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Leprosy

Diagnosis of Leprosy

HISTORY

- Leprosy or Hansen's disease.
- Discovered in 1873 by G. Hansen.

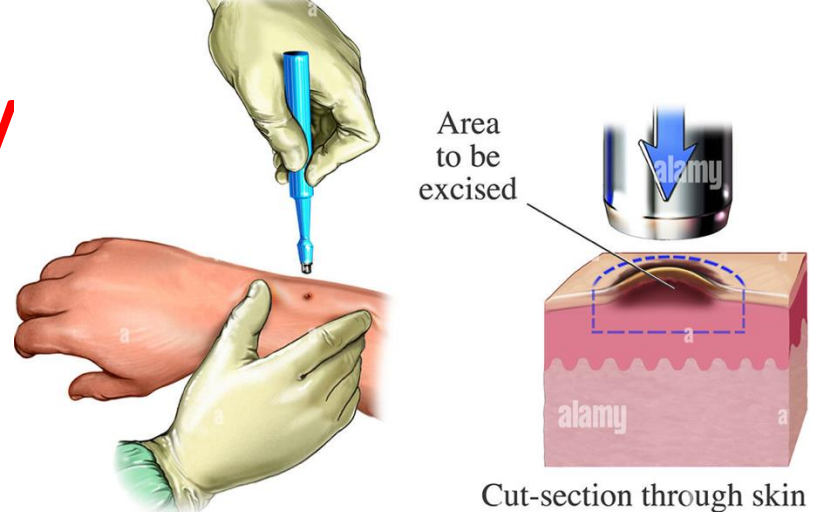
Lab Diagnosis Overview:

1. Specimens.
2. Acid fast staining.
3. Skin and nerve biopsy.
4. Animal inoculation.
5. Lepromin test.
6. PCR
7. Serodiagnosis.

Diagnosis of Leprosy

Specimen collection:

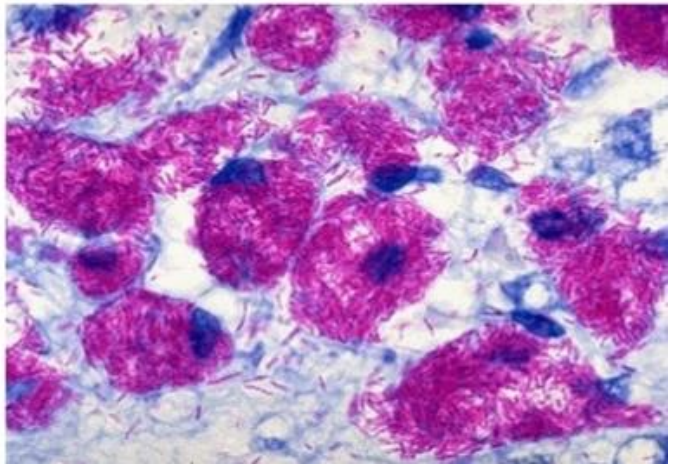
1. Nasal mucosa.
2. Skin: active edges of the patches
3. Nerve biopsy: from thickened nerves



Cut-section through skin

Procedure

- Lesion is cleaned with spirit
- 5mm long incision made by scalpel deep enough at edge of the lesion
- Scalpel blade is rotated transversely to get tissue pulp from below epidermis.
- Tissue pulp is used to produce smear on slide
- Stained with modified ZN stain.



The bacilli are present inside the foamy macrophages called Virchow's lepra cells or foamy cells.

The interpretation of Staining results:

- Positive or negative results.
- The positive results are then used to measure the **BI and MI**
- **Live bacilli:** uniformly stained parallel ends (rounded ends)
- **Dead bacilli-** Less uniform staining (Fragmented appearance).
- **Bacteriological index (BI)** = Total number of bacilli (Live + dead) (per oil immersion field)
- **Morphological index (MI)** =live bacilli / Live + dead bacilli × 100%
- During successful treatment the MI is decreased but BI is constant

Diagnosis of Leprosy

Lepromin test

- The lepromin test is used to study host immunity to *M. leprae*.
- The test is an intradermal skin test performed by using lepromin antigen, which is a suspension of killed *M. leprae* obtained from infected human or armadillo tissue.
- The lepromin test is not used to confirm the diagnosis of leprosy.
- It is not useful to indicate prior contact of the person with leprae bacilli.

Diagnosis of Leprosy

- Lepromin test is an intradermal test helping in classifying lesion of leprosy.
- **Principle :** Delayed hypersensitivity reaction of leprosy Ag.
- **Procedure**: 0.1 ml of lepromin Ag is injected I/D in forearm. Lepromin Ag is obtained from killed M. leprae.

Intradermal injection

Readings are taken on two occasions

A. Fernandez reaction (After 48hr): Positive if induration >10mm size indicates previous exposure to lepra bacilli

Fernandez reaction

B . Mitsuda reaction (At 21 days): Positive if enduration >5mm (This reaction is indicative of the host's ability to give a granulomatous response to antigens of M. leprae.

Diagnosis of Leprosy

Intradermal injection

Lepromin test

Interpretation.

1- Positive

- Tubercolid leprosy (CMI intact): good prognosis
- After BCG vaccination

2- Negative

- Lepromatous leprosy (Bad prognosis).
- Healthy

Fernandez reaction

Diagnosis of Leprosy

Serodiagnosis

- Serodiagnosis of leprosy is based on demonstration of antibodies to *M. leprae*, specific Phenolic glycolipid - **1** (PGL-1) antigens.
- Enzyme linked immunosorbent assay (ELISA) and latex agglutination test are used to detect serum antibodies.
- The serology is useful primarily in patients with untreated lepromatous leprosy, as most of patients have higher levels of serum antibodies.

Laboratory Diagnosis of Rabies

In man:

- Specimens: Saliva, CSF, Urine
- Immunofluorescence of skin biopsy.
- Isolation by inoculating saliva in mice.
- Detection of antibodies by serology.
- RNA detection by PCR in blood

Laboratory Diagnosis of Rabies

Antemortem testing

- Antemortem samples
- To rule out rabies before death, all four of the listed samples must be collected. Without all samples, a definitive rule-out cannot be provided.

1- Saliva

- Saliva is intermittently shed in patients with rabies. Serial collection of 4 or more samples over 24 hours may be necessary. Using a sterile eyedropper pipette, collect saliva and place it in a small sterile container that can be sealed securely. No preservatives or additional material should be added.
- The laboratory should conduct tests to detect rabies RNA by (RT-PCR). Tracheal aspirates and sputum are not suitable for rabies tests, and contamination with blood can reduce test accuracy.

2- Skin Biopsy

- A section of skin 5 to 6 mm in diameter should be taken from the posterior region of the neck at the hairline. The biopsy specimen must contain a minimum of 10 hair follicles and be of sufficient depth to include the cutaneous nerves at the base of the follicle. Place the specimen on a piece of sterile gauze moistened with sterile water and place in a sealed container. Do not add preservatives or additional fluids.
- Laboratory tests to be performed include RT-PCR and immunofluorescent staining for viral antigen in frozen sections of the biopsy.

Laboratory Diagnosis of Rabies

3- Serum

- At least 0.5 ml of serum should be collected; no preservatives should be added. Do not send whole blood. If **NO** vaccine or rabies immune serum has been given, the presence of antibodies to rabies virus in the serum can confirm a diagnosis of rabies.
- Laboratory tests for antibodies include an indirect fluorescent antibody test and a virus neutralization test.

4- Cerebral Spinal Fluid (CSF)

- At least 0.5 ml of CSF should be collected; no preservatives should be added. Antibody to rabies virus in the CSF, regardless of the immunization history, suggests a rabies virus infection.
- Rabies virus, antigen, and RNA are rarely found in CSF; therefore antigen and molecular test methods are not routinely performed.

Postmortem testing

- In certain cases, human samples may need to be tested for rabies after the patient has died. Fresh tissue samples from the central nervous system (brain) should be submitted.
- Postmortem diagnosis of rabies is made by immunofluorescent staining of viral antigen.

Tetanus

Laboratory Diagnosis

Specimen

- Wound swab
- Exudate or tissue from the wound.

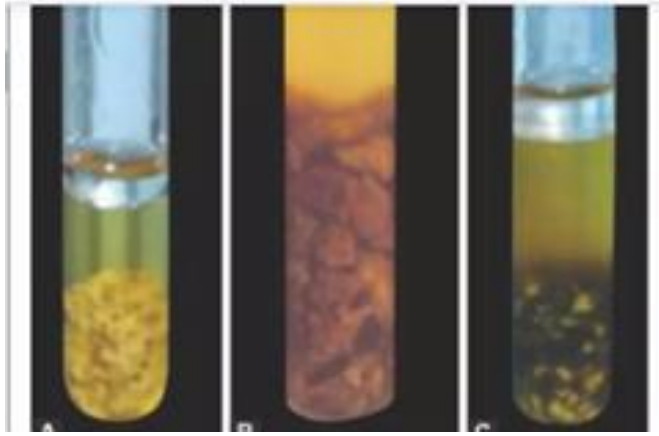
Gram Staining

- GPB with terminal and round spores (drum stick appearance).

Culture

- RCM (Reinforced Clostridial Medium):

Meat particle = black



- Blood agar with polymyxin B Swarming growth

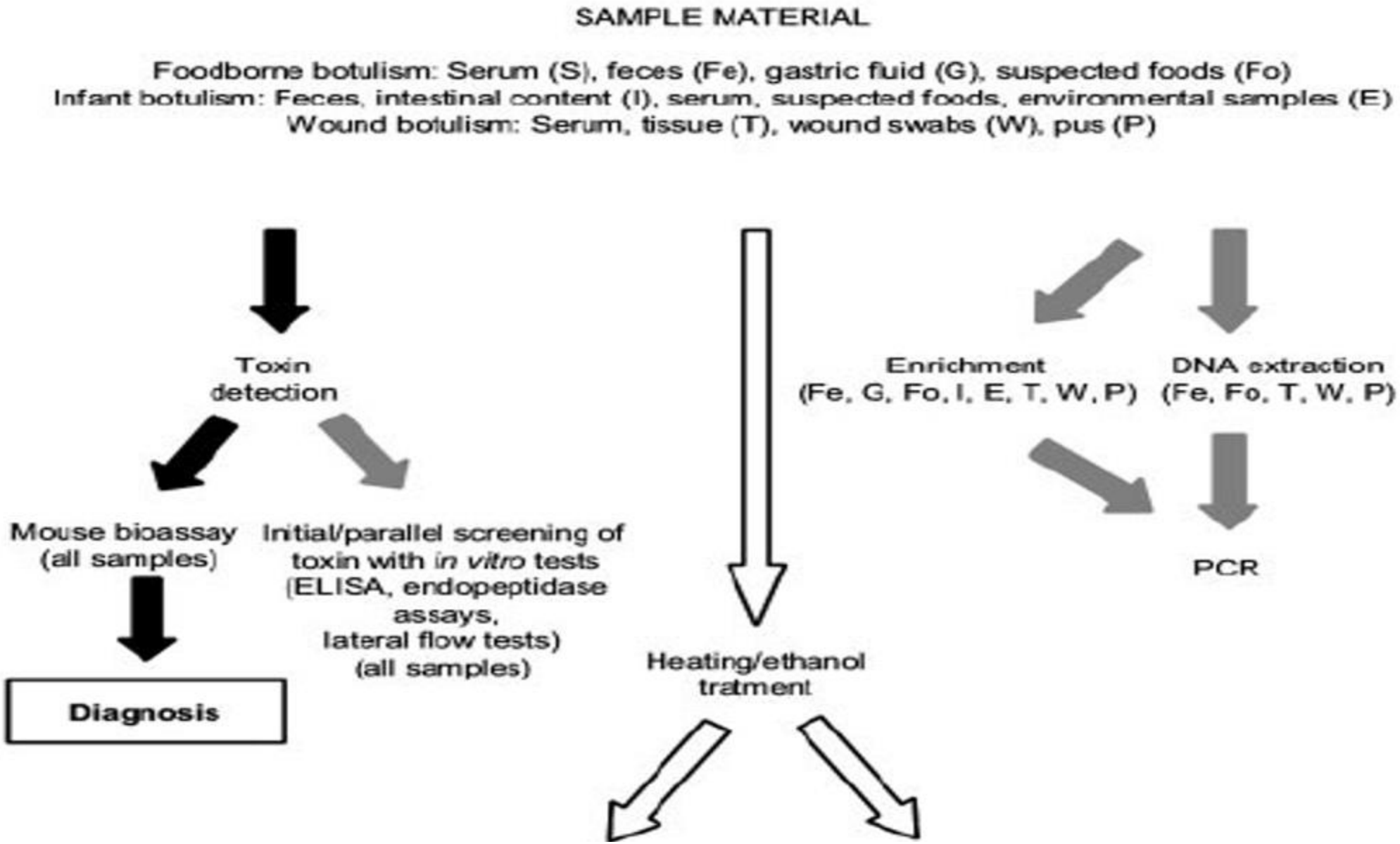
Botulism

Because testing for botulinum toxin is time consuming, diagnosis is heavily dependent on clinical presentation and examination.

If a clinician suspects botulism, antitoxin treatment and supportive therapy should start right away.

Laboratory confirmation of a botulism diagnosis should confirm clinical diagnoses

Botulism


SAMPLE MATERIAL
Foodborne botulism: Serum (S), feces (Fe), gastric fluid (G), suspected foods (Fo)
Infant botulism: Feces, intestinal content (I), serum, suspected foods, environmental samples (E)
Wound botulism: Serum, tissue (T), wound swabs (W), pus (P)
Toxin detection
Mouse bioassay (all samples)
Initial/parallel screening of toxin with in vitro tests (ELISA, endopeptidase assays, lateral flow tests) (all samples)
Diagnosis
Heating/ethanol tratment
Enrichment (Fe, G, Fo, I, E, T, W, P)
DNA extraction (Fe, Fo, T, W, P)
PCR

Botulism….cont