

NS-II Lab
Tetanus
Botulism
Rabies

Mathhar Ahmad Abu Morad MD

Mutah university

Faculty of Medicine

Diagnosis of Tetanus

Introduction

1. The usual route of infection is a soil-contaminated wound
2. Rarely done to diagnose tetanus
3. The laboratory diagnosis of tetanus involves the
 - A. Isolation and identification of *C. tetani*
 - B. Detection of toxigenicity in the isolate by mouse toxicity testing (The definitive test for the laboratory diagnosis of tetanus).

Diagnosis of Tetanus

Isolation and identification of *C. tetani*

1. Suitable specimens

- A. Wound swabs in Stuart's transport medium are the usual clinical specimens submitted for the laboratory diagnosis of tetanus.
- B. Surgical specimens also suitable when collected

2. Test details - Culture of *C. tetani*

- After incubation under anaerobic conditions at 35°C for 24 h, *C. tetani* produces a thin transparent film of swarming growth on the agar surface. Blood in blood agar plates is haemolysed. The thin film of growth may be difficult to detect.
- In Gram-stained smears of cultures at 24 h, the vegetative cells stain as gram-positive rods but sporing rods showing the typical round, terminal, distending spores (i.e. 'drumstick' spores)

Diagnosis of Tetanus

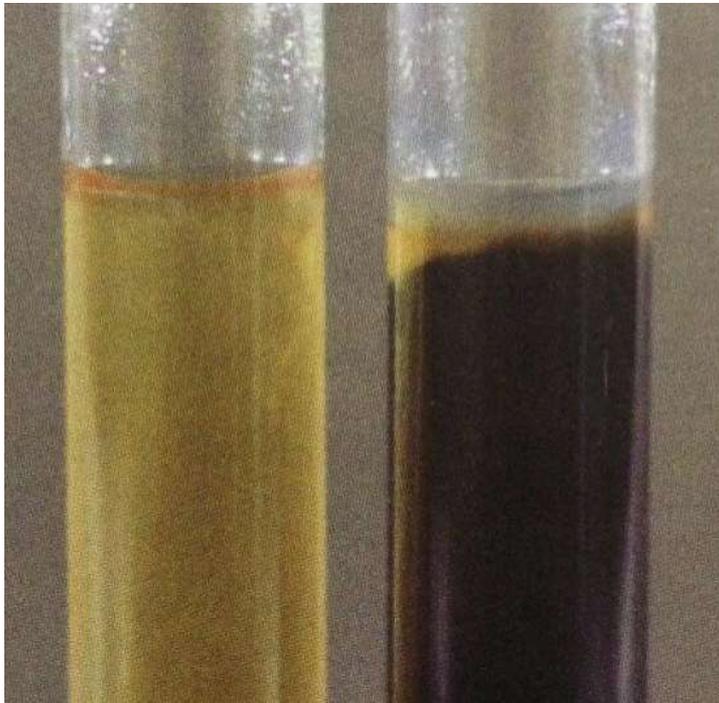
- Gram stain pattern of *C. tetanus*
- Anaerobic jar



Diagnosis of Tetanus

3. Biochemically

- C.tetani is an asaccharolytic.
- H₂S and DNase positive
- Nitrate reduction, aesculin , starch hydrolysis and lipase negative.



Diagnosis of Tetanus

Mouse testing for tetanus toxin (tetanospasmin)

Suitable specimens

- Cultures of *C. tetani* in Cooked Meat Medium (CMM) broth. The supernatant broth culture is filtered through a filter of 0.45 μm and small volumes of the filtrate are injected into mice.

Results

- Typical paralysis or death of the mice with prevention of these effects by the prior administration of tetanus antitoxin constitutes a positive test for tetanus toxin

Diagnosis of Tetanus

Culture of *C. tetani* produces a thin transparent film of swarming growth on the agar surface.



Diagnosis of Botulism

- To make a diagnosis, your doctor will ask questions and examine you to find out the cause of your symptoms.
- However, these clues are usually not enough to diagnose you because some diseases have symptoms similar to those of botulism, such as Guillain-Barré syndrome, stroke, myasthenia gravis.
- Your doctor may perform special tests to make a diagnosis. Some of these tests are:
 - Brain scan
 - Spinal fluid examination
 - Nerve and muscle function tests (nerve conduction study [NCS] and electromyography [EMG])
 - Test for myasthenia gravis
- If these tests don't determine what is making you sick, your doctor may order laboratory tests to look for the toxin and the bacteria that cause botulism.
- Start empirical therapy

Diagnosis of Botulism

- Botulism is confirmed in the laboratory by identifying botulinum neurotoxin in the serum, feces, vomitus, or gastric contents of patients, and/or in remnants of a food they consumed .
- Methods of identification:
 - I. Lab test
 - II. Mouse toxicity
 - III. Immunological methods.

Diagnosis of Botulism

Lab test

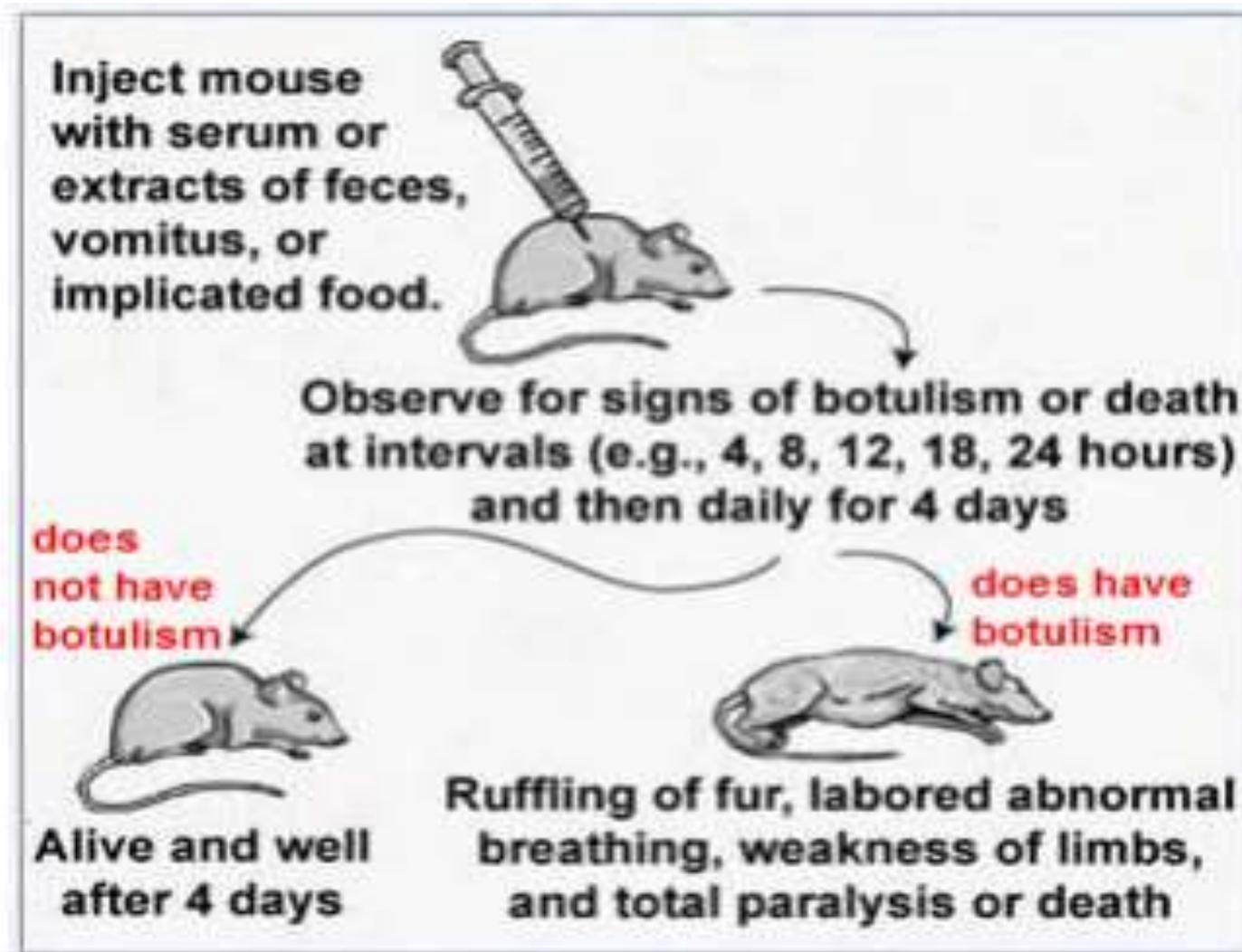
- Toxin and viable bacteria may be identified in serum, stool, vomitus, gastric aspirate, and suspected foods.
- *C botulinum* may be grown on selective media from samples of stool or foods.
- The specimens for toxin analysis should be refrigerated, but culture samples of *C botulinum* should not be refrigerated.
- Blood agar and egg yolk agar (EYA) are used for culturing samples.



Subterminal spore

Diagnosis of Botulism

Mouse toxicity



Diagnosis of Botulism

Immunological methods.

Compared with the mouse test, the immunoassays are technically simple and fast to perform and interpret.

- Radioimmunoassay
- Gel diffusion assay
- Passive hemagglutination assay
- Enzyme-linked immunosorbent assay (ELISA)

Laboratory Diagnosis of Rabies

In man:

- Specimens: Saliva, CSF, serum and urine.
- the direct fluorescent antibody (DFA) from corneal smears and skin biopsy.
- Isolation by inoculating saliva in mice or cell-culture.
- Detection of antibodies by serology.
- RNA detection by RT- PCR on clinical samples.

In Animals:

- Cytology of brain tissue for Negri inclusion bodies in nerve cells.
- Immunofluorescence to detect rabies antigens in the brain.
- Isolation by inoculating mice.
- Electron microscopy to detect the bullet-shaped virus.