

**NS-II Lab**  
**Tetanus**  
**Botulism**  
**Rabies**

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# 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

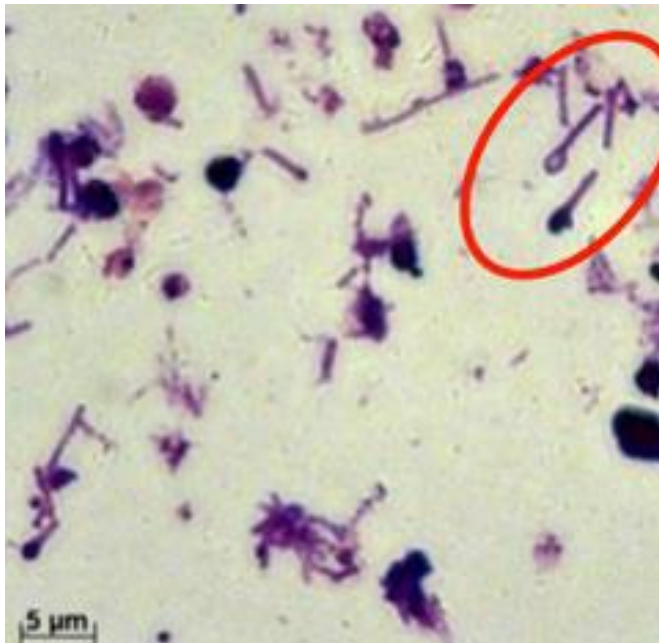
- A. Wound swabs in Stuart's transport medium are the usual clinical specimens submitted for the laboratory diagnosis of tetanus.
- B. Surgical specimens also suitable when collected

### 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

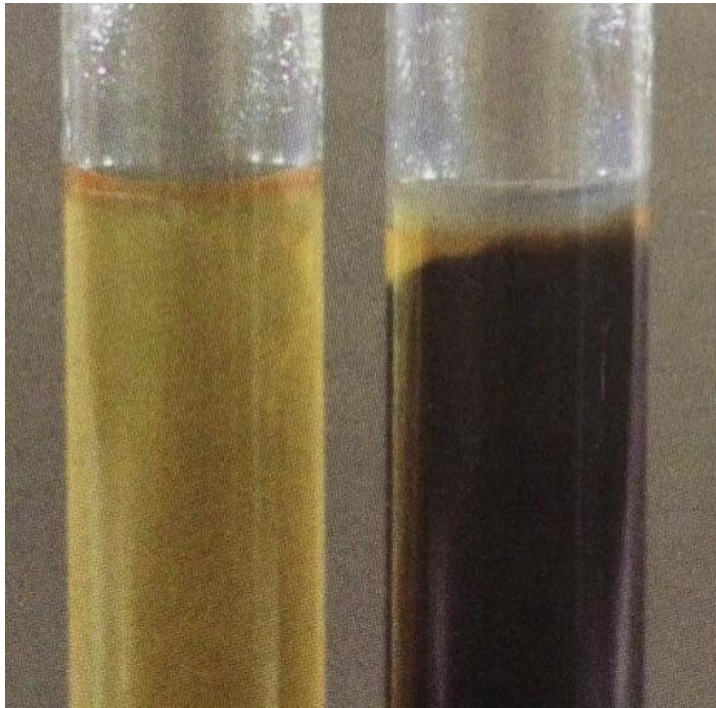
- Gram stain pattern of *C. tetanus*
- Anaerobic jar



# Diagnosis of Tetanus

## 3. Biochemically

- C.tetani is an asaccharolytic.
- H<sub>2</sub>S and DNase positive
- Nitrate reduction, aesculin and 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  $\mu\text{m}$  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



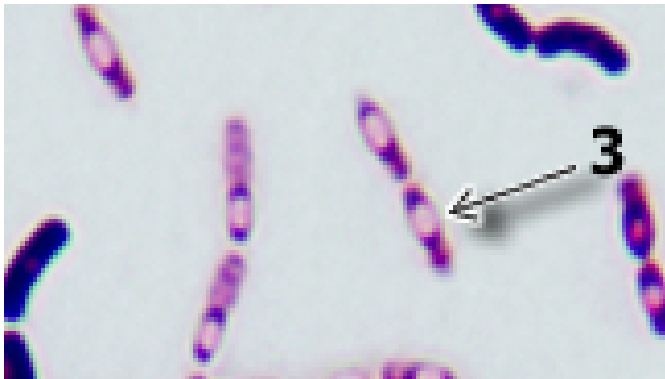
# Diagnosis of Botulism

- 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

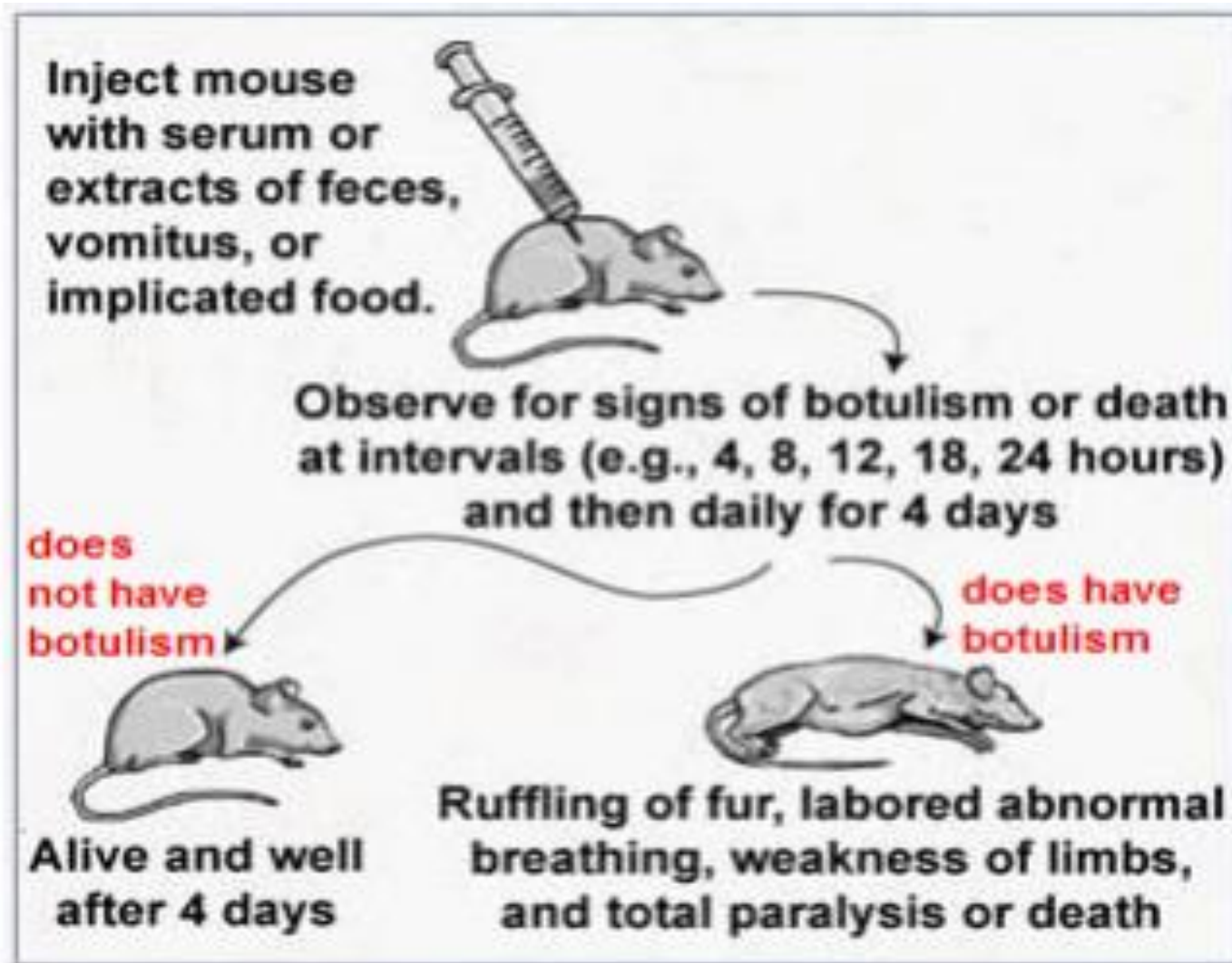
- Toxin and viable bacteria may be identified in serum, stool, vomitus, gastric aspirate, and suspected foods.
- *C botulinum* may be grown on selective media from samples of stool or foods.
- The specimens for toxin analysis should be refrigerated, but culture samples of *C botulinum* should not be 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, Urine
- the direct fluorescent antibody (DFA) from saliva, cornea smears and skin biopsy of neck or face
- Isolation by inoculating saliva in mice.
- Detection of antibodies by serology.
- RNA detection by RT- PCR in blood

## 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.