



PHLN

Public Health Laboratory Network

Tetanus (*Clostridium tetani*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *clostridium tetani*.

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1. Introduction

Tetanus is a rarely encountered but potentially fatal disease caused by a neurotoxin (tetanospasmin) produced by the anaerobic sporing bacterium, *Clostridium tetani*. The source/reservoir of infection is soil and the intestinal tracts of animals such as horses. The usual route of infection is a soil-contaminated wound, particularly a deep, penetrating wound with foreign material and necrotic tissue which promote multiplication of anaerobic *C. tetani* with production of toxin. However, tetanus may also develop after environmental contamination of trivial wounds. Prevention of tetanus depends on the presence of at least 0.01 U/ml of tetanus antitoxin in serum as the result of tetanus toxoid immunization.

The laboratory diagnosis of tetanus involves the isolation and identification of *C. tetani* and the detection of toxigenicity in the isolate by mouse toxicity testing. The latter is the definitive test for the laboratory diagnosis of tetanus. Serodiagnosis is not relevant because the clinical disease does not result in the production of tetanus antitoxin.

2. Tests

2.1 Isolation and identification of *C. tetani*

2.1.1 Suitable specimens

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

2.1.2 Test details - Culture of *C. tetani*

This species of *Clostridium* is an obligate anaerobe. 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) stain as gram-negative rods.

Biochemically, *C. tetani* is an asaccharolytic species of *Clostridium* that liquefies gelatin. The species also produces H₂S and DNAse but gives negative reactions for nitrate reduction, aesculin and starch hydrolysis and lipase and lecithinase activity. Gas liquid chromatographic analysis of metabolic products shows a predominance of acetic and butyric acids with a minor amount of propionic acid.

2.1.3 Test sensitivity

No quantitative data.

Anaerobic conditions in anaerobic jars must be assured by the use of catalysts that are regularly reactivated thermally and by monitoring with commercial redox indicators. Lack of an isolate does not preclude diagnosis of tetanus, which is clinical.

2.1.4 Test specificity

No quantitative data.

The morphological and biochemical characteristics described above identify an isolate as *C. tetani*. Presence of an isolate does not make the diagnosis, which is clinical.

2.1.5 Predictive values

As nontoxigenic strains of *C. tetani* have been reported, the identification of an isolate as *C. tetani* does not necessarily indicate that the causative agent of tetanus has been isolated and identified.

2.1.6 Suitable test acceptance criteria

An isolate is identified as *C. tetani* if it shows the typical growth requirements, colonial and microscopic morphological characteristics and gives the biochemical reactions as described in standard texts for this species.

2.1.7 Suitable internal controls

Documented testing of growth media, biochemical substrates and biochemical reagents with suitable control cultures.

2.1.8 Suitable original test validation criteria

Auditors should have available:

- detailed documentation of the characterization of the isolate;
- records of quality control on the batches of growth media, biochemical substrates and biochemical reagents used;
- records of the regular maintenance and proper functioning of the laboratory equipment used; and
- evidence of the competence of staff in the area of anaerobic bacteriology.

2.1.9 Suitable external Quality Control (QC) program

NATA/RCPA to ensure general competency. Nil specific.

2.1.10 Special considerations

The thin film of swarming growth of *C. tetani* on the agar surface may be missed altogether unless the bacteriologist is aware of the need to detect the growth of this (or other swarming species of *Clostridium*) by tracking with an inoculating loop across the agar surface.

2.1.11 References

1. Allen S D, Emery C L, Siders J A. *Clostridium*. In: Murray P R, Baron E J, Pfaller M A, Tenover F C, and Tenover F C, and Tenover F C editors. Manual of clinical microbiology, 7th ed. Washington D C: ASM Press, 1999: 654-671.
2. Cato E P, George W L, Finegold S M Genus *Clostridium*. In: Sneath P H A, Mair N S, Sharpe M E, Holt J G editors. Bergey's manual of systematic bacteriology, vol. 2. Baltimore: Williams & Wilkins, 1986: 1141-1200.

2.2 Mouse testing for tetanus toxin (tetanospasmin)

2.2.1 Suitable specimens

Cultures of *C. tetani* in Cooked Meat Medium (CMM) broth. The supernatant broth culture is filtered through a filter of 0.45 mm APD rating and small volumes of the filtrate are injected into mice. Broth cultures are tested for toxigenicity after incubation at 35°C for 18-24 h; and, if negative, are re-tested after incubation for up to 4 days.

2.2.2 Test details - Mouse toxicity testing

Small mice, preferably of body weight 15-18 g, are inoculated intramuscularly in the thigh with 0.1 and 0.3 ml volumes of filtrate. Control mice are inoculated intraperitoneally with 0.5 ml (1500 U) of tetanus antitoxin 1 h before injection of the filtrate. The tetanus antitoxin will specifically neutralize the neurotoxic effects of tetanospasmin. Smaller volumes/lesser amounts of tetanus toxin produce stiff paralysis in the leg of the mouse while larger volumes/more toxin tends to kill the animal within 18-24 h. These effects are prevented by the prior injection of mice with tetanus antitoxin.

2.2.3 Test sensitivity

No quantitative data.

The use of small mice of low body weight (e.g. 15-18 g) increases the sensitivity of tetanus toxin detection but larger animals can be used.

Tetanus toxin is released from *C. tetani* with autolysis and this release increases exponentially with time. Detection of the toxin in mice may require up to 4 days of incubation of *C. tetani* in CMM broth.

2.2.4 Test specificity

A stiff paralysis in the leg of the mouse on the side corresponding to the inoculated thigh is typical of tetanospasmin. Neutralisation of this paralytic effect or prevention of death in the mice by the prior administration of tetanus antitoxin to the control mice establishes specificity for tetanospasmin.

2.2.5 Predictive values

No quantitative data.

2.2.6 Suitable test acceptance criteria

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.

2.2.7 Suitable internal controls

Mice injected with filtered, uninoculated CMM broth supernatant and mice protected by prior administration of tetanus antitoxin provide the internal controls.

2.2.8 Suitable original test validation criteria

Auditors should have available:

- documented standard operating procedures for tetanus toxin testing in mice;
- detailed documentation of the test for tetanus toxin;
- evidence of suitable qualifications, training and experience of staff in the handling of mice; and
- approval of the relevant animal ethics experimentation committee for toxin testing in mice.

2.2.9 Suitable external QC program

None available.

2.2.10 Special considerations

A toxigenic strain of *C. tetani* may be wrongly identified as nontoxigenic if the time allowed for the autolytic release of tetanus toxin from the bacterium in CMM broth culture is inadequate. A time period of up to 4 days must be allowed.

2.2.11 References

1. Mayall B C, Snashall E A, Peel M M. Isolation of *Clostridium tetani* from anaerobic empyema. *Pathology* 1998; 30: 402-404.
2. Mitsui K, Mitsui N, Kobashi K, Hase J. High-molecular-weight haemolysins of *Clostridium tetani*. *Infect Immun* 1982; 35: 1086-1090.

3. Peel M M. Measurement of tetanus antitoxin. II. Toxin neutralization. J Biol Stand 1980; 8: 191–207.

3. Typing & Subtyping Methods

Not applicable

4. Laboratory Nomenclature for National Data Dictionary

4.1 Organism name(s) list

Clostridium tetani

4.2 Typing/subtyping nomenclature list(s)

Not applicable

5. PHLN Summary Laboratory Definition

Tetanus

5.1.1 Definitive Criteria

Isolation of *C. tetani* from a wound in a compatible clinical setting; or
(2) Prevention of positive tetanospasmin mouse test from such an isolate using specific tetanus antitoxin.

5.1.2 Suggestive Criteria

Nil

5.1.3 Guide for use

Diagnosis is primarily clinical

5.1.4 Parent PHLN Document Number (00-16)