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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.
IDENTIFICATION OF LISTERIA SPECIES, AND OTHER NON-SPORING GRAM-POSITIVE RODS (EXCEPT CORYNEBACTERIUM)

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the identification of Listeria species and other non-sporing Gram-positive rods (except Corynebacterium species) isolated from clinical specimens to genus or species level.

INTRODUCTION

For the identification of Corynebacterium species refer to BSOPID 2 - Identification of Corynebacterium diphtheriae, Corynebacterium ulcerans and Corynebacterium pseudotuberculosis.

A systematic approach is used to differentiate clinically encountered, morphologically similar, aerobic and facultatively anaerobic, non-sporing Gram-positive rods. The true branching organisms such as Actinomyces, Nocardia and Streptomyces species and those which produce spores are not described in this NSM. Rapidly growing Mycobacterium species may also be isolated on the media described in this document and acid-fast bacilli should be referred to the Reference Laboratory.

Taxonomy

Listeria

There are currently seven species in the genus Listeria: L. monocytogenes; L. ivanovii; L. seeligeri; L. innocua; L. welshimeri; L. grayi and L. murrayii.

Other non-sporing Gram-positive rods

The organisms classified as non-sporing Gram-positive rods are very diverse not only morphologically, but also metabolically and structurally.

Characteristics

Listeria species

Listeria species are short Gram-positive rods 0.5 x 0.5-3.0 μm, with rounded ends, occurring singly or in short chains and occasionally appearing filamentous. Colonies on blood agar are non-pigmented and may resemble those of β-haemolytic streptococci. Members of the genus are facultative anaerobes, non-sporing, non-acid fast and do not possess a capsule. Listeria species are motile by peritrichous flagella when grown at <30°C and display a characteristic “tumbling” motility. They are catalase positive, oxidase negative and ferment carbohydrates1,2. Listeria species are widely distributed in the environment and can be isolated from soil, decaying vegetable, food and feed. Listeria monocytogenes is an important pathogen which may affect pregnant patients, neonates, elderly individuals, and individuals who are immunocompromised. The most common clinical manifestation is diarrhoea. A mild presentation of fever, nausea, vomiting, and diarrhoea may thus resemble gastrointestinal illness. In pregnant women, L. monocytogenes often causes an influenza like illness that if untreated may result in abortion, stillbirth or premature birth. In non-pregnant human adults, L. monocytogenes may cause meningitis, encephalitis or bacteremia. L. monocytogenes is more usually isolated from blood, CSF, amniotic fluid, placenta, fetal tissue and food samples3,4.
Other non-sporing Gram-positive rods

**Arcanobacterium species**

*Arcanobacterium pyogenes* (formerly *Corynebacterium* or *Actinomyces pyogenes*) is a Gram-positive rod which may show branching. Colonies on blood agar produce sharp zones of β-haemolysis after 48 hours incubation. *A. pyogenes* is facultatively anaerobic, non-motile, and catalase-negative. Differentiation between *A. pyogenes* and *A. haemolyticum* may prove difficult but they may be distinguished by fermentation of α-mannose, pyrazinamidase and gelatin tests.

*Arcanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*) is a Gram-positive rod. Colonies on blood agar after 48 hours produce zones of β-haemolysis and are similar in appearance to *A. pyogenes*. *A. haemolyticum* is non-motile, facultatively anaerobic and, unlike *Corynebacterium* species, is catalase-negative.

*Arcanobacterium bernardiae* formerly (*Actinomyces bernardiae*) exhibits variable haemolysis.

**Aureobacterium species**

*Aureobacterium* species are Gram-positive, irregular, short rods and are catalase-positive. They are obligate aerobes, which produce acid from carbohydrates by oxidation rather than by fermentation. Strains may be vancomycin resistant and can be distinguished from *C. aquaticum* by casein and gelatin hydrolysis.

**Bifidobacterium species**

*Bifidobacterium* species vary in shape and may be curved, clubbed or branched rods or occasionally coccoid, Gram-positive forms, 0.5 - 1.3 x 1.5-8 µm. Cells often stain irregularly. Growth is anaerobic but some species can grow in air enriched with 10% CO₂. *Bifidobacterium* species are non-sporing, non-acid fast and non-motile. *Bifidobacterium* species ferment carbohydrates and are catalase-negative.

**Brevibacterium species**

*Brevibacterium* species are Gram-positive rods, which show a marked rod-coccus cycle. On fresh subculture, cells appear as bacilli but become coccoidal in older cultures. Colonies on blood agar are non-haemolytic and may turn a yellow to green colour after 48 hours incubation. *Brevibacterium* species are non-motile, aerobic, urease-negative and catalase-positive.

**Corynebacterium species**

(See BSOPID 2 - Identification of *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*)

**Cellulomonas species**

*Cellulomonas* species produce yellow or orange-pigmented colonies. They are catalase-positive and some species are motile. *Cellulomonas* species differ from *Oerskovia* species in that they lack hyphal growth.

**Dermabacter hominis**

*Dermabacter* species are very short Gram-positive rods that may be mis-interpreted as cocci. *Dermabacter hominis*, currently the only member of the genus, is non-haemolytic, non-motile and catalase-positive. *Dermabacter* species are fermentative and produce acid from glucose, lactose, sucrose and maltose. They hydrolyse aesculin and do not reduce nitrate or produce pyrazinamidase or DNase.

**Erysipelothrix rhusiopathiae**

*E. rhusiopathiae* is a Gram-positive rod, which produces α-haemolysis on blood agar. It is facultatively anaerobic, non-motile and catalase-negative. *Erysipelothrix* species can be distinguished from *Lactobacillus* species by its ability to produce H₂S in a triple sugar iron agar slant.
Gardnerella vaginalis

*Gardnerella vaginalis* is a pleomorphic, Gram-variable rod. It is facultatively anaerobic and non-motile. *G. vaginalis* is non-sporing, non-encapsulated and both oxidase-negative and catalase-negative.

**Lactobacillus species**

*Lactobacillus* species are long Gram-positive rods. Colonies are small and often α-haemolytic on blood agar after 48 h. They are facultatively anaerobic, rarely motile and catalase-negative.

**Microbacterium species**

*Microbacterium* species are Gram-positive slender rods. They may produce a yellow or orange pigment. The optimum growth temperature is 30°C. *Microbacterium* species are aerobic and some species are motile. All species are catalase-positive.

**Mycobacterium species**

*Mycobacterium* species other than *Mycobacterium tuberculosis* (MOTT) may be isolated on primary culture within 48 h for identification and/or susceptibility (see BSOP 40 - Investigation of specimens for *Mycobacterium* species). Refer to the Reference Laboratory.

**Oerskovia species**

*Oerskovia* species are Gram-positive branching rods. They form a mycelium, an extensively branching substrate hypha that breaks up to form rod-shaped or coccoid-rod elements. Most strains produce a yellow pigment. They are facultatively anaerobic, motile and catalase-positive.

**Propionibacterium species**

*Propionibacterium* species are Gram-positive pleomorphic rods (short “Y” forms). Strains generally grow better anaerobically, particularly on primary isolation, producing small colonies after 48 hours.

*Propionibacterium* species are facultatively anaerobic and are non-motile. They are catalase-positive except *Propionibacterium propionicum* (formerly known as *Arachnia propionica*), which is catalase-negative.

**Rhodococcus species**

*Rhodococcus* species usually stain Gram-positive. Cells form as cocci or short rods which grow in length, and may form an extensively branched vegetative mycelium which may fragment. They are usually partially acid-fast. Colonies may be rough, smooth or mucoid and are colourless, cream, beige, yellow, orange or red.

**Rothia dentocariosa**

*R. dentocariosa* is a Gram-positive irregular rod which may show branching in young cultures. In older broth cultures cells may be coccoid, which distinguishes them from *Actinomyces* species. It grows well on simple media and colonies may be creamy, dry, crumbly or mucoid. *Rothia* are facultatively anaerobic, non-motile, catalase-positive and ferment carbohydrates.

**Turicella otiitis**

The genus comprises a single species, *Turicella otiitis*. Microscopically it resembles a coryneform but has longer cells. It may be distinguished by colonial morphology from *C. afermentans* and *C. auris*. *T. otiitis* colonies are convex, whitish, creamy and non-haemolytic compared with the flat, grey-white and non haemolytic colonies of *C. afermentans* and the convex, dry, adherent, yellowish colonies of *C. auris*. *T. otiitis* is non-fermentative and occurs either alone or with Gram-negative rods. Isolates exhibit a strong CAMP reaction and are catalase-positive. *T. otiitis* may be misidentified, often as *Corynebacterium* species, by some commercial identification systems.
**Principles of identification**

**Listeria species**
Colonies on blood agar or *Listeria* selective agar are identified by colonial appearance, Gram stain, catalase production and tumbling motility at ambient temperature. If confirmation of identification is required, isolates should be sent to the Reference Laboratory. All identification tests should ideally be performed from non-selective agar.

**Other non-sporing Gram-positive rods**
Colonies on blood agar are identified by colonial appearance, Gram stain, catalase-production and motility. Identification is confirmed by further biochemical tests and/or referral to a Reference Laboratory. All identification tests should ideally be performed from non-selective agar.

**TECHNICAL INFORMATION**

N/A
1 SAFETY CONSIDERATIONS

Hazard Group 2 organisms
Pregnant staff should be prohibited from working with known or suspected cultures of *Listeria* species.

Refer to current guidance on the safe handling of all organisms documented in this NSM.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

2 TARGET ORGANISMS

*Listeria* species reported to have caused human infection

*Listeria monocytogenes*
*Listeria ivanovii*
*Listeria seeligeri*

Species morphologically similar to *Listeria* species known to have caused human infection

*Arcanobacterium* species
*Aureobacterium* species
*Bifidobacterium* species
*Brevibacterium* species
*Cellulomonas* species
*Dermabacter hominis*
*Erysipelothrix rhusiopathiae*

Other Gram-positive rods have been implicated in Human infection including *Corynebacterium* species (BSOPID 2 - Identification of *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*).

3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

Gram stain (BSOPTP 39 - Staining Procedures)

Gram-positive rods. Microscopic appearance varies with the species.
Listeria
Gram-positive rods approximately 0.5 x 0.5 - 3 µm with rounded ends, occurring singly or sometimes in pairs and may resemble 'coryneforms' or diplococci. Non-sporing, non-branching and non-capsulated.

3.2 PRIMARY ISOLATION MEDIA
Blood agar incubated in 5 - 10% CO₂ at 35°C – 37°C for 16 - 48 h.
Listeria selective agar incubated in O₂ at 35°C – 37°C for 40 - 48 h.

3.3 COLONIAL APPEARANCE
Listeria species
All Listeria species produce non-pigmented colonies on blood agar, which have a characteristic ground glass appearance. The appearance of L. monocytogenes, L. ivanovii, and L. seeligeri is described below. On Listeria Selective Agar (containing aesculin), Listeria species appear as black colonies with a surrounding black zone produced by hydrolysis of the aesculin.

L. monocytogenes
Blood agar: colonies are 0.5 - 1.5 mm in diameter, smooth, translucent and emulsifiable, with zones of hazy β-haemolysis. Colonies resemble those of Group B streptococci. Non-haemolytic colonies occasionally occur.

L. ivanovii
Blood agar: colonies are similar to L. monocytogenes but develop larger zones of complete haemolysis with outer zones of partial haemolysis.

L. seeligeri
Blood agar: zones of β-haemolysis are produced.

All other non-sporing Gram-positive rods
Appearance varies with species on blood agar, after aerobic incubation at 35 – 37 °C for 16 – 48 h.

Arcanobacterium species
β-haemolysis, which may not be present after 24 h. Haemolysis of A. bernardiae is variable.

Aureobacterium species
Non-haemolytic, yellow pigment.

Bifidobacterium species
Colonies are low, greyish-brown, ovoid with a brown opaque centre and a translucent crenated edge.

Brevibacterium species
Non-haemolytic, may turn yellow to green after 48 h.

Cellulomonas species
Non-haemolytic, yellow- or orange-pigmented.

Dermabacter hominis
Non-haemolytic, small grey/white convex colonies.
**Erysipelothrix rhusiopathiae**
At 48 h two distinct colony types appear: a small smooth form, 0.3 -1.5 mm, transparent, convex and circular with entire edges. The large rough form is flatter, more opaque, with a matt surface and an irregular edge. Both exhibit α-haemolysis

### 3.4 TEST PROCEDURES

**Catalase activity test** [BSOPTP 8 - Catalase Test]

*Listeria* species are catalase-positive in contrast to Group B streptococci which are catalase negative. The catalase test results for other non-sporing Gram-positive rods varies.

### Summary table of results

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</tr>
<tr>
<td><em>L. ivanovii</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><em>L. seeligeri</em></td>
<td>+</td>
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<td>–</td>
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</table>

**Motility test** - (see [BSOPTP 21 - Motility Test](#))

Performed at 37°C and <30°C for all organisms.

All *Listeria* species exhibit tumbling motility at ambient temperatures (<30°C) but not at 37°C. For other organisms results vary.

**Commercial identification system**

### 3.5 CONFIRMATION

Following motility, biochemical identification and/or the Reference Laboratory results.

### 3.6 STORAGE AND REFERRAL

If required, save the pure isolate on a blood or nutrient agar slope for referral to the Reference Laboratory.

### 3.7 TECHNICAL INFORMATION

Listeria colonies resemble those of Group B streptococci, and the catalase test is a rapid, easily performed test which will help differentiate *Listeria* species from Group B, BHS. Listeria are catalase-positive whereas Group B streptococci are catalase-negative.
4 IDENTIFICATION OF \textit{LISTERIA} SPECIES, AND OTHER NON-SPORING GRAM-POSITIVE RODS (EXCEPT \textit{CORYNEBACTERIUM})

- Clinical specimens
  - Primary isolation plate

- Blood agar
- \textit{Listeria} selective agar containing Aesculin

  Non-pigmented translucent haemolytic colonies
  (Non-haemolytic strains of \textit{L. monocytogenes} occur occasionally)

  Black colonies with a black/brown zone around colonies (\textit{Listeria})
  (if required, subculture to blood agar to view haemolysis)

  Colonial morphology and haemolysis varies with other Gram-positive rods (see 5.1)

  Gram's stain on pure culture

  Gram-positive rods
  If there is a different Gram's stain appearance refer to the appropriate NSM

  Catalase (Appendix)

  Positive
  - Tumbling motility
    - Tumbling motility
      - Positive
        - \textit{Listeria} species
          - All \textit{Listeria} species
          - Commercial identification system

  Negative
  - Other motility
    - Further identification if clinically indicated

  If required, save pure isolate on a blood or nutrient agar slope for referral to the Reference Laboratory
5 REPORTING

5.1 PRESUMPTIVE IDENTIFICATION
If appropriate growth characteristics, colonial appearance, Gram stain of the culture and catalase results are demonstrated.

5.2 CONFIRMATION OF IDENTIFICATION
N/A

5.3 MEDICAL MICROBIOLOGIST
Inform the medical microbiologist of all presumptive and confirmed L. monocytogenes isolates and Listeria species when the request card bears relevant information e.g.
- the patient is pregnant, immunocompromised or newborn
- suspicion of septicaemia and/or meningo-encephalitis in persons with alcoholism, substance abuse, and other serious underlying disorders such as cancer, or patients receiving treatments for cancer which induce neutropenia and/or mucositis
- investigation of outbreaks

Inform the medical microbiologist of presumptive and confirmed non-sporing Gram-positive rods when the request card bears relevant information e.g.
- cases of suspected endocarditis
- infection of indwelling medical devices (prosthetic valves, pacemakers, peritoneal and vascular catheters, CSF shunts)
- history of substance abuse, alcoholism, immunodeficiency or other serious underlying disorder such as cancer, or patients receiving treatment for cancer, inducing neutropenia and/or mucositis

Follow local protocols for reporting to clinician

5.4 CCDC
Refer to local Memorandum of Understanding.

5.5 CENTRE FOR INFECTION
Refer to current guidelines on CDSC and COSURV reporting.

5.6 INFECTION CONTROL STAFF
Inform the infection control team of presumptive and confirmed isolates of L. monocytogenes.
6 REFERRALS

6.1 REFERENCE LABORATORY

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to:
http://www.hpa.org.uk/cfi/reference_tests_index.htm

Suspected *Listeria* isolates for confirmation:
Food Safety and Microbiology Laboratory
Centre for Infections
61 Colindale Avenue
London
NW9 5HT
http://www.hpa.org.uk/cfi/fsml/default.htm
Contact Centre for Infections main switchboard: Tel. +44 (0) 20 8200 4400

Gram-positive rods for further characterisation:
Laboratory of Hospital Infection
Health Protection Agency
Specialist and Reference Microbiology Division
61 Colindale Avenue
London
NW9 5HT
http://www.hpa.org.uk/cfi/lhcai/default.htm
Contact Centre for Infections main switchboard: Tel. +44 (0) 20 8200 4400
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For further information please contact us at:

Standards Unit
Evaluations and Standards Laboratory
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ
E-mail: standards@hpa.org.uk
APPENDIX: CHARACTERISTICS TO DISTINGUISH AMONG BETWEEN NON-SPORING GRAM-POSITIVE RODS ON BLOOD AGAR – GUIDANCE

The flowchart is for guidance only
Consider Mycobacterium species (refer to the Reference Laboratory)
The flowchart is for guidance only

IDENTIFICATION OF LISTERIA SPECIES AND OTHER NON-SPORING GRAM-POSITIVE RODS (EXCEPT CORYNEBACTERIUM)
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Email: standards@hpa.org.uk
REFERENCES


