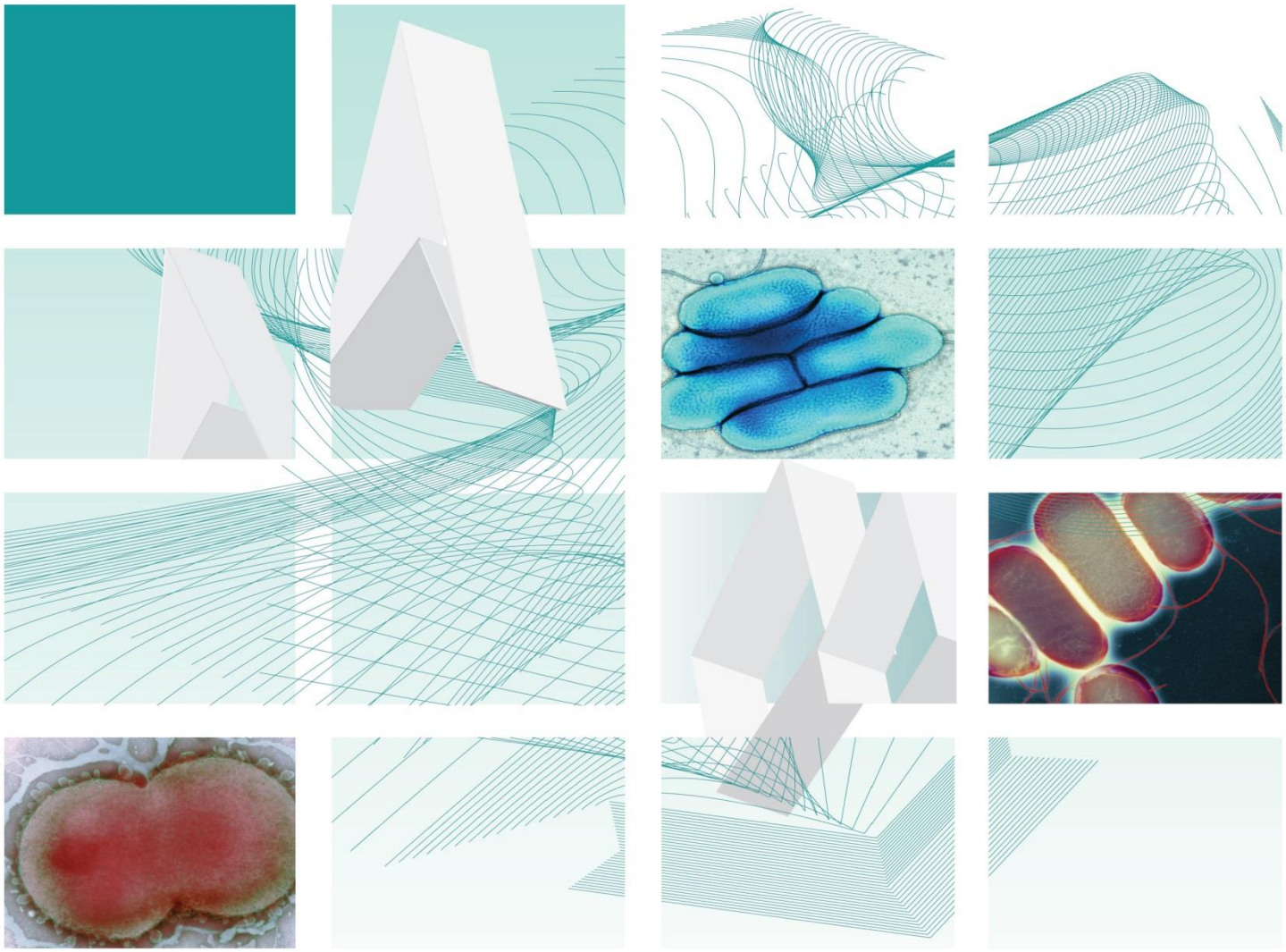
UK Standards for Microbiology Investigations

Identification of *Listeria* species, and other non-sporing Gram positive rods (except *Corynebacterium*)



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Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	12/02.06.20
Issue number discarded	4
Insert issue number	4.1
Anticipated next review date*	24.03.23
Section(s) involved	Amendment
Whole document.	To section 8.3 sentence “Some strains of *L. monocytogenes* can be non-haemolytic” added. Section 8.6 “if required” removed Section 9.3 amended with the link to reference laboratory user manual and request forms.

Amendment number/date	11/24.03.20
Issue number discarded	3.2
Insert issue number	4
Anticipated next review date*	24.03.23
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format Taxonomy of *Listeria* species updated. Minor amendments to characteristics of organisms. Information on whole genome sequencing added Phenotypic characteristics of *Listeria* and other non-sporing gram positive represented in a table. Tryptose media no longer in regular use in the laboratories and has been removed from this document.

*Reviews can be extended up to five years subject to resources available.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK Standard for Microbiology Investigation (UK SMI) 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.

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 UK SMI. Rapidly growing *Mycobacterium* species may also be isolated on the media described in this document. Acid-fast bacilli should be referred to the Reference Laboratory.

For the identification of *Corynebacterium* species, refer to ID 2 - Identification of *Corynebacterium* species.

This UK SMI includes both biochemical tests and automated methods for the identification of microorganisms. Some biochemical tests may not be done routinely in laboratory except in cases where confirmation by an alternative technique is required or automated methods are not available.

This UK SMI should be used in conjunction with other relevant UK SMIs.

4 Introduction

4.1 Taxonomy/characteristics

Listeria species[1-4]

There are currently nineteen validly named species in the genus *Listeria*: *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, L. grayi, L. fleischmannii, L. marthii, L. rocourtiae L. weihenstephanensis*, *L. aquatica, L. booriae, L. cornellensis, L. floridensis, L. grandensis, L. newyorkensis, L. costaricensis, L. murrayi and L. riparia.* Of these nineteen species, the first six can potentially cause infections in humans, albeit rarely in some cases.

The type species is *Listeria monocytogenes*.

Listeria species are short Gram positive rods, 0.4 to 0.5 × 0.5 to 2.0 µm, with rounded ends, occurring singly or in short chains. Filaments of 6 to 20 µm in length may occur singly or in short chains. Members of the genus are facultative anaerobes, non-sporing, non-acid fast and do not possess a capsule. The optimum growth temperature (but not for motility) is 30-37 °C.

Colonies on blood agar are non-pigmented and may resemble those of β-haemolytic streptococci but can be distinguished by a positive catalase test. Colonies will vary in colour and form depending on which commercial chromogenic agar is being used.

Listeria species are catalase positive, oxidase negative and ferment carbohydrates, they are widely distributed in the environment; some species are pathogenic for humans and animals.

The medically important species are:

***L. monocytogenes*[3]**

Microscopically, they appear as small rods, which are sometimes arranged in short chains. In direct smears they may be coccoid and may be mistaken for streptococci. Longer rods may resemble Corynebacterium. Haemolytic activity on blood agar has been used as a marker to distinguish *Listeria monocytogenes* from other *Listeria* species, but it is not an absolutely definitive criterion. Further biochemical characterisation may be necessary to distinguish between the different *Listeria* species. They are catalase positive and oxidase negative.

L. monocytogenes is the agent of listeriosis, a serious infection caused by eating food contaminated with the bacterium such as soft cheese, milk, pâtés, cooked sliced meats and prepacked sandwiches. Listeriosis has been recognised as an important public health problem and the disease affects primarily pregnant women, neonates, elderly people, and those with weakened immune systems.

***L. ivanovii*[5]**

The species has been divided into 2 subspecies. These are; *Listeria ivanovii* subsp. *ivanovii* and *Listeria ivanovii* subsp. *londoniensis*[6]. They are facultative anaerobes and have been isolated from healthy animals and human carriers and from the environment.

***L. seeligeri*[7]**

Cells are small (0.4 to 0.8 × 0.5 to 2.5µm), gram positive rods. Growth occurs at 4 °C within 5 days. They are facultative anaerobes and have been isolated from plants, soil, and animal faeces (sheep) in Europe.

***L. innocua*[8,9]**

L. innocua are mesophilic, operating at an optimal temperature range of 30-37 °C with a very complex metabolism. They are capable of metabolising methane, sulphur and nitrogen, among many other organic and inorganic compounds. These organisms also carry out numerous biosynthetic pathways, including peptidoglycan synthesis.
L innocua, like other members of their genus, are facultative anaerobes, which means that they can metabolise glucose (and other simple sugars) in both aerobic and anaerobic conditions. Under the aerobic metabolism of glucose, they form lactic acid and acetic acid. However, under anaerobic conditions, the metabolism of glucose yields only lactic acid.

The species is widespread in the environment and in food and has also been associated with one reported case of fatal bacteraemia.

***L. welshimeri*[7]**

Cells are small (0.4 to 0.5 × 0.5 to 2.0µm) rods which are motile by means of peritrichous flagella.

Metabolism is facultatively anaerobic. Acid, but no gas, is produced from D-glucose, D-xylose, and a-methyl-D-mannoside. Acid may or may not be produced from L-rhamnose. Acid is not produced from D-mannitol. They are positive for catalase, aesculin hydrolysis, Voges-Proskauer and methyl red tests, and negative for oxidase, urea, gelatin hydrolysis, indole and H_2S production as well as reduction of nitrates.

This species has been isolated from decaying plants and soil.

L. grayi[10]

According to Rocourt *et al.* (1992), *Listeria grayi* is an earlier heterotypic synonym of *Listeria murrayi* and so both were assigned to a single species, *Listeria grayi*.

Cells are small peritrichous rods which are motile.

Metabolism is facultatively anaerobic and catalase positive. Oxidase, urea and gelatin hydrolysis, H_2S and indole production is negative. Voges-Proskauer and methyl red tests are positive. Reduction of nitrates to nitrites is variable. Acid, without gas, is produced from glucose, mannitol, and other sugars. Sheep erythrocytes are not haemolysed.

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.

Arcanobacterium species[11,12]

There were 13 species of which 5 have been re-assigned to the genus *Trueperella* and of the remaining 8 species, only one is known to infect humans, *Arcanobacterium haemolyticum*.

Both rod-shaped and coccoid cells are Gram positive, non-acid-fast and non-motile. They are facultative anaerobes. Growth is considerably enhanced in an atmosphere of CO_2. Growth is sparse on ordinary media but enhanced on blood or serum containing media. Optimum temperature for growth is 37 °C. They are unable to withstand heating at 60 °C for 15 min.

Arcanobacterium species may give variable catalase reactions and are positive for CAMP-test. See species below.

Arcanobacterium haemolyticum[13]

Species have been isolated from the throat of infected individuals. After 48 hr colonies on blood agar produce zones of β-haemolysis and are similar in appearance to *Trueperella pyogenes.*

Aureobacterium species[14,15]

Aureobacterium species 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[1,16]

Bifidobacterium species vary in shape and may be curved, clubbed or branched rods or occasionally coccoid, Gram positive forms, 0.5 to 1.3 × 1.5 to 8µm. Cells often stain irregularly. Growth is anaerobic but some species can grow in air enriched with 10%

CO_2. *Bifidobacterium* species are non-sporing, non-acid fast and ferment carbohydrates.

Brevibacterium species[13,17]

Brevibacterium species are non-motile, salt tolerant (>6.5% NaCl), aerobic, urease negative and catalase positive.

Cellulomonas species[13,18]

There are over 20 *Cellulomonas* species, of which only *C. hominis* and *C. denverensis* have been isolated from humans. They may be non-motile or motile due to single or sparse lateral flagella. One of their main distinguishing features is their ability to degrade cellulose, using enzymes such as endoglucanase and exoglucanase. They are both oxidative and fermentative in their metabolism. *Cellulomonas* species differ from *Oerskovia* species in that they lack hyphal growth.

Cutibacterium species (formerly *Propionibacterium* species)[19,20]

The genus *Cutibacterium* has only 3 species which have been re-assigned from the genus *Propionibacterium* and they are *C. acnes*, *C. avidum* and C*. granulosum*. *Cutibacterium* species are Gram positive pleomorphic non-motile rods and generally grow better anaerobically.

The type species is *Cutibacterium acnes*.

Dermabacter hominis[13]

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 produce alkaline phosphatase, pyrrolidonyl arylamidase, leucine aminopeptidase and DNase. They do not reduce nitrate or produce pyrazinamidase.

Erysipelothrix rhusiopathiae[21]

E. rhusiopathiae produces a narrow zone of α-haemolysis on blood agar. It is facultatively anaerobic, non-motile and catalase negative. All colonies are clear, circular and very small increasing in size and tending towards a pale blue opacity with further incubation or age. *Erysipelothrix* species can be distinguished from *Lactobacillus* species by its ability to produce H_2S in a triple sugar iron agar slant.

Gardnerella vaginalis[1,16]

Gardnerella vaginalis is a pleomorphic, Gram variable rod or coccobacilli. It is facultatively anaerobic and non-motile. *G. vaginalis* is non-sporing, non-encapsulated and both oxidase negative and catalase negative. Acid is produced from glucose and other carbohydrates but not gas. It hydrolyses hippurate and does not reduce nitrate.

Lactobacillus species[1,16]

Lactobacillus species are long Gram positive rods. Colonies can vary from small to medium, grey and often α-haemolytic on blood agar after 48hr. They are facultatively anaerobic, rarely motile and catalase negative. They produce lactic acid as a major end product.

Microbacterium species[13,18]

Microbacterium species are small, slender, irregularly shaped Gram positive rods. The optimum growth temperature is 30 °C. The species are primarily oxidative and aerobic in their metabolism, but some species may be fermentative. They may be non-motile or motile by means of 1 to 3 flagella. Most species are catalase positive but catalase negative strains may be observed.

Mycobacterium species

Mycobacterium species other than *Mycobacterium tuberculosis* (MOTT) may be isolated on primary culture within 48hr for identification and/or susceptibility (B 40 - Investigation of specimens for *Mycobacterium* species). Refer to the National Mycobacterium Reference Services-South.

Oerskovia species[13]

Oerskovia species are non-spore forming Gram positive bacteria. They form a mycelium, an extensively branching substrate hypha that breaks up to form rod-shaped motile or non-motile coccoid-rod elements. No aerial hyphae are formed. They are facultatively anaerobic, fermentative and catalase positive. Motility is variable.

Propionibacterium species[13]

Propionibacterium species are Gram positive pleomorphic rods (short “Y” forms). Strains generally grow better anaerobically, particularly on primary isolation, producing small colonies after 48hr. *Propionibacterium* species are facultatively anaerobic and are non-motile. They are catalase positive except *Propionibacterium propionicum* (now known as *Pseudopropionibacterium propionicum*), which is catalase negative.

Note: It should be noted that within this genus, some of the species have been reclassified to other genera.

Rhodococcus species[22]

Rhodococcus species are usually 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 due to the mycolic acid in their cell walls. Incubation at 30 °C also increases recovery.

Although other biochemical tests help to distinguish *Rhodococcus* from other organisms, differentiation from other aerobic actinomycetes can be difficult. *Rhodococcus* species typically react positively in catalase, nitrate reduction, and urea hydrolysis tests and negatively with oxidase, gelatin hydrolysis, and carbohydrate reduction. They are non-motile and non-sporulating. Their inability to ferment carbohydrate is important in distinguishing them from *Corynebacteria*.

Trueperella species[11,23]

There are currently five validly named species in the genus *Trueperella* and two of these cause infections in humans.

Cells are, non-motile, non-spore-forming coccobacilli. They vary in shape and size (0.2 to 0.9 × 60.3 to 2.5µm) in different media. Cells from 24hour old broth cultures are Gram positive, but may be Gram variable in older cultures. Colonies of *Trueperella* species grow as pinpoint β-haemolytic colonies. This occurs on sheep’s blood agar after 24hr of incubation. They are aerobic and facultatively anaerobic. Members are

strictly fermentative. Lactic acid is the primary metabolic product in glucose yeast extract broth but acetate and succinate are minor products.

The type species is *Trueperella pyogenes*.

***Trueperella pyogenes*[24]**

Trueperella pyogenes is a rod which may show branching.*T. pyogenes* is facultatively anaerobic, non-motile, and catalase negative but one strain has been reported as positive. Metabolism is strictly fermentative. Differentiation between *T. pyogenes* and *A. haemolyticum* may prove difficult but they may be distinguished by fermentation of α-mannose, pyrazinamidase and gelatin tests.

This organism is frequently isolated from a wide variety of pyogenic disease conditions in many species of domestic animals and in humans.

***Trueperella bernardiae*[25]**

Trueperella bernardiae is a rod with coccobacilli predominating. Primary branching is not observed. *T. bernardiae* is facultatively anaerobic, non-motile, catalase negative and unable to produce acid from glucose. after 48hr incubation. Colony diameters range from 0.2mm to 0.5mm after 48hr incubation.

This species has been isolated from human blood as well as abscesses from ear and chest.

***Turicella otitidis*[13,18,26]**

The genus comprises a single species, *Turicella otitidis*. Microscopically it resembles a coryneform but has longer cells. *T. otitidis* colonies are convex, whitish and creamy in colour and range from 1.0 to 2.0 mm in diameter after 48h of incubation. It is non-fermentative and occurs either alone or with Gram negative rods. Isolates exhibit a strong CAMP reaction and are DNase and catalase positive.

T. otitidis may be misidentified, often as *Corynebacterium* species, by some commercial biochemical identification systems. This organism has been isolated from middle-ear fluids of patients with otitis media.

5 Technical information/limitations

Differentiation of *Listeria* species from Group B streptococci

Colonies of *Listeria* species 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 streptococci. *Listeria* species are catalase positive whereas Group B streptococci are catalase negative.

Motility test

Motility is one of many parameters used in the characterisation of *Listeria* species. It should be used in conjunction with other tests. This test should not be used for primary identification of *Listeria* species or purposes other than the investigation of motility.

Listeria species are motile by peritrichous flagella when at 20 to 25 °C and non-motile at 35 °C and above. Therefore, an appropriate temperature must be chosen for incubation to avoid false negative results. There have been occasional non-motile strains[27].

6 Safety consideration[28,29]

Hazard Group 2 organisms

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

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[29].

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

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

Compliance with postal and transport regulations is essential

7 Target organisms

***Listeria* species and other morphologically similar Gram positive rods reported to have caused human infection[13,30]**

Listeria monocytogenes, Arcanobacterium species*, Erysipelothrix rhusiopathiae*

Other *Listeria* species reported to have caused occasional or single human infections[9,31]

Listeria ivanovii, Listeria seeligeri, Listeria innocua, Listeria grayi

Other species morphologically similar to *Listeria* species known to have caused human infection[13,28,29]

Aureobacterium species, *Bifidobacterium* species, *Brevibacterium* species, *Cellulomonas* species, *Dermabacter hominis*

Other Gram positive rods have been implicated in human infections[13,18].

8 Identification

8.1 Microscopic appearance

Gram stain (TP 39 - Staining procedures)

Gram positive rods. Microscopic appearance varies with the species.

***Listeria* species**

Gram positive rods approximately 0.5 × 0.5 – 3µm with rounded ends, occurring singly or sometimes in pairs and may resemble 'coryneforms' or diplococci. They are non-sporing, non-branching and non-capsulated.

***Arcanobacterium* species**

Gram positive rod-shaped and coccoid cells; they are slender, irregular and predominately rod-shaped or arranged at an angle to give V-formations during the first 18hr of growth, becoming granular and segmented, resembling small, irregular cocci over time.

***Aureobacterium* species**

Cells are Gram positive irregular short rods.

***Bifidobacterium* species**

These species are Gram positive, vary in shape and may be curved, clubbed or branched rods or occasionally coccoid forms, 0.5 to 1.3 × 1.5 to 8µm. Cells often stain irregularly. Colonies are convex, entire and cream to white, smooth, glistening and soft.

***Brevibacterium* species**

Gram positive rods and they show a marked rod-coccus cycle. On fresh subculture, cells appear as bacilli but become coccal in older cultures.

***Cellulomonas* species**

Cells are Gram positive slender irregular rods that produce yellow or orange pigmented colonies

***Cutibacterium* species**

Gram positive pleomorphic rods. Colonies appear as small, round, opaque, white to off-white colonies which are non-haemolytic but sometimes are ß haemolytic after 5 to 6 days.

Dermabacter hominis

They are very short Gram positive rods that may be misinterpreted as cocci.

For all the other non-sporing Gram positive rods, see the section for “Characteristics”.

Erysipelothrix rhusiopathiae

Cells are slender Gram positive non-sporulating rods occurring in short chains, in pairs, in a "V" configuration or even grouped randomly. This organism can appear Gram variable because of their tendency to decolourise rapidly.

***Trueperella* species**

Cells are Gram positive, coccobacilli and short rods that occur singly, in pairs (V, T and palisade formations) or in clusters.

8.2 Primary isolation media

Blood agar incubated in 5 to 10% CO_2 at 35 °C to 37 °C for 16 to 48hr.

Listeria selective agar incubated in O_2 at 35 °C to 37 °C for 40 to 48hr.

Note: *Listeria* species are also capable of growth at 2 °C to 43 °C.

8.3 Colonial appearance

Table 1: Summary of the organisms and their appearances on blood agar plate.

Organism	**Characteristics of growth on blood agar after incubation at 35 to 37 °C for 16 to 48hr**
L. monocytogenes	Colonies are 0.5 to 1.5mm in diameter, smooth, translucent with a characteristic ground glass appearance able to be emulsified and with a zone of hazy β-haemolysis extending 1 to 2mm from the edge of the colony. Some strains of *L. monocytogenes* can be non-haemolytic.

L. ivanovii	Colonies are similar to *L. monocytogenes* but develop larger zones of complete haemolysis with outer zones of partial haemolysis. Cells are small, motile rods. Colonies on sheep or horse blood (5%) agar are strongly β-haemolytic. Growth occurs at 4 °C within 5 days.
L. seeligeri	Cells are small (0.4 to 0.8 × 0.5 to 2.5µm) rods. Colonies are similar to *L. monocytogenes* but zones of β- haemolysis are produced.
L. innocua	Cells are small rods occurring singly or in short chains. Cream colonies, no haemolysis.
L. grayi	Colonies are small, regular, smooth and are 1 to 2mm in diameter after 1 to 2 days of incubation at 37 °C.
L. welshimeri	Cells are small (0.4 to 0.5 × 0.5 to 2.0µm) rods. Sheep erythrocytes are not haemolysed. Growth occurs at 4 °C within 5 days.
Arcanobacterium species	Cells are slender, irregular and predominately rod-shaped or arranged at an angle to give V-formations during the first 18hr of growth on blood agar, becoming granular and segmented, resembling small, irregular cocci over time. After 48hr incubation, colonies produce zones of β-haemolysis.
Aureobacterium species	Non-haemolytic, yellow pigmented colonies.
Bifidobacterium species	Colonies are low, greyish-brown, and ovoid with a brown opaque centre and translucent crenated edges.
Brevibacterium species	Colonies are opaque, grey-white, 2mm or more in diameter after 24hr, convex and have a smooth shiny surface. They are non-haemolytic and may turn yellow to green after 48hr.
Cellulomonas species	Non-haemolytic, yellow- or orange-pigmented colonies.
Cutibacterium acnes	Colonies are round, opaque, white to off-white sometimes β-haemolytic colonies after 5 to 6 days.
Dermabacter hominis	Non-haemolytic, small grey/white convex colonies with entire edges.
Erysipelothrix rhusiopathiae	After 48hr incubation, two distinct colony types appear: a small smooth (S) form, 0.5 to 1mm in diameter, transparent, convex and circular with entire edges. The large rough (R) form is flatter, more opaque, with a matt surface and an irregular edge. Most strains exhibit a narrow zone of α-haemolysis but the R – form does not cause haemolysis.
Gardnerella vaginalis	Growth is enhanced by the addition of 5 to 10% CO_2. Colonies are small, circular, convex and grey. It also produces diffuse β-haemolysis on rabbit blood agar but not on sheep blood agar. Haemolysis on horse blood agar is variable.
Lactobacillus species	Colonies are small and often α-haemolytic on blood agar after 48hr.
Microbacterium species	Colonies are circular, convex with entire margins, moist, shiny and may produce a yellow or orange pigment.
Oerskovia species	Most strains produce a yellow pigment.
Propionibacterium species	They produce small colonies after 48hr incubation.
Rhodococcus species	Colonies may be rough, smooth or mucoid and are colourless, cream, beige, yellow, orange or red.

Trueperella species	Pinpoint, β-haemolytic colonies occur on blood agar after 24hr of incubation and after 48 to 72hr of incubation, colonies are 0.5 to 1.5mm in diameter, convex, circular and translucent with entire edges. *T. pyogenes* produces sharp zones of β-haemolysis after 48hr incubation. Haemolysis of *T. bernardiae* is variable.
Trueperella bernardiae	Colonies exhibit variable haemolysis, appear circular, smooth and slightly convex with a glassy appearance.
Trueperella pyogenes	Colonies produce sharp zones of β-haemolysis after 48hr incubation. They appear convex, white, smooth, translucent and soft with entire edges.
Turicella otitidis	Colonies are convex, whitish, creamy and non-haemolytic in appearance.

Other *Listeria* species show similar colonial appearance and are either haemolytic or non-haemolytic: these species are very rarely isolated from normally sterile sites and should be submitted to the Reference Laboratory for identification.

For all other non-sporing Gram positive rods[13,18]

Appearance varies with species on blood agar, after aerobic incubation at 35 to 37 °C for 16 to 48hr. See Table 1 above.

8.4 Test procedures

Catalase test (TP 8 - Catalase test)

Listeria species are catalase positive.

Arcanobacterium species may give variable catalase reactions.

Erysipelothrix rhusiopathiae is catalase negative.

For the other non-sporing rods, see Table 2 and Appendix.

Motility test (TP 21 - Motility test)

This is performed at 20 °C – 25 °C for *Listeria* species and above 30 °C for all other organisms.

All *Listeria* species exhibit tumbling motility at 20 °C to 25 °C but not at above 30 °C. Other organisms may be motile but do not exhibit tumbling motility.

For the other non-sporing rods, see Table 2 and Appendix.

Commercial identification systems

Laboratories should follow manufacturer's instructions for rapid tests and kits.

Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)[32]

This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis.

MALDI-TOF has been developed and validated to determine species and lineages of *Listeria* species using isolates from a variety of sources.

It has been known to be used to identify other non sporing Gram positive rods such as *T. bernardiae, E.rhusiopathiae, A.haemolyticum* and *G.vaginalis*[33,34].

Refer to UK SMI TP 40 - MALDI TOF MS test procedure for further information.

Nucleic Acid Amplification Tests (NAATs)[35,36]

PCR is now established as a rapid, reliable and reproducible technique for identification of *Listeria* species and, more importantly, for the differentiation of *L. monocytogenes* from other species using primers targeting genes encoding virulence factors or RNA sub-unit genes.

The Multiplex PCR-based serotyping assay, such as the use of group-specific PCR primers, has equally provided additional tools for the identification and grouping of *L. monocytogenes*, however consideration should be given to the type of laboratory and samples received.

Table 2: Phenotypic characteristics of *Listeria* and other non-sporing gram positive rods (except Corynebacterium)

Organisms	Gram stain	Catalase	Motility
Listeria Species	Positive	Positive	Positive
Arcanobacterium haemolyticum	Positive	Negative	Negative
Aureobacterium species	Positive	Positive	Variable
Bifidobacterium species	Positive	Negative	Negative
Brevibacterium species	Positive	Positive	Negative
Cellulomonas species	Positive	Positive	Variable
Cutibacterium	Positive	Positive	Negative
Dermabacter hominis	Positive	Positive	Negative
Erysipelothrix rhusiopathiae	Positive	Negative	Negative
Gardnerella vaginalis	Variable	Negative	Negative
Lactobacillus species	Positive	Negative	Rarely motile
Microbacterium species	Positive	Variable	Variable
Oerskovia species	Positive	Positive	Variable
Propionibacterium species	Positive	Positive	Negative
Rhodococcus species	Positive	Positive	Negative
Trueperella pyogenes	Positive	Negative	Negative
Trueperella bernardiae	Positive	Negative	Negative
Turicella otitidis	Positive	Positive	Negative

8.5 Further identification

Following the colonial morphology, catalase test, motility test and biochemical identification results, if further identification is required, send isolate to the Reference Laboratory.

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Fluorescent Amplified Fragment Length Polymorphism (AFLP) and Pulsed Field Gel Electrophoresis (PFGE). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

Whole genome sequencing (WGS)[37]

Whole genome sequencing (WGS) also known as full genome sequencing, complete genome sequencing or entire genome sequencing is a laboratory method that determine the complete DNA sequence of an organism's genome at a single time.

WGS has been used successfully to explore the genome of organisms as well as to identify candidate genes responsible for pathogenesis, and to develop better methods of strain identification and to advance the understanding of evolution of organisms. WGS analysis can provide phylogenetic data, which helps to perform real-time or retrospective surveillance of *L. monocytogenes* and related outbreak investigations[38].

Single nucleotide polymorphism (SNP)[39]

The single nucleotide polymorphism (SNP) based approach is most commonly used principle for the retrieval of subtyping information from WGS data. SNPs are flexible, do not require a redefined scheme and provides an exceptionally high subtyping resolution.

SNP is used for the highly discriminative typing of *L. monocytogenes*.

16S rRNA gene sequencing[40]

A genotypic identification method, 16S rRNA gene sequencing is widely recognised as a useful method for the molecular subtyping of bacterial species and strains.

This 16S rRNA sequence method is used for the rapid, specific and sensitive speciation of *Listeria* isolates as well as differentiation within species for isolates of *L.monocytogenes, L.innocua* and *L.welshimeri*.

8.6 Storage and referral

Save the pure isolate on a blood or nutrient agar slope for referral to the Reference Laboratory.

9 Reporting

9.1 Infection Specialist[41]

Infection is most commonly acquired from consumption of contaminated food (including that served in hospital) although cross-infection in delivery suites are well documented. Isolation of the bacterium is most common from blood or CSF. Inform the infection specialist of all preliminary and confirmed *Listeria monocytogenes* and other *Listeria* species isolated from sterile sites when the request card bears relevant information eg:

- The patient is >60 years old, immunocompromised, pregnant, or neonate
- Suspicion of sepsis, meningitis and/or meningo-encephalitis in persons with alcoholism, other substance abuse, or immunocompromised. Also, patients with other serious underlying disorders such as cancer, or patients receiving treatments which induce neutropenia and/or mucositis
- Investigation of outbreaks

Inform the infection specialist of presumptive and confirmed non-sporing Gram positive rods. Typically, these will include:

- 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, which induces neutropenia and/or mucositis

Follow local protocols for reporting to clinician.

9.2 Preliminary identification

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

9.3 Confirmation of identification

For confirmation and identification please see Specialist and reference microbiology: laboratory tests and services page on GOV.UK for reference laboratory user manuals and request forms.

9.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

9.5 Public Health England[42]

Refer to current guidelines on SGSS reporting.

9.6 Infection prevention and control team

Inform the Infection Prevention and Control team of presumptive and confirmed isolates of *L. monocytogenes* according to local protocols.

10 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or an anomaly that requires investigation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

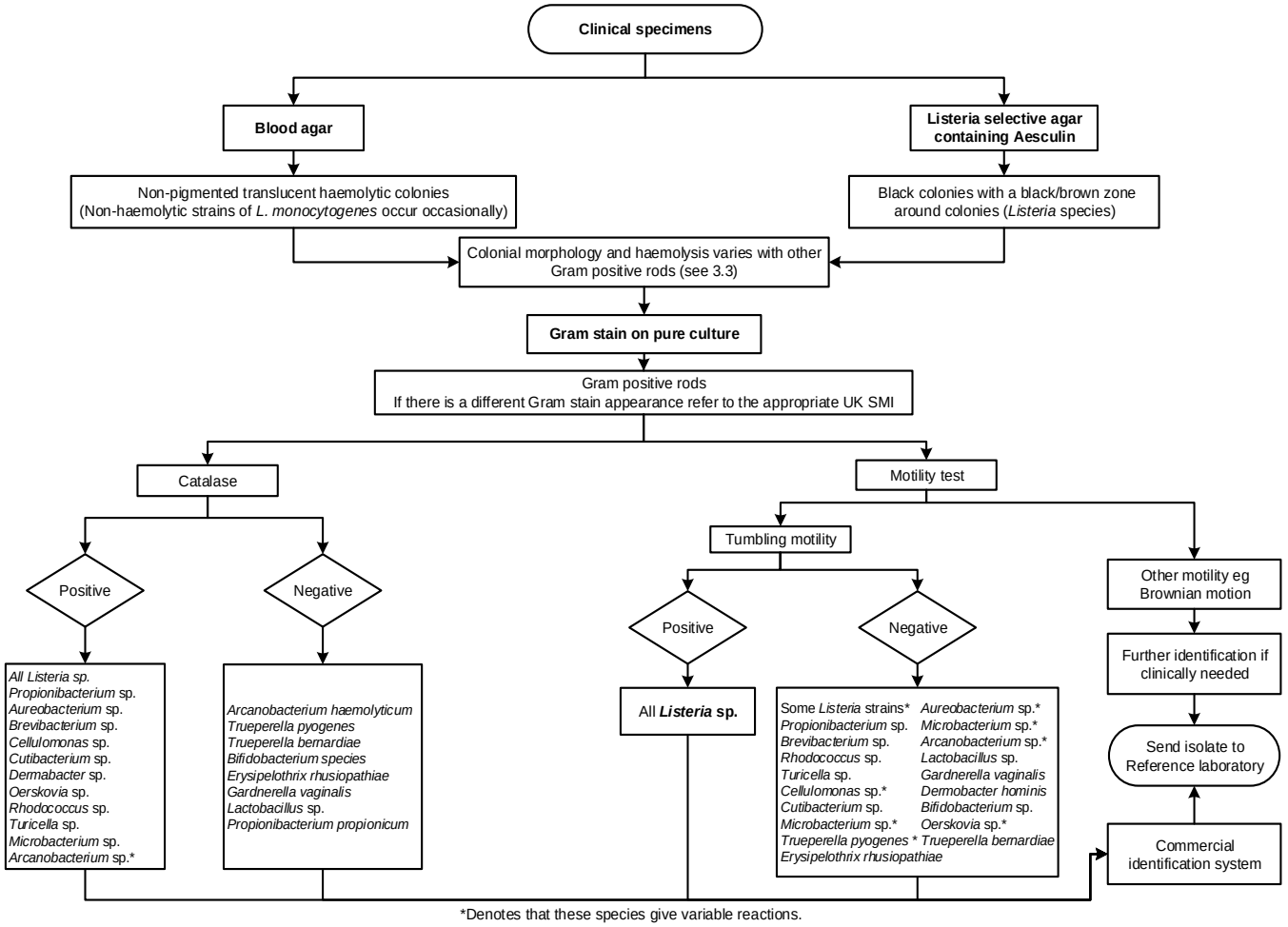
Scotland

https://www.hps.scot.nhs.uk/a-to-z-of-topics/reference-laboratories/

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Appendix: Identification of *Listeria* species and other non-sporing Gram positive rods (except *Corynebacterium*)



The flowchart is for guidance only.

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For the information for the evidence grade ratings given, refer to the scientific information link above in section 2.

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