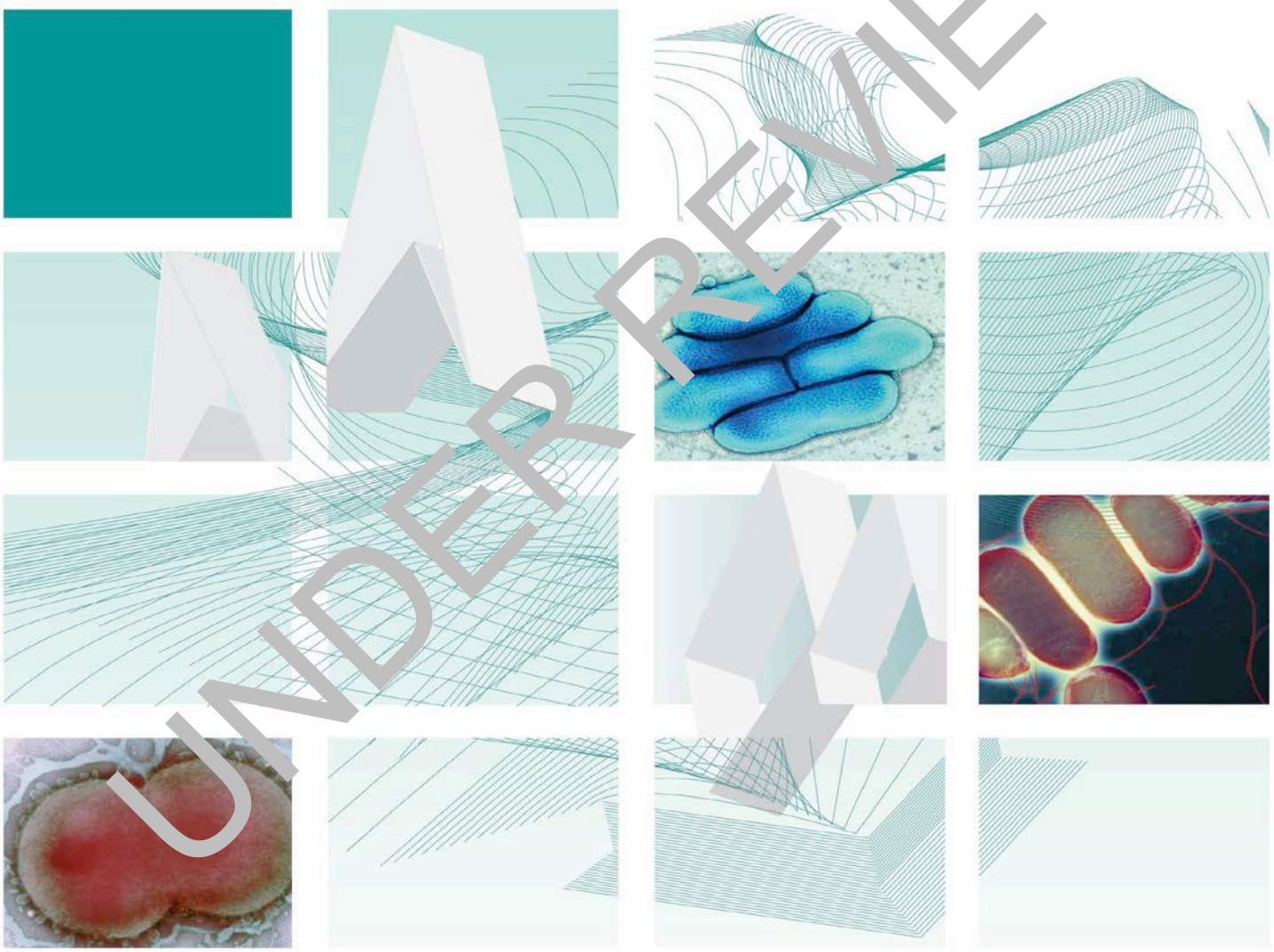
Public Health England

NHS

UK Standards for Microbiology Investigations

Identification of *Listeria* species, and other Non-Spoing Gram Positive Rods (except *Corynebacterium*)



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Acknowledgments

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UK Standards for Microbiology Investigations are produced in association with:

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Contents

Amendment Table

Each 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 No/Date.	10/01.07.16
Issue no. discarded.	3.1
Insert Issue no.	3.2
Section(s) involved	**Amendment**
Introduction.	Sentence on *Arcanobacterium* species updated. No substantive changes made.
Identification.	Subsection 3.4.1 has been updated with information on catalase test for *Arcanobacterium* species.

Amendment No/Date.	9/29.10.13
Issue no. discarded.	3
Insert Issue no.	3.1
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Identification.	Updates have been done on 3.4 and 3.5 to reflect standards in practice. Subsection 3.5 has been updated to include the Rapid Molecular Typing Methods.

Amendment No/Date.	8/18.06.14
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	**Amendment**

Scope of document.	The scope has been updated to give a link to the document for identification of *Corynebacterium* species.
Introduction.	Taxonomy updated. More information has been added to the Characteristics section. Addition of medically important *Listeria* species. Other non-sporing rods that are medically important are also mentioned and their characteristic described. References updated in the relevant subheadings. Section on Principles of Identification has been updated to reflect the correct temperatures used for tumbling motility in identification of *Listeria* species.
Technical Information/Limitations.	Addition of information regarding motility test and differentiation of *Listeria* species from Group B streptococci.
Safety considerations.	This section has been updated to include the references.
Target Organisms.	The section on the Target Organisms has been updated.
Identification.	Updates have been done on 3.1, 3.3 and 3.4 to reflect standards in practice. It also includes all the non-sporing Gram positive rods apart from *Listeria* species. Section 3.4 has been updated accordingly. The table in 3.3 has been updated with references. Subsection 3.5 has been updated to include the Rapid Molecular Methods. 3.7 has been removed and put under the section "Technical Information/Limitation".
Identification Flowchart.	Modification of flowchart for identification of *Listeria* species has been done for easy guidance.
Reporting.	Subsections 5.2, 5.3, 5.5 and 5.6 have been updated to reflect reporting practice.
Referral.	The addresses of the Reference Laboratories have been updated.
Whole document.	Document presented in a new format.

References.	Some references updated.
Appendix.	The flowchart in the appendix titled "Characteristics to distinguish between non-sporing Gram positive rods on blood agar" has been deleted from this document and merged with the flowchart in section 4.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under effective conditions.

The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

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The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

Suggested Citation for this Document

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Scope of Document

This 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.

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

This SMI should be used in conjunction with other SMIs.

Introduction

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 SMI. 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*[1,2]**

There are currently ten 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* and *L. weihenstephanensis*. Of these ten species, the first six can potentially cause infections in humans, albeit rarely in some cases.

The type species is *Listeria monocytogenes*.

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, 0.4-0.5 x 0.5-2.0µm, with rounded ends, occurring singly or in short chains and occasionally appearing filamentous. 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 20°C - 25°C and display a characteristic “tumbling” motility. 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. If using commercial chromogenic agar, follow manufacturer’s instructions.

They are catalase positive, oxidase negative and ferment carbohydrates[1,3].

Listeria species are widely distributed in the environment; some species are pathogenic for humans and animals.

The medically important species are:

L. monocytogenes

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 corynebacteria. 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 characterization may be necessary to distinguish between the different *Listeria* species. *L. monocytogenes* is catalase positive and oxidase negative.

L. monocytogenes is the agent of listeriosis, a serious infection caused by eating food contaminated with the bacterium. Listeriosis has been recognized as an important public health problem and the disease affects primarily pregnant women, neonates, elderly people, and those with weakened immune systems.

L. ivanovii[4]

Cells are small, motile rods. Colonies on tryptose agar are very small (0.5 to 1mm in diameter after 1 or 2 days of incubation at 37°C), regular, and smooth and appear bluish green when they are viewed by obliquely transmitted light. Colonies on sheep or horse blood (5%) agar are strongly β-haemolytic. Growth occurs at 4°C within 5 days. They are facultatively anaerobic.

L. ivanovii are positive for catalase, Voges-Proskauer and methyl red tests, and aesculin hydrolysis. They are negative for oxidase, urea and gelatin hydrolysis; and reduction of nitrates. Neither indole nor H_2S is produced. Acid but no gas is produced from glucose and D-xylose. No acid is produced from D-mannitol, L-rhamnose, or α-methyl-D-mannoside.

The species has also been isolated from healthy animals and human carriers and from the environment. Subsequently, this species has been divided into 2 subspecies. They are; *Listeria ivanovii* subsp. *ivanovii* and *Listeria ivanovii* subsp. *Londoniensis*[5]. Most of the characteristics are similar to those of *L. ivanovii*, except that *L. ivanovii subsp. londoniensis* does not produce acid from ribose, but produces acid from N-acetyl-P-D-mannosamine after 1 to 24hr of incubation at 37°C[4].

L. seeligeri[6]

Cells are small (0.4 to 0.8 x 0.5 to 2.5µm) rods which are motile by means of peritrichous flagella. Colonies on tryptose agar are similar to that of *L. welshimeri*. Growth occurs at 4°C within 5 days. They are facultatively anaerobic.

They are positive for catalase, Voges-Proskauer and methyl red tests; and aesculin hydrolysis. They are negative for oxidase, reduction of nitrates, urea hydrolysis and indole production. Acid, but no gas, is produced from D-glucose and D- xylose. Acid is not produced from D-mannitol or L-rhamnose. Most strains do not produce acid from α-methyl-D-mannoside.

They have been isolated from plants, soil, and animal faeces (sheep) in Europe.

L. innocua

Cells are small rods occurring singly or in short chains. They are motile by means of peritrichous flagella. They are mesophilic, operating at an optimal temperature range of 30-37°C.

Listeria innocua have a very complex metabolism. They are capable of metabolizing 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 metabolism glucose (and other simple sugars) in under 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[7].

This species is widespread in the environment and in food and has also been associated with one reported case of fatal bacteraemia[8].

L. welshimeri[6]

Cells are small (0.4 to 0.5 by 0.5 to 2.0µm) rods which are motile by means of peritrichous flagella. Colonies on tryptose agar are small (1 to 2mm in diameter after 1 or 2 days of incubation at 37°C), regular, and smooth with a blue-green colour when they are examined with obliquely transmitted light. Sheep erythrocytes are not haemolysed. Growth occurs at 4°C within 5 days.

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.

They have been isolated from decaying plants and soil.

L. grayi

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*[9].

Cells are small (0.4 to 0.5 x 0.5 to 2µm) peritrichous rods which are motile. Colonies on tryptose agar are small (1 to 2mm in diameter after 1 to 2 days of incubation at 37°C), regular, and smooth. Growth occurs at 4°C within 5 days.

Metabolism is facultatively anaerobic. They are positive for catalase, aesculin hydrolysis, Voges-Proskauer and methyl red tests and negative for the oxidase, urea and gelatin hydrolysis, H_2S and indole production. 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[10-12]

Arcanobacterium species[13]

There were 11 species of which 5 have been re-assigned to the genus *Trueperella* and of the remaining 6 species, only one is known to infect humans, *Arcanobacterium haemolyticum*[14].

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. Both rod-shaped and coccoid cells are Gram positive, non-acid-fast and non-motile. Endospores are not formed. They are facultatively anaerobic. 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* (formerly *Corynebacterium haemolyticum*)**

Colonies on blood agar after 48hr produce zones of β-haemolysis and are similar in appearance to *Trueperella pyogenes. A. haemolyticum* is non-motile, facultatively anaerobic and, unlike *Corynebacterium* species, is catalase negative[15].

They have been isolated from the throat of infected individuals.

Arcanobacterium pyogenes

This has been reclassified to a new genus. See *Trueperella* species below.

Arcanobacterium bernardiae

This has been reclassified to a new genus. See *Trueperella* species below.

***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[16,17].

***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 1.5-8µm. 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 non-motile. *Bifidobacterium* species ferment carbohydrates and are catalase negative[1].

***Brevibacterium* species**

Brevibacterium species are Gram positive rods, which show a marked rod-coccus cycle. On fresh subculture, cells appear as bacilli but become coccal in older cultures. Colonies on blood agar are non-haemolytic and may turn a yellow to green colour after 48hr incubation. *Brevibacterium* species are non-motile, salt tolerant (>6.5% NaCl), aerobic, urease negative and catalase positive[15,18].

***Cellulomonas* species**

Cellulomonas species are Gram positive slender irregular rods that produce yellow or orange pigmented colonies. They are catalase positive and 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[19]. *Cellulomonas* species differ from *Oerskovia* species in that they lack hyphal growth[15].

Dermabacter hominis

Dermabacter species are very short Gram positive rods that may be misinterpreted 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 produce alkaline phosphatase, pyrrolidonyl arylamidase, leucine aminopeptidase and DNase. They do not reduce nitrate or produce pyrazinamidase[15].

Erysipelothrix rhusiopathiae

E. rhusiopathiae is a non-sporulating Gram positive rod, which 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[20].

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. Acid is produced from glucose and other carbohydrates but not gas. It hydrolyses hippurate and does not reduce nitrate[1].

Lactobacillus species

Lactobacillus species are long Gram positive rods. Colonies are small and often α-haemolytic on blood agar after 48hr. They are facultatively anaerobic, rarely motile and catalase negative[1].

Microbacterium species

Microbacterium species are small, slender, irregularly shaped Gram positive rods. They may produce a yellow or orange pigment. 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[15,19]. All species are catalase positive.

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 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 motile or non-motile coccoid-rod elements. Most strains produce a yellow pigment. No aerial hyphae are formed. They are facultatively anaerobic, fermentative and catalase positive[15].

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 48hr. *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[15].

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 due to the mycolic acid in their cell walls. Colonies may be rough, smooth or mucoid and are colourless, cream, beige, yellow, orange or red. Incubation at 30°C also increases recovery[21].

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. Their inability to ferment carbohydrate is important in distinguishing them from corynebacteria.

Trueperella species[13,22]

There are currently five validly named species in the genus *Trueperella* and two of these cause infections in humans[23].

Cells are Gram positive, non-motile, non-spore-forming coccobacilli and short rods that occur singly, in pairs (V, T and palisade formations) or in clusters. Cells vary in shape and size (0.2–0.9 x 60.3–2.5µm) in different media. Cells from 24 hour old broth cultures are Gram positive, but may be Gram variable in older cultures. Pinpoint, β-haemolytic colonies occur on sheep's blood agar after 24hr of incubation. After 48–72hr of incubation, colonies are 0.5–1.5mm in diameter, convex, circular and translucent with entire edges. 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 (formerly *Arcanobacterium pyogenes*) is a rod which may show branching. Colonies on blood agar produce sharp zones of β-haemolysis after 48hr incubation. They appear convex, white, smooth, translucent and soft with entire edges.

T. pyogenes is facultatively anaerobic, non-motile, and catalase negative but one strain has been reported as positive. Metabolism is strictly fermentative[24]. 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 (formerly *Arcanobacterium bernardiae*) is a rod with coccobacilli predominating. Primary branching is not observed. *T. bernardiae* is facultatively anaerobic, non-motile and catalase negative. Colonies on blood agar exhibit variable haemolysis, appear circular, smooth and slightly convex with a glassy appearance after 48hr incubation. Colony diameters range from 0.2mm to 0.5mm after 48hr incubation[25].

This has been isolated from human blood as well as abscess from ear or chest[25].

Turicella otitidis

The genus comprises a single species, *Turicella otitidis*. Microscopically it resembles a coryneform but has longer cells. It may be distinguished by colonial morphology from *C. afermentans* and *C. auris*. *T. otitidis* 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. otitidis* is non-fermentative and occurs either alone or with Gram negative rods. Isolates exhibit a strong CAMP reaction and are DNase positive and catalase positive.

T. otitidis may be misidentified, often as *Corynebacterium* species, by some commercial identification systems[15,19].

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 20-25°C but not at 35°C. 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/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 (see below)

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 isolation of *Listeria* species or purposes other than the investigation of motility.

Listeria species are motile at 20-25°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[3].

1 Safety Considerations[10-12,26-39]

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 SMI.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[12].

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 and other morphologically similar Gram positive rods reported to have caused human infection[15,40,41]**

Listeria monocytogenes, Arcanobacterium species, *Erysipelothrix rhusiopathiae*

Other *Listeria* species reported to have caused occasional or single human infections[8,42-47]

Listeria ivanovii, Listeria seeligeri, Listeria innocua, Listeria grayi

Other species morphologically similar to *Listeria* species known to have caused human infection[10-12,15]

Aureobacterium species, *Bifidobacterium* species, *Brevibacterium* species, *Cellulomonas* species, *Oerskovia xanthineolytica*, *Rothia dentocariosa*

Other Gram positive rods have been implicated in human infections[15,19,48].

3 Identification

3.1 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 x 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.

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 negative 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.

***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, 0.5-1.3 x 1.5-8µm. Cells often stain irregularly.

***Brevibacterium* species**

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

***Cellulomonas* species**

Cells are Gram positive slender irregular rods.

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”.

3.2 Primary Isolation Media

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

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

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

3.3 Colonial Appearance

This table is a summary of the organisms and their appearances on blood agar plate.

Organism	**Characteristics of growth on blood agar after incubation at 35-37°C for 16-48hr**
L. monocytogenes	Colonies are 0.5-1.5mm in diameter, smooth, translucent with a characteristic ground glass appearance able to be emulsified and with a zone of hazy β-haemolysis extending 1-2mm from the edge of the colony.
Arcanobacterium species	After 48hr incubation, colonies produce zones of β-haemolysis.
Erysipelothrix rhusiopathiae	After 48hr incubation, two distinct colony types appear: a small smooth (S) form, 0.5-1mm 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.
L. ivanovii	Colonies are similar to *L. monocytogenes* but develop larger zones of complete haemolysis with outer zones of partial haemolysis.
L. seeligeri	Colonies are similar to *L. monocytogenes* but zones of β-haemolysis are produced.
L. innocua	Cream colonies, no haemolysis.
L. grayi	Colonies are small, regular, smooth and are 1 to 2mm in diameter after 1–2 days of incubation at 37°C.
Trueperella species	Pinpoint, β-haemolytic colonies occur on blood agar after 24hr of incubation and after 48–72hr of incubation, colonies are 0.5–1.5mm 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.
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.
Dermabacter hominis	Non-haemolytic, small grey/white convex colonies with entire edges.
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.
Gardnerella vaginalis	Growth is enhanced by the addition of 5-10% 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.

Rhodococcus species	Colonies may be rough, smooth or mucoid and are colourless, cream, beige, yellow, orange or red.
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[15,19]

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

3.4 Test Procedures

3.4.1 Biochemical tests

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 the flowchart on section 4.

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 - 25°C but not at above 30°C. Other organisms may be motile but do not exhibit tumbling motility.

3.4.2 Commercial identification systems[49]

Laboratories should follow manufacturer's instructions. Rapid tests and kits and should be validated and be shown to be fit for purpose prior to use.

3.4.3 Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)

This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis[50]. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use.

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 *T. bernardiae* and thus will help in its future identification and in elucidating the role that this rarely isolated species plays in infection of humans[51].

3.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR is usually considered to be a good method as it is simple, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes.

This 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[52].

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*[53].

3.5 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.

Rapid Molecular Methods

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.

Fluorescent Amplified Fragment Length Polymorphism (AFLP)

Fluorescent Amplified Fragment Length Polymorphism is a high-resolution whole genome methodology used as a tool for rapid and cost-effective analysis of genetic diversity within bacterial genomes. It is useful for a broad range of applications such as identification and subtyping of microorganisms from clinical samples, for identification of outbreak genotypes, for studies of micro and macro-variation, and for population genetics. It has been used successfully for studying of *L. monocytogenes*[54-56].

FAFLP has numerous advantages over other DNA fingerprinting techniques because it assesses the whole genome for both conserved and rapidly evolving sequences in a relatively unbiased way. The number of fragments obtained for comparative purposes between isolates is significantly greater than pulsed-field gel electrophoresis (PFGE), thus making it more discriminatory than PFGE and the FAFLP results are highly reproducible due to stringent PCR cycling parameters.

Pulsed Field Gel Electrophoresis (PFGE)

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in

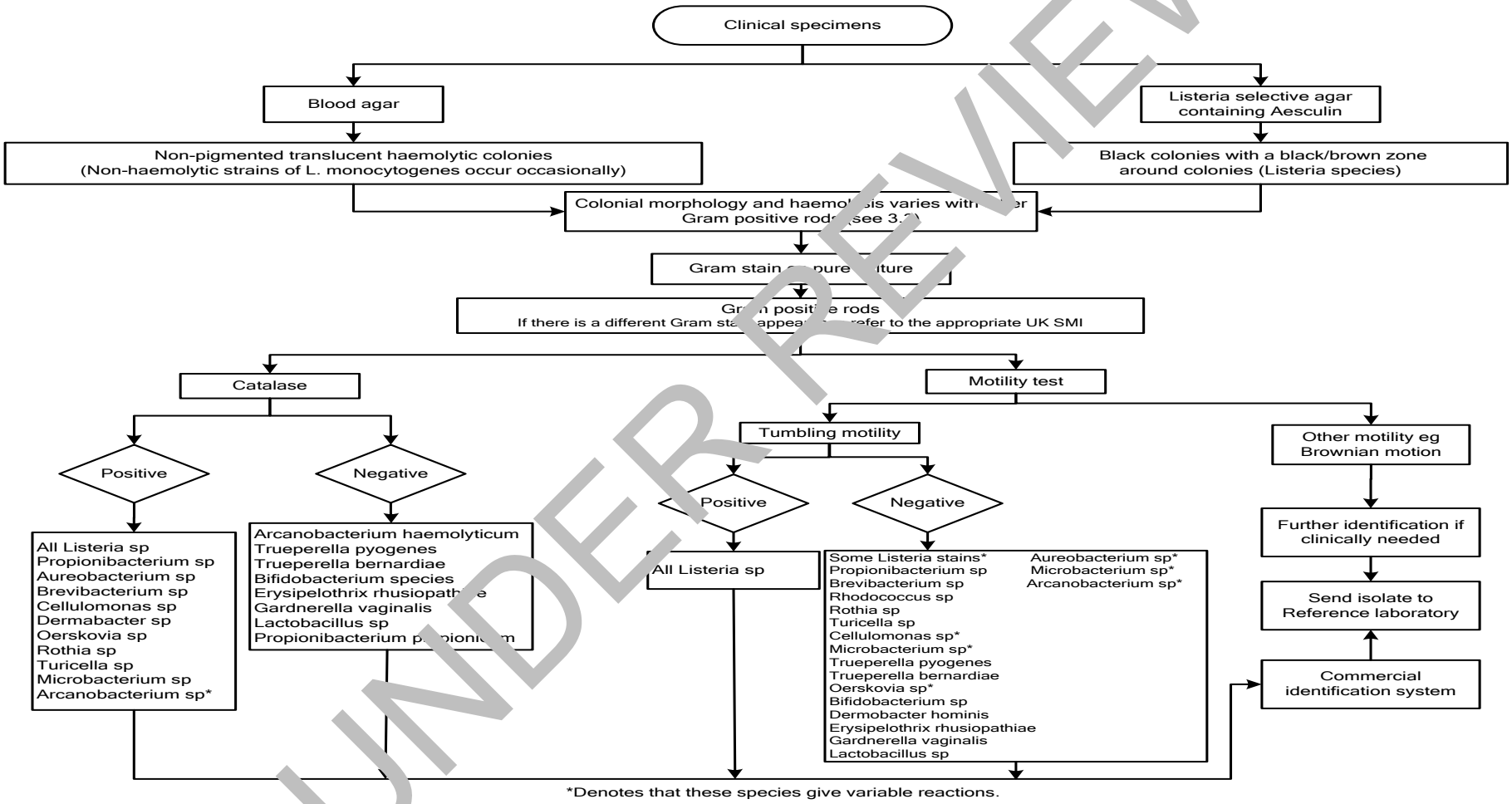
characterising epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories[55,57].

It is a highly reproducible, discriminatory and effective molecular typing method for identifying and classifying *L. monocytogenes* into subtypes that is considered the reference standard.

3.6 Storage and Referral

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

4 Identification of *Listeria* species and other Non-Sporing Gram Positive Rods (except *Corynebacterium*)



The flowchart is for guidance only.

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

Confirmation of identification and toxigenicity of non-sporing Gram positive rods are undertaken only by the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI) while confirmation of identification for *L. monocytogenes* isolates and *Listeria* species are undertaken by the Foodborne Pathogens Reference Services at the Gastrointestinal Bacteria Reference Unit, Colindale.

5.3 Medical Microbiologist

Infection is most commonly acquired from consumption of contaminated food (including that served in hospital) although cross-infection in delivery suites are well documented[40]. Isolation of the bacterium is most common from blood or CSF. Inform the medical microbiologist of all presumptive 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 septicaemia, 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 medical microbiologist of presumptive and confirmed non-sporing Gram positive rods when the request card bears relevant information eg:

- 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.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 Public Health England[58]

Refer to current guidelines on CIDSC and COSURV reporting.

5.6 Infection Prevention and Control Team

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

6 Referrals

6.1 Reference Laboratory

Contact appropriate devolved nation Reference Laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

Suspected Listeria isolates for confirmation:

Gastrointestinal Bacteria Reference Unit
Bacteriology Reference Department
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5EQ

Gram positive rods for further characterisation:

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5EQ

https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services

Contact PHE's main switchboard. Tel. +44 (0) 20 8200 4400

England and Wales

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

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm

7 Notification to PHE[58,59] or Equivalent in the Devolved Administrations[60-63]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance 2010 includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[60,61], Wales[62] and Northern Ireland[63].

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