PHLN
Public Health Laboratory Network

Listeriosis (*Listeria sp.*)

Laboratory case definition

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

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1 PHLN summary laboratory definition

1.1 Condition:

Listeriosis

1.1.1 Definitive Criteria

- Isolation of *Listeria* species from normally sterile sites OR
- Isolation of *Listeria* species from faeces

1.1.2 Suggestive Criteria

- Isolation of Gram positive bacilli from normally sterile site, demonstrating tumbling motility at room temperature

1.1.3 Special Considerations

- Isolation of *Listeria* species from a non-sterile body surface are not necessarily indicative of invasive infection, but may be used to prompt further definitive investigations such as blood culture, if clinically indicated

1.1.4 Links to related documents

- CDNA (clinical) case definition for listeriosis: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-cd_listera.htm
- Fact Sheet on *Listeria*: http://www.health.gov.au/internet/main/publishing.nsf/Content/ageing-clinicians-type.htm/$File/Listeria.pdf

2 Introduction

The genus *Listeria* contains a number of distinct species; however, only two members of the genus are regarded as pathogenic to humans, namely *Listeria monocytogenes* and *Listeria ivanovii. Listeria monocytogenes* has risen to prominence in recent years with frequent isolation from a wide range of foods. There is direct causal evidence of infection following consumption of food heavily contaminated by this species. *L.ivanovii* causes rare infections. Disease attributed to L.innocua has been reported.

L. monocytogenes is a risk to pregnant mothers, the very young and the aged, and patients on immunosuppressive therapy. Given the widespread distribution of this organism in food, education targeted at these risk groups has been used as the most effective means to decrease the risk of infection.

L. monocytogenes causes a transient flu-like illness. Invasion of the blood stream can lead to bacteraemia and/or meningitis, and in the case of pregnant women, the organism can cross the placental barrier and infect the unborn child, resulting in premature abortion and/or stillbirth. In rare cases, it can cause symptoms typical of food poisoning (vomiting and diarrhoea) some 19 hours after consumption of contaminated food.

Though the genus *Listeria* has not changed since the first edition of this Laboratory Case Definition, developments in molecular biology have led to improvements in molecular laboratory methods including hybridisation assays, PCR assays and molecular typing. The availability of a complete, annotated genome for both the pathogenic and some of the non-pathogenic *Listeria* species is likely to result in a range of new molecular methods for *Listeria* detection and characterisation.

3 Tests

3.1 Culture for *Listeria* species

Listeria species are small, facultative anaerobic, non-spore forming, Gram positive bacilli, that grow readily on blood agar. In addition, a number of selective agars have been developed for use in food microbiology. On blood agar *L monocytogenes* produces incomplete β-haemolysis.

3.1.1 Media

For optimal isolation of *Listeria* species, direct plating onto blood agar is recommended. This may be direct from clinical specimens or from subcultures of blood culture medium. In addition, when foodborne cases are being investigated, culture from faeces is often useful. In such cases, selective media such as Oxford agar or PALCAM are recommended, as well as enrichment in a *Listeria* selective broth.

3.1.2 Suitable specimens:

- *Blood* – Blood samples should be inoculated immediately into blood culture bottles. These may be stored at room temperature during transport to the laboratory and subsequently incubated at 37oC.
- *Brain/Abscess/foetal material swab*s – Swabs should be placed in an appropriate transport medium and transported to the laboratory quickly.

- *Faeces/rectal swab* – Faeces collected in an appropriate sterile container, transported to the laboratory ASAP (if longer than 2h keep at 4°C), may be stored at 4° C for 24 hours before culture. Rectal swabs may be put into a tube of transport medium containing modified Stuart's medium, transported to the laboratory ASAP. May be stored at RT for 24 hours before culture.

3.1.3 Test sensitivity

No mathematical data available.

Depends on the quality and type of the specimen, the type of media chosen, and whether enrichment is used prior to direct plating. Antibiotic treatment may inhibit the growth of *Listeria* species.

3.1.4 Test specificity

No mathematical data available.

The isolation of a Listeria-like organism from a normally sterile site, confirmed by specific biochemical and phenotypic techniques. The isolation of *Listeria monocytogenes* is notifiable in most States of Australia.

3.1.5 Predictive values

A negative blood or other culture does not exclude the diagnosis of listeriosis.

3.1.6 Suitable acceptance criteria

On Blood agar, small β-haemolytic colonies which demonstrate characteristic tumbling motility at room temperature (optimal at 25oC) in broth cultures and which biochemically fits an identification of *L. monocytogenes. L. ivanovii* can be distinguished by its distinctive CAMP test reaction and different biochemical reactions.

3.1.7 Suitable internal controls

Properly documented, relevant, quality control program for each type and batch of medium used.

3.1.8 Suitable test validation criteria

Isolation of *L. monocytogenes*, confirmed by biochemical and other phenotypic parameters, is the reference standard.

3.1.9 Suitable external QC programme

Royal College of Pathologists of Australia Quality Assurance Programs Pty. Ltd. (RCPA)

3.1.10 Special considerations

L. monocytogenes can be differentiated from *L. ivanovii* by the CAMP test. It is important that laboratories correctly identify the species present in a specimen.

3.2 Identification of *Listeria* species

There are two levels of action in the identification of *Listeria* sp.:

A. **Diagnostic laboratories** – Most diagnostic laboratories should be capable of biochemically confirming the identity of *Listeria monocytogenes*.

B. **Reference laboratories** – Isolates, particularly where a cluster is involved, should be forwarded to a State reference laboratory for serotyping, molecular characterisation and molecular comparison with food isolates.

3.2.1 Conventional biochemical tests

3.2.1.1 Suitable specimen

A pure culture on solid medium.

3.2.1.2 Media

Various specific biochemical substrates

3.2.1.3 Test sensitivity

This depends on the biochemical tests performed. The demonstration of tumbling motility at 25oC is usually a reliable characteristic for presumptive recognition of a *Listeria* species. Many laboratories also use Aesculin hydrolysis as an additional screening test. Sugar fermentation tests may exhibit variable reactions. A number of commercial strip tests are available.

3.2.1.4 Test specificity

No mathematical data available. The CAMP test and acid formation from specific sugars are used to differentiate between isolates of *L.monocytogenes* and *L. ivanovii*.

3.2.1.5 Predictive values

A negative result in one test does not preclude *L. monocytogenes*. Further tests may be necessary to confirm the identification.

3.2.1.6 Suitable test criteria

An isolate that exhibits biochemical characteristics consistent with documented reactions for a *Listeria* species. The major distinguishing feature of *L.monocytogenes* is fermentation of Rhamnose, lack of fermentation of Xylose and hydrolysis of aesculin.

3.2.1.7 Suitable internal controls

Each batch of biochemical substrate tested with positive and negative control strains. Results of all testing recorded and the records maintained.

3.2.1.8 Suitable validation criteria

Correct biochemical reactions exhibited by a standard *Listeria* strain.

3.2.1.9 Suitable external QC program

RCPA Quality Assurance Programs Pty. Ltd.

3.2.2 Kits/automated systems for biochemical identification

Various kits and automated machines exist for the identification of *Listeria* species. Of the numerous kits available in Australia, the API *Listeria* and the MICRO-ID *Listeria* are most commonly used. MicroScan (Baxter, Baxter Healthcare Corporation, West Sacramento, USA), Vitek (bioMerieux,

Marcy- l'Etoile, France), MicroStation (Biolog, Heyward, USA) and Cobas (Becton Dickinson Instrument Systems, Sparks, USA) are the major systems in use.

The information stated in sections 3.2.1.1 - 3.2.1.9 applies to both kits and automated identification systems.

3.2.2.1 Special considerations

For accurate results, follow the manufacturer's procedure.

3.2.3 Molecular identification

Molecular probes have been developed for the detection and identification of *L.monocytogenes*, particularly for detection in food samples. However, to date molecular probes are only in use in some reference laboratories and have not been made commercially available for use by clinical laboratories.

3.3 Serotyping of *L. monocytogenes*

Although there are at least 13 serotypes of L.monocytogenes, almost all disease is caused by serotypes 4b, 1/2a and 1/2b. Serotyping of *Listeria* isolates therefore has limited epidemiological value.

3.3.1 Suitable specimen

Pure bacterial culture.

3.3.2 Materials

L.monocytogenes is subdivided into 13 serovars based on cellular O and flagellar H antigens; 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. Serotyping is usually confirmed in reference laboratories with stocks of type-specific antisera.

3.3.3 Test sensitivity

Unless an isolate is a rough colony variant/phenotype, most *L.monocytogenes* isolates can be serotyped.

3.3.4 Test specificity

No data available.

3.3.5 Predictive values

Failure to serotype a strain does not mean it does not have a serotype.

3.3.6 Suitable test acceptance criteria

Agglutination against a type-specific antisera.

3.3.7 Suitable internal controls

Regular serotyping of a range of different, previously characterised L.monocytogenes serotypes.

3.3.8 Suitable validation criteria

Unequivocal demonstration of a recognised serotype.

3.3.9 Suitable External QC program

None

3.4 Further subtyping

Further typing for epidemiological purposes is available in a small number of reference laboratories. Their methods include molecular serotyping, binary molecular typing, MLVA, MLST, Ribotyping and DNA macrorestriction (often known as “PFGE”). The methods chosen for use in specific jurisdictional laboratories differ according to public health operational priorities but can be regarded as complementary. In the event of multi-jurisdictional laboratory-based investigations, isolates or DNA extracts can be exchanged between reference laboratories within the PHLN. *L.monocytogenes* isolates should be forwarded to the nearest reference laboratory for confirmation, further analysis and storage.

3.5 Serodiagnosis

Serology is unreliable and is not recommended.

4 Agreed typing & subtyping methods

A standard nomenclature has yet to be agreed. Other than for binary genotyping systems such as MLST, where the naming is strictly arbitrary, genotyping methods are prone to multiple sources of variation. Even when binary genotyping systems are used, there may still be an implicit bias in genotype data interpretation by the specific mathematic algorithm adopted by interpretive software. The performance of specific *Listeria* genotyping methods should be discussed with the public health laboratory performing the analysis. The scientific interpretation of genotyping results is best done by the scientists who did the genotyping.

4.1 Laboratory Nomenclature for National Database Dictionary

4.1.1 Organism Name(s) List

- *Listeria monocytogenes*
- *Listeria ivanovii*
- *Listeria species*

4.1.2 Typing/Subtyping Nomenclature List(s)

Varies by jurisdiction.

5 References

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