

NATIONAL STANDARD METHOD

IDENTIFICATION OF *LEGIONELLA* SPECIES

BSOP ID 18

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections



Association of Medical Microbiologists
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Page 1 of 11

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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

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The HPA aims to be a fully 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 secure conditions¹.

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Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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IDENTIFICATION OF LEGIONELLA SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory

Page 2 of 11

Reference no: BSOP ID 18i2

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www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

INDEX

STATUS OF NATIONAL STANDARD METHODS	2
INDEX	3
AMENDMENT PROCEDURE	4
SCOPE OF DOCUMENT	5
INTRODUCTION	5
TECHNICAL INFORMATION	5
1 SAFETY CONSIDERATIONS	6
2 TARGET ORGANISMS	6
3 IDENTIFICATION	6
3.1 MICROSCOPIC APPEARANCE.....	6
3.2 PRIMARY ISOLATION MEDIA.....	6
3.3 COLONIAL APPEARANCE.....	6
3.4 TEST PROCEDURES.....	7
3.5 FURTHER IDENTIFICATION.....	7
3.6 STORAGE AND REFERRAL.....	7
4 IDENTIFICATION OF LEGIONELLA SPECIES - FLOW CHART	8
5 REPORTING	9
5.1 PRESUMPTIVE IDENTIFICATION.....	9
5.2 CONFIRMATION OF IDENTIFICATION.....	9
5.3 MEDICAL MICROBIOLOGIST.....	9
5.4 CCDC.....	9
5.5 CENTRE FOR INFECTIONS.....	9
5.6 INFECTION CONTROL STAFF.....	9
6 REFERRALS	9
6.1 REFERENCE LABORATORY.....	9
7 ACKNOWLEDGEMENTS AND CONTACTS	10
REFERENCES	11

IDENTIFICATION OF *LEGIONELLA* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory

Page 3 of 11

Reference no: BSOP ID 18i2

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AMENDMENT PROCEDURE

Controlled document reference	BSOP ID 18
Controlled document title	Identification of <i>Legionella</i> species

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

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
2/ 12.11.07	1.1	2	1	Front Page	Northern Ireland logo added
			5	Technical information	<i>L. oakridgensis</i> added and in flow chart
			8	Flow chart	Title changed and flowchart put in to Visio format. Contents of flow chart updated.
			9	6 Referrals	Links to reference laboratory user manuals inserted.
			11	References	References reviewed and updated
			All	All	PDF links inserted to cross-reference NSM documents

IDENTIFICATION OF *LEGIONELLA* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory

Page 4 of 11

Reference no: BSOP ID 18i2

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IDENTIFICATION OF *LEGIONELLA* SPECIES

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the presumptive identification of *Legionella* species isolated from clinical specimens to genus level. For information on which specimens to use for the investigation of *Legionella* species refer to [BSOP 47 - Investigation of specimens for Legionella species](#)

Full identification of *Legionella* species is not cost-effective in most routine clinical microbiology laboratories and isolates should be sent to the Reference Laboratory.

INTRODUCTION

Taxonomy

The family Legionellaceae includes 52 species and in excess of 60 serogroups². *Legionella pneumophila* is responsible for 95 per cent of infections with *Legionella* species. There are 16 serogroups of *L. pneumophila*, but serogroup 1 accounts for the majority of strains from human infections³.

Characteristics

The family Legionellaceae consists of faintly staining, Gram-negative, pleomorphic rods. They generally appear as coccobacilli in tissue or secretions, but may become filamentous in culture. The organisms are aerobic and will not grow on blood agar or buffered charcoal yeast extract agar without cysteine. The presence of soluble iron in the media also helps to support their growth².

Members of the genus are relatively inert biochemically, catalase-positive (some may be only weakly catalase-positive), oxidase-variable and possess polar flagella.

Some species other than *L. pneumophila* fluoresce blue-white under long-wave UV light (360nm ± 20nm) whilst others fluoresce dull yellow⁴ or brick red.

Principles of identification

Colonies isolated on Legionella selective agar are identified by colonial morphology, Gram's stain and by the requirement of L-cysteine for growth. All isolates from clinical specimens should be sent to the Reference Laboratory for confirmation and further identification.

TECHNICAL INFORMATION

Laboratories should be aware that some laboratory-adapted strains of *L. oakridgensis* lose the requirement for L-cystine.

1 SAFETY CONSIDERATIONS⁵⁻¹⁵

Legionella species are in Hazard Group 2 although in some cases the nature of the work with *L. pneumophila* may dictate full Containment Level 3 conditions.

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

The organism infects primarily by the respiratory route.

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

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

Compliance with postal and transport regulations is essential.

2 TARGET ORGANISMS

Legionella species

3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

Gram stain (see [BSOFTP 39 - Staining Procedures](#))

Gram-negative poorly staining rods, which may be filamentous in older cultures. Gram stain from cysteine containing agar only.

3.2 PRIMARY ISOLATION MEDIA

Buffered-charcoal-yeast extract (BCYE) agar base supplemented with ACES buffer with L-cysteine and the selective agar BMPA α (cefamandole, polymyxin B and anisomycin) incubated for up to 10 days in a moist atmosphere at 35 - 37°C ([BSOP 47 - Investigation of specimens for *Legionella* species](#)).

3.3 COLONIAL APPEARANCE

This requires the use of a low power binocular microscope with incident light illuminating the agar surface at an acute angle.

Legionella species produce a characteristic ground glass colony. However, it takes a minimum of 36 hours incubation before colonies can be seen, even with a low power microscope. A plate viewed at 24 hours will provide information of the location, number and morphology of contaminants. This will assist in eliminating 'suspect' colonies which might be further investigated if the plates are not read until 3 days. Colony edges are entire and tend to have speckled green or pinkish purple iridescent edges. The colour of the colonies may be a variety of shades of purple or green or a range of colours depending on the thickness of the agar plate and the age of the culture (colonies become grey with age).

IDENTIFICATION OF *LEGIONELLA* SPECIES

3.4 TEST PROCEDURES

Subculture to legionella selective and non-selective agar.

Legionella species will grow on legionella agar base (BCYE) supplemented with ACES buffer, L-cysteine. *Legionella* species will not grow on the same medium from which L-cysteine has been omitted. Growth on both plates indicates that the organism is not *Legionella* species. Please see Technical Information.

Inoculate each agar plate and for the isolation of individual colonies, spread inocula with a sterile loop (see [QSOP 52 - Inoculation of culture media \(formerly BSOP 54\)](#))

Colonies may be examined under long-wave UV light (360 nm ± 20nm) to reveal yellow pigment on BCYE or auto-fluorescence of Legionella colonies.

Following subculture, Gram's stain and catalase, cultures at this stage should be regarded as presumptive until confirmed by the Reference Laboratory.

3.5 FURTHER IDENTIFICATION

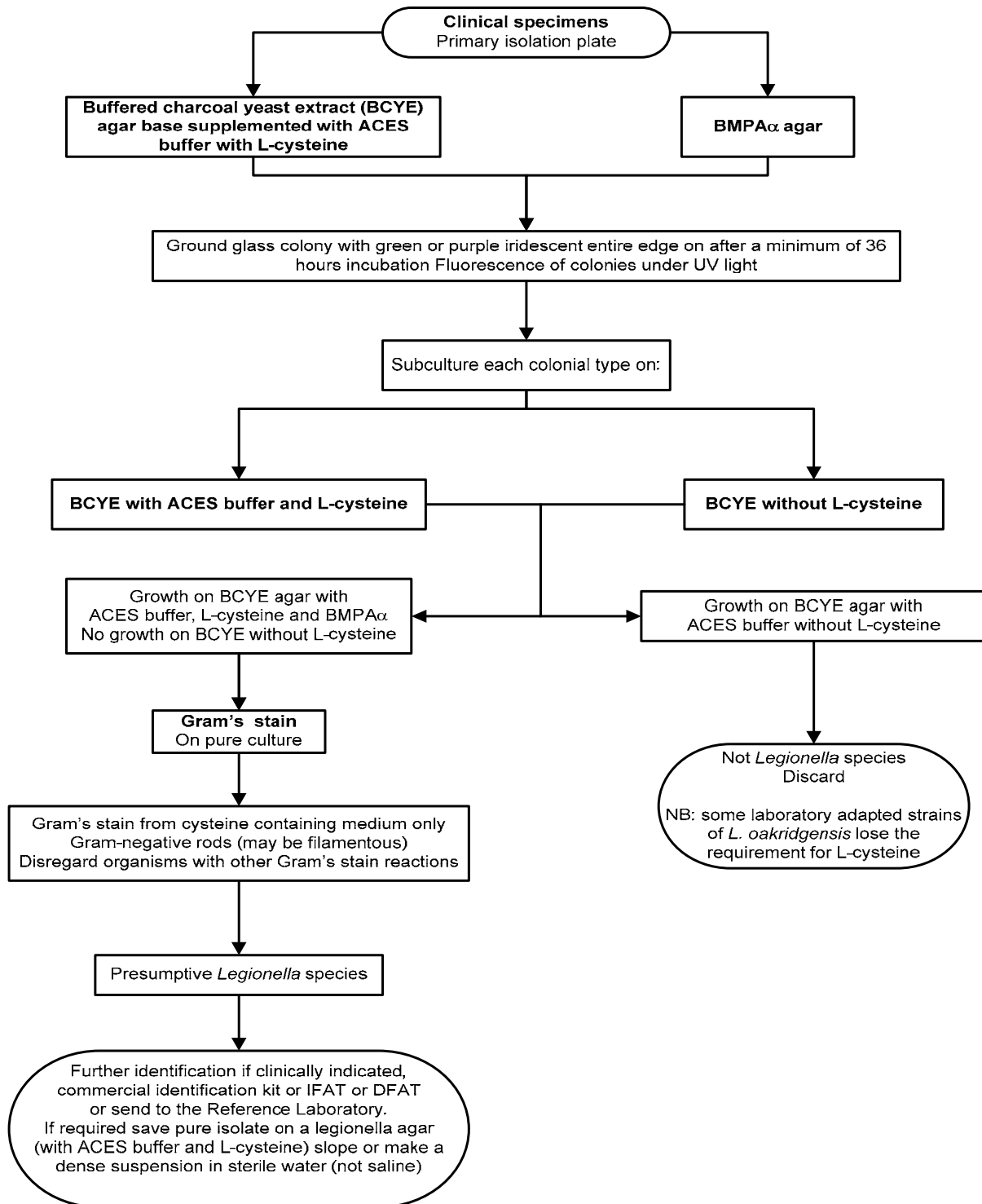
Use a commercial kit (latex or DFA) on a 'presumptive' isolate to get a basic identification this should be supported by a Reference Laboratory report.

3.6 STORAGE AND REFERRAL

Save pure isolate on legionella agar base with ACES buffer and L-cysteine slope or as a dense suspension in sterile water (not saline) for referral to the Reference Laboratory.

IDENTIFICATION OF *LEGIONELLA* SPECIES

4 IDENTIFICATION OF LEGIONELLA SPECIES - FLOW CHART



IDENTIFICATION OF LEGIONELLA SPECIES

5 REPORTING

5.1 PRESUMPTIVE IDENTIFICATION

If appropriate growth characteristics, colonial appearance, catalase and Gram's stain are demonstrated.

5.2 CONFIRMATION OF IDENTIFICATION

N/A

5.3 MEDICAL MICROBIOLOGIST

Inform the medical microbiologist of any presumptive *Legionella* species.

The medical microbiologist should be informed of all confirmed legionella isolates.

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 CENTRE FOR INFECTIONS ¹⁶

Refer to current guidelines on CDSC and COSURV reporting.

5.6 INFECTION CONTROL STAFF

Inform the infection control team of new and presumptive isolates of *Legionella* species.

6 REFERRALS

6.1 REFERENCE LABORATORY

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

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7 ACKNOWLEDGEMENTS AND CONTACTS

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Page 11 of 11

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