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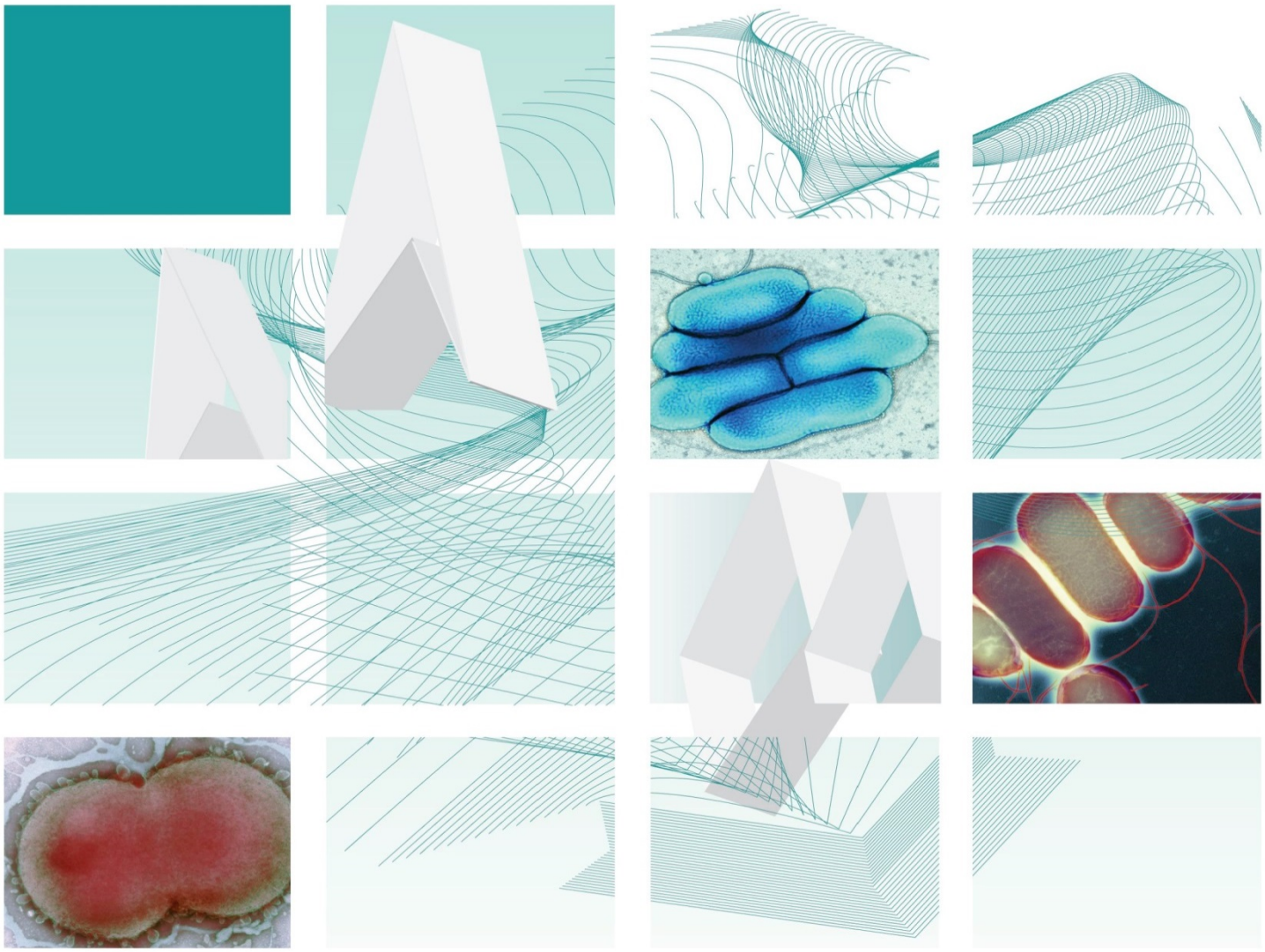
UK Standards for Microbiology Investigations

Identification of *Vibrio* and *Aeromonas* species



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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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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.	5/14.04.15
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Title Change.	The title has been updated to include *Aeromonas* species.
Scope of Document.	The scope has been updated to include *Aeromonas* species.
Introduction.	The taxonomy of *Vibrio* species has been updated. *Aeromonas* species have been added into this document. More information has been added to the Characteristics section. The medically important species of *Aeromonas* and *Vibrio* are mentioned. Section on Principles of Identification has been updated to include the MALDI-TOF.
Technical Information/Limitations.	Addition of information regarding serology, oxidase test, Gram stain, commercial identification systems and differentiation between *Aeromonas* and *Vibrio* species.
Safety Considerations.	This section has been updated on the handling of *Aeromonas* and *Vibrio* species as well as laboratory acquired infections.
Target Organisms.	The section on the Target organisms has been updated and presented clearly for both organisms.
Identification.	Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice. Section 3.4.2, 3.4.3 and 3.4.4 has been updated to include Commercial Identification Systems,

	MALDI-TOF MS and NAATs with references. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification Flowchart.	Modification of flowchart for identification of *Vibrio* species has been done for easy guidance. A new flowchart for identification of *Aeromonas* species has also been done.
Reporting.	Subsections 5.3, 5.5 and 5.6 have been updated to reflect the information required on reporting practice.
Referral.	The addresses of the reference laboratories have been updated.
References.	Some references updated.

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 secure 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.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

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.

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Scope of Document

This SMI describes the identification of *Vibrio* and *Aeromonas* species.

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The genus *Vibrio* is a member of the family Vibrionaceae and consists of 103 recognised species. Twelve species have been reclassified to other genera within the family[1]. Currently, only 10 species of the genus *Vibrio* have been incriminated in gastrointestinal and extra-intestinal diseases in man; the most important of these being *Vibrio cholerae*, the cause of cholera.

The genus *Aeromonas* now belongs to the family Aeromonadaceae (which is currently made up of *Oceanimonas, Aeromonas, Tolumonas, Zobellella* and *Oceanisphaera*) after being relocated from the family Vibrionaceae because they were not closely related to the vibrios upon phylogenetic analyses. The current classification of the genus *Aeromonas* is based on DNA-DNA hybridisation and 16S rDNA relatedness. It consists of 31 recognised species and 12 subspecies[2]. Of these, only 17 are currently known to cause infections in humans ranging from gastroenteritis to wound infections and septicaemia. They are *A. aquariorum, A. bestiarum, A. caviae*, *A. diversa, A. fluvialis, A. hydrophila, A. jandaei, A. media, A. popoffii*, *A. salmonicida, A. sanarellii, A. schubertii, A. sobria, A. taiwanensis, A. tecta, A. trota and A. veronii*[3-5].

Characteristics

Vibrio species

Vibrio species are straight or curved Gram negative non-spore forming rods, 0.5-0.8µm wide x 1.4-2.6µm long in size. They all grow at 20°C and most at 30°C. On blood agar, colonies are greyish and circular, 2-3mm in diameter and colonies on thiosulphate citrate bile salt sucrose (TCBS) agar are either yellow or green. *Vibrio* species are facultative anaerobes and are motile by polar flagellum with sheaths. *V. cholerae* has a single polar flagellum with sheath. Some species, such as *V. parahaemolyticus* and *V. alginolyticus*, have both a single polar flagellum with sheath and thin flagella projecting in all directions, and the other species, such as *Aliivibrio fischeri* (formerly known as *V. fischeri*), have tufts of polar flagella with sheath. They are also mesophilic and chemoorganotrophic, and have a facultatively fermentative metabolism[6].

All members of the genus *Vibrio*, with the exceptions of *V. metschnikovii* and *V. gazogenes* (non-human), are oxidase positive and reduce nitrates to nitrites[7]. They are usually sensitive to the vibriostatic agent O129 (2, 4-diamino-6, 7-diisopropylpteridine phosphate-150µg disc). Growth is stimulated by sodium ions (halophilic) - the concentration required is reflected in the salinity of their natural environment. *V. cholerae* (the causative agent of cholera) is not halophilic[7].

Vibrio species are sea-dwelling organisms, and some species have been known to cause fatal infections in humans. In humans, *Vibrio* species has been isolated from stool, vomitus, blood, or wound infections[8,9].

The type species is *V. chloerae*.

The medically important *Vibrio* species are:

V. cholerae

Cells are comma shaped gram negative, non-spore forming rods. The bacterium is 1- 3µm x 0.5-0.8µm and is motile. It has a single polar flagellum. They grow at several temperatures - 4°C, 20°C, 30°C and 35 – 37°C. On blood agar, colonies are strongly haemolytic except for strains of the classical biotype of *V. cholerae*, which are non-haemolytic. On TCBS agar, colonies are yellow and at least 2 mm in diameter after 18 – 24hr incubation[10].

Vibrio cholerae can be serogrouped into 155 groups on the basis of somatic O antigens. Serogroups O1 (classical and El Tor biotypes) and O139 are primarily responsible for cholera outbreaks. Epidemic strains of *V. cholerae* O1 can be differentiated into El Tor and classical biotypes, which are further subdivided into Inaba, Ogawa and Hikojima subtypes. Worldwide, *V. cholerae* El Tor is currently the predominant biotype and Ogawa the predominant subtype. Strains not belonging to serogroup O1 are generally referred to as *V. cholerae* non-O1 and can still cause illness in humans. In 1993 an outbreak of epidemic cholera began in Bengal caused by a new serogroup of non-O1 *V. cholerae*[11]. Although initial isolates of this serogroup (O139) were resistant to vibriostatic agent O129, recently isolated strains are sensitive[11].

V. cholerae O1 depends on the detection of the O1 antigen on the surface of the bacterium, and therefore does not identify *V. cholerae* O139 strains.

V. cholerae O1 classical biotype is Voges-Proskauer (VP) negative and is sensitive to polymyxin (50 IU disc). *V. cholerae* O1 El Tor biotype is VP positive and is resistant to polymyxin[12]. They are oxidase positive, reduce nitrates, grow at 40°C, as well as utilize sucrose, α-ketoglutarate and also grow in the absence of Na^+. These distinguish them from other species of *Vibrio*[10].

The source of some outbreaks have been linked with contaminated shellfish, including raw oysters and crabs[8].

V. parahaemolyticus

They have similar characteristics to the *V. cholerae.* However on TCBS agar, colonies are green and at least 2 mm in diameter after 18 – 24hr incubation.
V. parahaemolyticus is also associated with the Kanagawa phenomenon, in which strains isolated from human hosts (clinical isolates) are haemolytic on blood agar plates, while those isolated from non-human sources are non-haemolytic.

They are also catalase and oxidase positive. They do not ferment sucrose.

V. parahaemolyticus may spread into humans orally via contaminated food, particularly molluscs such as oysters leading to the development of acute gastroenteritis with diarrhoea[8].

V. vulnificus

They have similar characteristics to the *V. cholerae*. However on TCBS agar, colonies are green and at least 2mm in diameter after 18 – 24hr incubation.

They are also catalase and oxidase positive. They give variable results for sucrose fermentation although are usually negative.

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