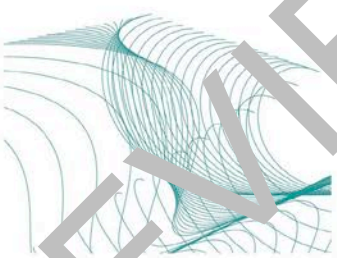
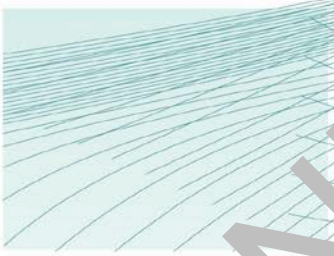
Public Health England

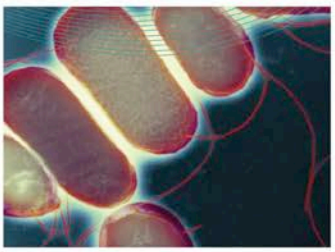
NHS

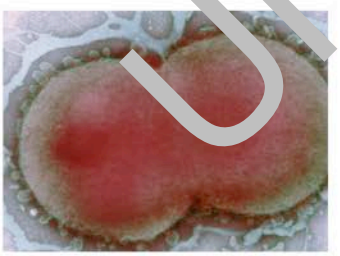
UK Standards for Microbiology Investigations

Identification of *Vibrio* 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 http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

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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UK Standards for Microbiology Investigations are produced in association with:

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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.	4/11.03.14
Issue no. discarded.	2.1
Insert Issue no.	2.2
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	3/21.10.11
Issue no. discarded.	2
Insert Issue no.	2.1
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format.
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 http://www.hpa.org.uk/SMI/Partnerships. 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 http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. 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.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2014). Identification of *Vibrio* species. UK Standards for Microbiology Investigations. ID 19 Issue 2.2. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

This SMI describes the identification of *Vibrio* 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 at least 34 recognised species. *Vibrio cholerae* can be serogrouped into 155 groups on the basis of somatic antigens. Epidemic strains usually belong to serogroup O1, which can be further subdivided into Inaba, Ogawa and Hikojima subtypes. Epidemic strains of *V. cholerae* O1 can be further differentiated into El Tor and Classical biotypes. Strains not belonging to serogroup O1 are generally referred to as *V. cholerae* non-O1. In 1993 an outbreak of epidemic cholera, caused by a new serogroup of non-O1 *V. cholerae,* began in Bengal[1]. Although initial isolates of this serogroup (O139) were resistant to vibriostatic agent O129, recently isolated strains are sensitive[1].

Characteristics

Vibrio species are curved, Gram negative rods. They produce colonies 2-3mm in diameter on blood agar, and colonies on thiosulphate citrate bile salt sucrose (TCBS) are either yellow or green. *Vibrio* species are facultative anaerobes, motile by a single polar flagellum, and are oxidase positive (except *Vibrio metschnikovii*[2]). 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[2].

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 VP-negative and is sensitive to polymyxin (50 IU disc). *V. cholerae* O1 El Tor biotype is VP-positive and is resistant to polymyxin[3].

Twelve species of the genus *Vibrio* have been incriminated in gastrointestinal and extra intestinal diseases in man; the most important of these is cholera.

Principles of Identification

Isolates from primary culture are identified by colonial appearance, Gram stain, serology (agglutination with specific antisera) and biochemical testing. 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. The oxidase test may give false negative results if performed from TCBS agar.

It should be noted that *V. hollisae* will not grow on TCBS[4].

Technical Information/Limitations

N/A

1 Safety Considerations[5-21]

Hazard Group 2 organisms.

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.

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

Compliance with postal and transport regulations is essential.

2 Target Organisms

***Vibrio* species reported to have caused human disease[22]**

Vibrio alginolyticus	*Vibrio furnissii*
Vibrio carchariae	*Vibrio hollisae*
Vibrio cholerae	*Vibrio metschnikovii*
Vibrio cincinnatiensis	*Vibrio mimicus*
Vibrio damsela	*Vibrio parahaemolyticus*
Vibrio fluvialis	*Vibrio vulnificus*

Any species of Vibrio may be found in faeces after the ingestion of seafood or water that contains them.

3 Identification

3.1 Microscopic Appearance

Gram stain (refer to TP 39 - Staining Procedures)

Gram negative rods are characteristically curved or comma-shaped. This characteristic appearance is not always observed when the organism is Gram stained from solid media.

3.2 Primary Isolation Media

Blood agar incubated in air at 35-37°C for 18-24hr

TCBS agar incubated in air at 35-37°C for 18-24hr

3.3 Colonial Appearance

On blood agar, colonies are 2-3mm in diameter. Some strains may be haemolytic. After 18-24hr incubation colonies on TCBS are at least 2mm in diameter, and yellow in the case of sucrose fermenters, and green non-sucrose fermenters. Cultures should be examined quickly after removal from the incubator as the yellow colouration of the colonies may revert to a green colour when left at room temperature. Organisms other than *Vibrio* species grow on TCBS.

Organism	Colour of colonies on TCBS
V. cholerae	yellow
V. alginolyticus	yellow
V. cincinnatiensis	yellow
V. damsela	green
V. carchariae	yellow/green
V. fluvialis	yellow
V. furnissii	yellow
V. hollisae (Note: has been shown not to grow on TCBS[4])	green
V. parahaemolyticus	green
V. metschnikovii	yellow
V. vulnificus	green
V. mimicus	green
Aeromonas species	yellow
Pseudomonas species	blue/green*
Proteus species	yellow/green*
Enterococcus species	yellow

* The colonies are smaller than those produced by *Vibrio* species

3.4 Test Procedures

Oxidase Test (TP 26 - Oxidase Test)

Vibrio species are oxidase positive (oxidase tests may give false negative results on media containing carbohydrates - subculture to nutrient or blood agar before testing).

Sensitivity to pteridine O129 (10µg and 150µg discs)

Most *Vibrio* species are sensitive with 150µg but species differ with 10µg discs (some strains of *V. cholerae* O1 and O139 may be resistant to both disc contents).

Serology

Commercial identification kit

These tests may require supplementation with NaCl. Refer to the manufacturer's instructions.

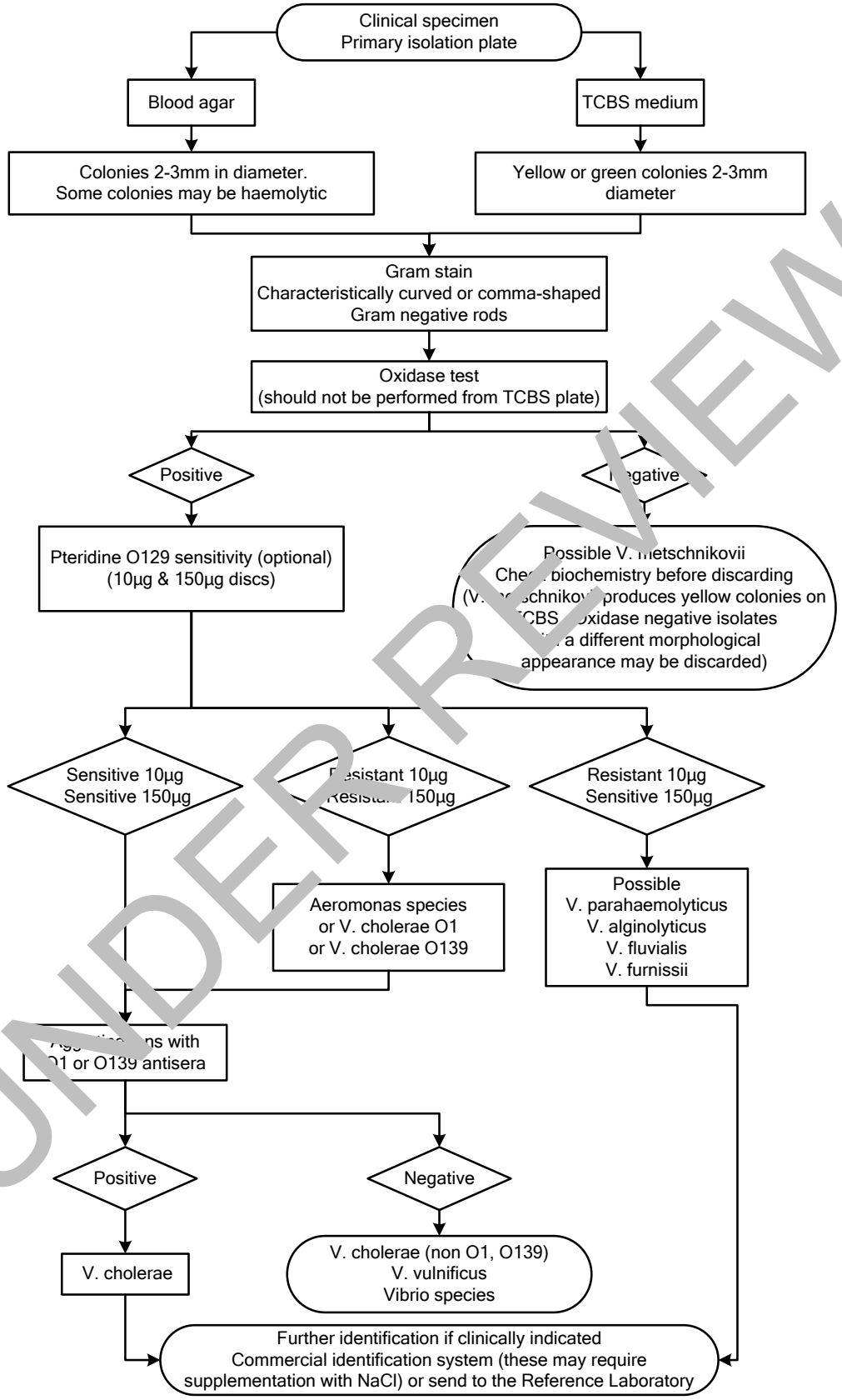
3.5 Further Identification

Following O129 sensitivity testing (optional), serology and commercial identification system results.

3.6 Storage and Referral

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

4 Identification of *Vibrio* species



The flowchart is for guidance only

5 Reporting

5.1 Presumptive Identification

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

5.2 Confirmation of Identification

Further biochemical tests and/or molecular methods and/or reference laboratory report.

5.3 Medical Microbiologist

Inform the medical microbiologist of all positive cultures from normally sterile sites of all presumed and confirmed *Vibrio* species that are known to be pathogenic or potentially pathogenic, and all isolates in outbreaks.

Inform the medical microbiologist if the request card bears information which suggests infection with *V. cholerae* or *V. parahaemolyticus*, according to local protocols, eg:

- Severe watery diarrhoea
- Suspected cholera
- History of foreign travel, or laboratory work
- Suspected food poisoning (especially cases involving consumption of seafood)

The medical microbiologist should also be informed of presumed or confirmed *Vibrio* species in association with:

- Wound infection or (necrotising) myofasciitis
- Septicaemia
- History of foreign travel
- Contact with (brackish) water, fishing/eating fish or seafood (suggestive of infection with *V. vulnificus*, *V. damsela* or *Aeromonas hydrophila sensu lato)*
- Medicinal use of leeches, as in plastic surgery (suggestive of infection with *Aeromonas hydrophila sensu lato*)
- Alcoholism, substance abuse, immunodeficiency
- Other serious medical condition such as cancer, or persons receiving treatment for cancer 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[23]

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

Inform the infection control team of presumptive and confirmed isolates of *Vibrio* species.

6 Referrals

6.1 Reference Laboratory

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

Gastrointestinal Infections Reference Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5EQ
http://www.hpa.org.uk/cfi/lep/default.htm

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

England and Wales
http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370

Scotland

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

Northern Ireland

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

7 Notification to PHE[23,24] or Equivalent in the Devolved Administrations[25-28]

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'.

Other arrangements exist in Scotland[25,26], Wales[27] and Northern Ireland[28].

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