

NATIONAL STANDARD METHOD

IDENTIFICATION OF *VIBRIO* SPECIES

BSOP ID 19

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



Association of Medical Microbiologists
Association of Medical Microbiologists
Association of Medical Microbiologists



IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 1 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

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.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

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.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

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.

Suggested citation for this document:

Health Protection Agency (2007). *Identification of Vibrio species*. National Standard Method BSOP ID 19 Issue 2. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

IDENTIFICATION OF VIBRIO SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 2 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

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 <i>VIBRIO</i> – 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	10
6.1 REFERENCE LABORATORY.....	10
7 ACKNOWLEDGEMENTS AND CONTACTS.....	11
REFERENCES	12

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 3 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

AMENDMENT PROCEDURE

Controlled document reference	BSOP ID 19
Controlled document title	Identification of <i>Vibrio</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
			8	Flow chart	Title changed and flowchart put in to Visio format. Contents of flow chart updated.
			6	6 Referrals	Links to reference laboratory user manuals inserted.
			12	References	References reviewed and updated
			All	All	PDF links inserted to cross- reference NSM documents

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 4 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

IDENTIFICATION OF *VIBRIO* SPECIES

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the identification of *Vibrio* species.

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 began in Bengal caused by a new serogroup of non-O1 *V. cholerae*². Although initial isolates of this serogroup (O139) were resistant to vibriostatic agent O129, recently isolated strains are sensitive².

Characteristics

Vibrio species are curved, Gram-negative rods. They produce colonies 2 - 3 mm 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*³). They are usually sensitive to the vibriostatic agent O129 (2,4-diamino-6, 7-diisopropylpteridine phosphate – 150 µg 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 halophilic³.

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 polymyxin⁴.

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's 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 TCBS⁵.

TECHNICAL INFORMATION

N/A

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 5 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

1 SAFETY CONSIDERATIONS⁶⁻¹⁶

Hazard Group 2 organisms.

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

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 disease¹⁷**

Vibrio alginolyticus

Vibrio carchariae

Vibrio cholerae

Vibrio cincinnatiensis

Vibrio damsela

Vibrio fluvialis

Vibrio furnissii

Vibrio hollisae

Vibrio metschnikovii

Vibrio mimicus

Vibrio parahaemolyticus

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

Gram-negative rods 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 – 24 h

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

3.3 COLONIAL APPEARANCE

On blood agar colonies are 2 - 3 mm in diameter. Some strains may be haemolytic. After 18 - 24 hours incubation colonies on TCBS are at least 2 mm 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.

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 6 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

Organism	Colour of colonies on TCBS
<i>V. cholerae</i>	yellow
<i>V. alginolyticus</i>	yellow
<i>V. cincinnatiensis</i>	yellow
<i>V. damsela</i>	green
<i>V. carchariae</i>	yellow/green
<i>V. fluvialis</i>	yellow
<i>V. furnissii</i>	yellow
<i>V. hollisae</i> (NB has been shown not to grow on TCBS ⁵)	green
<i>V. parahaemolyticus</i>	green
<i>V. metschnikovii</i>	yellow
<i>V. vulnificus</i>	green
<i>V. mimicus</i>	green
<i>Aeromonas</i> species	yellow
<i>Pseudomonas</i> species	blue/green*
<i>Proteus</i> species	yellow/green*
<i>Enterococcus</i> species	yellow

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

3.4 TEST PROCEDURES

Oxidase (see [BSOPTH 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 (10 µg and 150 µg discs)

Most *Vibrio* species are sensitive with 150 µg but species differ with 10 µg 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.

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 7 of 13

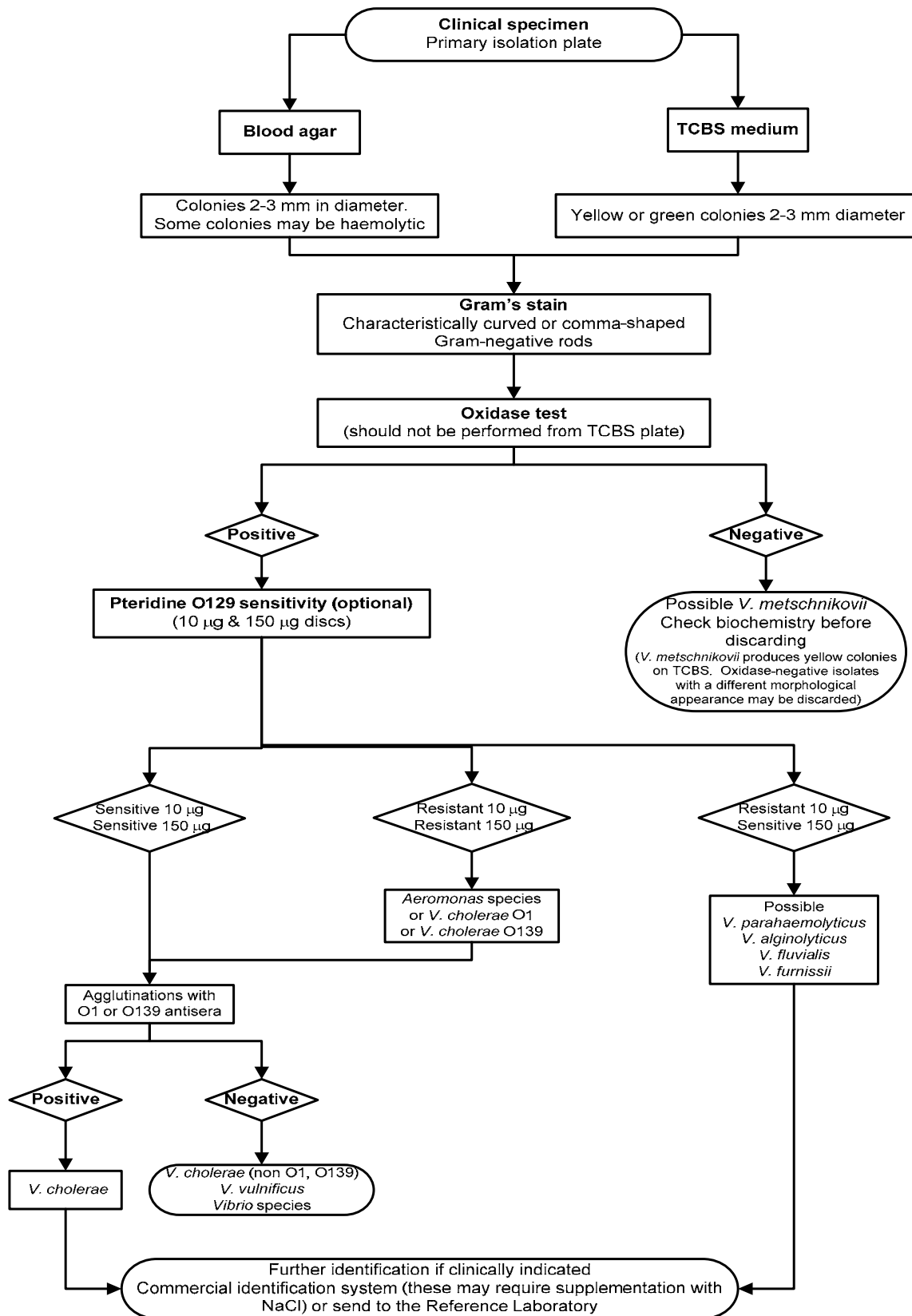
Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

4 IDENTIFICATION OF *VIBRIO* – FLOW CHART



The flowchart is for guidance only

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 8 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

5 REPORTING

5.1 PRESUMPTIVE IDENTIFICATION

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

5.2 CONFIRMATION OF IDENTIFICATION

N/A

5.3 MEDICAL MICROBIOLOGIST

Inform the medical microbiologist of all positive cultures from normally sterile sites, of all presumptive 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 presumptive 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 CENTRE FOR INFECTIONS¹⁸

Refer to current guidelines on CDSC and COSURV reporting.

5.6 INFECTION CONTROL STAFF

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

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 9 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

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/lep/default.htm>

Laboratory of Enteric Pathogens
Centre for Infections
Health Protection Agency
61 Colindale Avenue
London
NW9 5HT

Contact CFI main switchboard: Tel. +44 (0) 20 8200 6173

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 10 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

7 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency London.

For further information please contact us at:

Standards Unit
Evaluations and Standards Laboratory
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 11 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Sack DA, Sack RB, Nair GB, Siddique AK. Cholera. *Lancet* 2004;363:223-33.
3. Tantillo GM, Fontanarosa M, Di Pinto A, Musti M. Updated perspectives on emerging vibrios associated with human infections. *Lett Appl Microbiol* 2004;39:117-26.
4. Color Atlas and Textbook of Diagnostic Microbiology. In: Konemann EW, Allen SD, Janda WM, Schreckenberger PC, Winn WJ, editors. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 1997. p. 346-8.
5. Hickman FW, Farmer JJ, III, Hollis DG, Fanning GR, Steigerwalt AG, Weaver RE, et al. Identification of *Vibrio hollisae* sp. nov. from patients with diarrhea. *J Clin Microbiol* 1982;15:395-401.
6. Advisory Committee on Dangerous Pathogens. 2004 Approved List of Biological Agents. <http://www.hse.gov.uk/pubns/misc208.pdf>. p. 1-17.
7. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety Precautions: Notes for Guidance. 4th ed. London: Public Health Laboratory Service (PHLS); 1993.
8. Control of Substances Hazardous to Health Regulations 2002. General COSHH. Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002.
9. Health and Safety Executive. 5 steps to risk assessment: a step by step guide to a safer and healthier workplace, IND (G) 163 (REVL). Suffolk: HSE Books; 2002.
10. Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law, IND (G) 218 (L). Suffolk: HSE Books; 2002.
11. Health Services Advisory Committee. Safety in Health Service laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2nd ed. Suffolk: HSE Books; 2003.
12. NHS Estates. Health Building Note 15. Facilities for pathology services. 2nd ed. London: The Stationary Office; 2005.
13. BS EN 12469: 2000. Biotechnology - performance criteria for microbiological safety cabinets. London: British Standards Institution (BSI); 2000.
14. BS 5726: 1992. Microbiological safety cabinets. Part 2. Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation. London: British Standards Institution (BSI); 1992.
15. BS 5726: 1992. Microbiological safety cabinets. Part 4. Recommendations for selection, use and maintenance. London: British Standards Institution (BSI); 1992.
16. Advisory Committee on Dangerous Pathogens. The management, design and operation of microbiological containment laboratories. Suffolk: HSE Books; 2001.
17. Farmer JJ, III, Hickman-Brenner FW. The genera *Vibrio* and *Photobacterium*. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH, editors. *The Prokaryotes*. 2nd ed. Vol 3. New York: Springer-Verlag; 1992. p. 2952-3011.

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 12 of 13

Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

18. Health Protection Agency. Laboratory Reporting to the Health Protection Agency. Guide for diagnostic laboratories. February. 2007.

IDENTIFICATION OF *VIBRIO* SPECIES

Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 13 of 13
Reference no: BSOP ID 19i2

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk