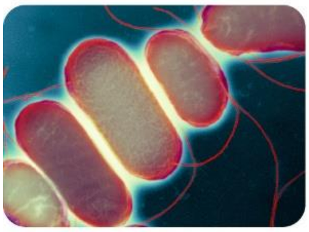
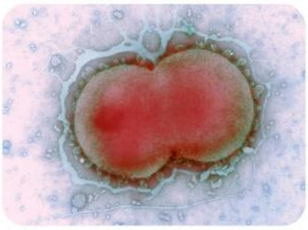
UK Health Security Agency

UK Standards for Microbiology Investigations

Identification of *Vibrio* and *Aeromonas* species





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Acknowledgments

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Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	6/31.07.25
Issue number discarded	3
Insert issue number	3.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 14/04/2015.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms. Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template. 'Public Health responsibilities for diagnostic laboratories' section added.

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 MS.
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.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

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

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

4.1 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].

4.2 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, 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.

In humans, *V. vulnificus* has been associated with a small but increasing number of serious life-threatening conditions, many stemming from wound infections which become septicaemic after exposure in infected waters or via puncture wounds from the spines of fish such as tilapia or stingrays[8,13].

It has been isolated from stool, wound, or blood culture in humans and in the environment - seawater, sediments, plankton, shellfish (oysters, clams and crabs).

Aeromonas species

The genus *Aeromonas* are made up of straight, coccobacillary to bacillary Gram negative bacteria with surrounding ends measuring 0.3 -1.0µm wide x 1.0 - 3.5µm long. They are non- spore formers. Most motile strains produce a single polar flagellum, while peritrichous or lateral flagella may be formed on solid media in some species. On blood agar, colonies appear distinctive with or without haemolysis after aerobic incubation at 35°C for 18 - 24hr. They also grow readily in blood culture media for isolation from normally sterile body sites as well as on MacConkey agar or cefsulodin-irgasan-novobiocin agar at 35°C for 24 - 48hr.

Aeromonas species are facultative anaerobic, catalase and oxidase positive, as well as chemoorganotrophic. They produce diverse kinds of extracellular hydrolytic enzymes such as arylamidases, esterases, amylase, elastase, deoxyribonuclease, chitinase, peptidases, and lipase. They also grow optimally at temperature ranges of between 22°C and 35°C, but growth can also occur at 0 - 45°C in a few species. Some species, such as *A. salmonicida* strains, do not grow at 35°C but rather at 22 - 28°C. Their optimum pH range is 5.5 – 9 and optimum sodium chloride concentration range is 0 - 4%[5].

Their resistance to vibrostatic compound 0/129 (150µg) and variable presence of ornithine decarboxylase activities differentiates the genus from *Plesiomonas* and *Vibrio*. All *Aeromonas* species are negative for ornithine decarboxylase hydrolysis (except for *A. veronii* biovar veronii)[14]. Other important distinguishing qualities include their inability to grow in the presence of 6.5% sodium chloride; ability to liquefy gelatin; inability to ferment i-inositol and their negative string test[5].

The aeromonads and Enterobacterialesshare many biochemical characteristics but are easily differentiated by oxidase test for which the aeromonads are positive.

Aeromonas species are found globally in surface water, ground water, chlorinated drinking water, non-chlorinated drinking water, bottled mineral water and broad range of foods. They are found in the intestinal tract of humans and animals, ticks and insects, raw sewage, sewage effluents, soil, sewage contaminated waters and activated sludge[5]. These species have also been introduced into humans via contaminated lines, such as catheters and transhepatic drainage devices as well as from replaceable contact lens wear[4].

Aeromonas species are known as causative agents of a wide spectrum of diseases in man and animals. Those capable of causing diseases in human are associated with a variety of infections including septicaemia, meningitis, wound infections, pneumonia, peritonitis, urogenital tract, ocular and hepatobiliary infections.

These species have been isolated from clinical specimens such as – sputum or other respiratory tract specimens, bronchoalveolar lavage, lung and pleural effusions, wounds (either through trauma, medicinal leech therapy, bites of various animal species or burns), faeces, skin lesions, gastrointestinal tract, urine and blood culture. They have also been isolated from foods like fish, shellfish, meats, dairy products and fresh vegetables[4,5].

The medically important *Aeromonas* species are:

A. hydrophila

Cells are straight rods with rounded ends usually from 0.3 - 1.0μm in width, and 1.0 - 3.0μm in length. They can grow at temperatures as low as 4°C. These bacteria are motile by a polar flagellum.

A. hydrophila cause disease in humans, such as gastroenteritis, myonecrosis, eczema, and in rare cases, septicaemia. It is also associated with cellulitis and spa bath folliculitis.

In humans, this organism has been isolated from specimens such as faeces, blood, throat and from infected wounds.

A. caviae

They have similar characteristics as *A. hydrophila.*

This organism has been isolated from rectal surveillance cultures and specimens such as faeces, infected surgical wounds and liver abscess[15].

A. veronii biovar sobria

They have similar characteristics as *A. hydrophila* except that they are negative for ornithine decarboxylase reaction which differentiates them from *A. veronii* biovar *veronii*.

In humans, *A. veronii* can cause diseases ranging from wound infections and diarrhoea to septicaemia in immunocompromised patients. *A. veronii* has also been isolated from stools, wounds, and the respiratory tract of humans.

4.3 Principles of Identification

Isolates from primary culture are identified by colonial appearance, Gram stain, serology (agglutination with specific antisera) and biochemical testing.

Full identification using for example, MALDI-TOF MS can be used to identify *Vibrio* and *Aeromonas* isolates to species level.

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.

5 Technical Information/Limitations

Oxidase Test

The oxidase test may give false negative results if performed from TCBS agar and so colonies should be sub-cultured to a non-selective medium such as blood agar or on any media without fermentable sugars before testing[9].

Gram Stain

Gram stain is a relative rapid and easy procedure for diagnosis. The morphology of *Vibrio* species should be curved Gram negative rods on microscopic examination. Based on this characteristics, a Gram stain can promptly help differentiate *Vibrio* species from *Pseudomonas* species[16].

Differentiation between *Aeromonas* species and *Vibrio* species

Aeromonas' resistance to vibrostatic compound O/129 (150µg) and variable presence of ornithine decarboxylase activities differentiates the genus from *Plesiomonas* and *Vibrio*. Other important distinguishing qualities include their inability to grow in the presence of 6.5% sodium chloride; ability to liquefy gelatin; inability to ferment I -inositol; negative string test[5].

Serology

Agglutination should be carried out with subcultures onto non-selective agar, because *Vibrio* colonies can auto-agglutinate from TCBS agar, giving false-positive results[11].

Serology assays are not considered reliable for detecting antibodies to *Aeromonas* because of their low sensitivity and specificity[14].

Commercial identification systems

Identification may be attempted using commercial systems but their results are not always reliable. However, some of these automated commercial identification systems do not have the ability to differentiate between closely related species such as *A. hydrophila* and *A. caviae* as well as between the two genera, *Aeromonas* and *Vibrio* resulting in very major errors[4,17].

Misidentification between *Photobacterium damselae* and *Vibrio* species

It is important that diagnostic laboratories are aware that failure to grow on TCBS agar is a common feature of many *Photobacterium damselae* (formerly known as *Vibrio damsela*e) isolates. Green colonies can be obtained by increasing the iron content with the addition of 0.3% ferric citrate, but this is unlikely to be used in a clinical laboratory. *Photobacterium* species produce plump straight rods unlike *Vibrio* species that produce straight or curved rods.

Photobacterium damselae has been described as a vibrio-like organism that is implicated in wound infections predominantly necrotizing fasciitis in healthy patients[18].

6 Safety Considerations[19-35]

V. cholerae and *V. parahaemolyticus* are Hazard Group 2 organisms, and in some cases the nature of the work may dictate full Containment Level 3 conditions. All laboratories should handle specimens as if potentially high risk.

V. cholerae and *V. parahaemolyticus* cause severe and sometimes fatal diseases. The infectious dose ranges between 10^6 and 10^{11} ingested *Vibrio* organisms. Laboratory-acquired infections have been reported[36-38]. Infection may be acquired either through ingestion, contact with non-intact skin or mucosa and accidental parenteral inoculation[39]. Vaccine is recommended for laboratory workers who may be regularly exposed to cholera in the course of their work. This would normally only include those working in reference laboratories or in laboratories attached to infectious disease units; guidance is given in the DH Green Book[40]. This vaccine confers protection specific to *V. cholerae* serogroup O1. Immunisation does not protect against *V. cholerae* serogroup O139 or other species of *Vibrio*[40].

Aeromonas species are Hazard Group 2 organisms. The infectious dose for humans is greater than 10^{10} organisms. No laboratory-acquired infections have been reported to date. However, care should be taken when working with animals (reptiles, or aquatic animals) in a laboratory environment. Infection may be acquired either through ingestion, accidental inoculation and direct contact with contaminated areas.

The most effective method for preventing laboratory-acquired infections is the adoption of safe working practices. Complying with these rules remains the top priority.

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

7 Target Organisms

Vibrio species commonly reported to cause human disease[13]

*V. cholerae (serogroups 01 and 0139 (*Bengal*)), V. parahaemolyticus*,
V. vulnificus

Other _Vibrio_ species reported to have caused human disease[41]

V. alginolyticus, V. carchariae, V. cholerae (serogroups other than 01 and 0139)*,*
V. cincinnatiensis, V. fluvialis, V. furnissii, V. metschnikovii, V. mimicus

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

Aeromonas species commonly reported to cause human disease[3,4,15,42,43]

A. hydrophila, *A. caviae, A. veronii* biovar sobria

Other _Aeromonas_ species reported to have caused human disease

A. diversa, A. fluvialis, A. taiwanensis, A. sanarellii, A. media, A. schubertii, A. jandaei, A. trota, A. bestiarum, A. popoffii, A. aquariorum, A. bestiarum, A. sobria, A. salmonicida, A. tecta, A. trota, A. taiwanensis and *A. veronii* biovar veronii

Aeromonas species has also been found in the stools of 1% to 4% of asymptomatic individuals with no underlying health disorders[4].

8 Identification

8.1 Microscopic appearance

Gram stain

(refer to UK SMI TP 39 - Staining Procedures)

Vibrio species

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

Aeromonas species

Cells are Gram negative rods characteristically straight, coccobacillary to bacillary, with rounded ends measuring 0.3 -1.0µm wide x 1.0 - 3.5µm long. They appear singly or in pairs and on occasions in short chains.

8.2 Primary isolation media

Vibrio species

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

Thiosulfate citrate bile salts sucrose (TCBS) agar incubated in air at 35-37°C for 18-24hr

Aeromonas species

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

Cefsulodin-irgasan-novobiocin (CIN) agar incubated in air at 35-37°C for 24 -48hr

MacConkey agar incubated in air at 35-37°C for 24 - 48hr

Other validated media may be used.

Note: TCBS, a selective agar for *Vibrio* species, is inhibitory to *Aeromonas* species and should not be used when *Aeromonas* gastrointestinal infections are suspected.

For isolation of aeromonads from faeces, cefsulodin-irgasan-novobiocin (CIN) agar can be used as a selective medium.

Some *Aeromonas* species, such as *A. salmonicida* strains, do not grow at 35 - 37°C but rather at 22 - 28°C.

8.3 Colonial Appearance

Vibrio species

On blood agar, colonies are 2-3mm in diameter. Some strains may be haemolytic.

On TCBS agar, colonies are at least 2mm in diameter and yellow in the case of sucrose fermenters and green non-sucrose fermenters after 18-24hr incubation. 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. See table below.

Organism	**Colour of colonies on TCBS agar**
V. cholerae	yellow
V. alginolyticus	yellow
V. cincinnatiensis	yellow
V. carchariae	yellow/green
V. fluvialis	yellow
V. furnissii	yellow
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.

Aeromonas species

On blood agar, colonies appear distinctively circular, large, round, raised, opaque with or without haemolysis and are 1 – 3mm in diameter after aerobic incubation at 35°C for 18- 24hr. The colonies start off greyish in colour as a result of β-haemolysis and after three days growth, the colonies turn dark green.

On MacConkey agar, colonies are typically non-lactose fermenting; however, some lactose fermenting *Aeromonas* species have been observed.

On CIN agar, *Aeromonas* form pink bull's eye colonies due to the fermentation of D-Mannitol similar to *Yersinia enterocolitica*.

8.4 Test Procedures

8.4.1 Biochemical tests

Oxidase Test

(UK SMI TP 26 - Oxidase Test)

Vibrio species are oxidase positive with the exceptions of *V. metschnikovii* and *V. gazogenes.*

Aeromonas species are oxidase positive.

Note: Oxidase test may give false negative results on media containing carbohydrates - subculture to nutrient or blood or MacConkey agar before testing.

Voges-Proskauer Test (optional)

The Voges-Proskauer test has been used to differentiate between the El Tor and classical biotype of *V. cholerae* O1. Classical biotypes usually give negative results; El Tor isolates are generally positive. A cherry red colour indicates a positive reaction.

This test can also be used to differentiate *Aeromonas* species.

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

Pteridine 0129 is useful in the differentiation of *Vibrio* from other gram-negative bacteria especially *Aeromonas*, which are characteristically resistant to 0129.

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

Aeromonas species are resistant to vibrostatic compound 0/129 (150µg discs).

Serology (agglutination with specific antisera)

Serotype identification is based on agglutination in antisera to type-specific O antigens. The use of specific antisera is one of the most rapid and specific methods of identifying *Vibrio* species.

Note: Agglutination should be carried out with subcultures onto non-selective agar, because *Vibrio* colonies can auto-agglutinate from TCBS agar, giving false-positive results[11].

Serology assays are not considered reliable for detecting antibodies to *Aeromonas* because of their low sensitivity and specificity[14].

8.4.2 Commercial identification systems

For most vibrios, these tests may require supplementation with sodium chloride (NaCl) while *Aeromonas* species do not require this. Laboratories should therefore follow manufacturer’s instructions. Rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

8.4.3 Matrix assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS)

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), which can be used to analyse the protein composition of a bacterial cell, has emerged as a new technology for species identification. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF MS as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use[44].

This has been utilized to aid in both the detection and species-level identification of *Vibrio* species - *Vibrio parahaemolyticus*[45]. It has also been used to discriminate between closely related species, such as *Photobacterium damselae* (formerly *Vibrio damselae*) and *Grimontia hollisae* (formerly *Vibrio hollisae*)[45].

MALDI-TOF MS has also been used to determine genus-level identification with 100% accuracy and species- level identifications with 90% accuracies for clinical *Aeromonas* isolates, However the database will need improving to accommodate unidentified and newer species not represented in the database[45].

8.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR is usually considered to be a good method for bacterial detection as it is simple, rapid, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. However, it does have limitations. Although the 16S rRNA gene is generally targeted for the design of species-specific PCR primers for identification, designing primers is difficult when the sequences of the homologous genes have high similarity.

PCR targeted to the *dnaJ* gene has been used successfully in the identification of *Vibrio* species – *V. cholerae, V. parahaemolyticus, V. vulnificus, V. mimicus*, and *V. alginolyticus*[46]. PCR has also been used to detect *V. vulnificus*- specific genes within 2hr in the blood of patients with skin and soft tissue infections[47].

PCR directed to two gene targets - cholesterol acyltransferase (*gcat*) and small subunit (16S) recombinant DNA (rRNA) has been used to identify pathogenic *Aeromonas* species and this has been found to be reproducible and specific. This will also allow between *Vibrio* and *Aeromonas* species in patients with in cholera-like symptoms[48].

8.5 Further Identification

Rapid Molecular Methods

Molecular methods have had an enormous impact on the taxonomy of *Vibrio* and *Aeromonas*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Vibrio*, *Aeromonas* and related organisms; and has resulted in the recognition of numerous new species. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable Number Tandem Repeat Analysis (MVLA), Fluorescent Amplified Fragment Length Polymorphism (FAFLP) and Whole Genome Sequencing (WGS). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

Pulsed Field Gel Electrophoresis (PFGE)

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories[49,50].

This has been used successfully to identify and discriminate between species of the genus *Vibrio* using the *NotI* and *SfiI* enzymes[4]. It has also been used to perform molecular subtyping of *Vibrio cholerae* 01 and 0139[51,52].

PFGE has also been used to differentiate *Aeromonas* strains employing restriction endonucleases – *XbaI, SpeI* and *SwaI*[4,53].

Multilocus Sequence Typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. The technique is highly discriminatory, as it detects all the nucleotide polymorphisms within a gene rather than just those non-synonymous changes that alter the electrophoretic mobility of the protein product. One of the advantages of MLST over other molecular typing methods is that sequence data are portable between laboratories and have led to the creation of global databases that allow for exchange of molecular typing data via the Internet[54].

MLST has been extensively used as one of the main typing methods for analysing the genetic relationships within the genus *Vibrio*[6]. It has been very useful in the typing of *V. cholerae*. MLST has also been suggested to have better discriminatory ability than PFGE[55].

This method has also been used for the detection of *V. parahaemolyticus* strains and in the recognition of evolutionary trends and emergence of *V. parahaemolyticus* clonal complexes, thus providing an early warning system[56].

This has also been used successfully to identify the *Aeromonas* species based on two housekeeping genes *rpoD* and *gyrB*[57].

The drawbacks of MLST are the substantial cost and laboratory work required to amplify, determine, and proof read the nucleotide sequence of the target DNA fragments, making the method hardly suitable for routine laboratory testing.

Fluorescent Amplified Fragment Length Polymorphism (FAFLP)

Fluorescent Amplified Fragment Length Polymorphism is a high-resolution whole genome methodology used as a tool for rapid and cost-effective analysis of genetic diversity within bacterial genomes. It is useful for a broad range of applications such as identification and subtyping of microorganisms from clinical samples, for identification of outbreak genotypes, for studies of micro and macro-variation, and for population genetics.

FAFLP has numerous advantages over other DNA fingerprinting techniques because it assesses the whole genome for both conserved and rapidly evolving sequences in a relatively unbiased way. The number of fragments obtained for comparative purposes between isolates is significantly greater than pulsed-field gel electrophoresis (PFGE), therefore making it more discriminatory than PFGE and the FAFLP results are highly reproducible due to stringent PCR cycling parameters.

This robust and reproducible fingerprinting technique has been used to distinguish between *V. cholerae* O1 and non-O1 and non-O139 strains[58]. This has also shown that clinical isolates closely resemble environmental isolates in their genomic patterns[59].

It has also been shown as an accurate method for the identification, classification and subtyping of aeromonads[3,60].

Multiple-Locus Variable Number Tandem Repeat Analysis (MVLA) also known as VNTR

Multiple-Locus Variable number tandem repeat Analysis (MLVA) is a method used to perform molecular typing of particular microorganisms. It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in many different loci in the genome of a variety of organisms. The molecular typing profiles are used to study transmission routes, to assess sources of infection and also to assess the impact of human intervention such as vaccination and use of antibiotics on the composition of bacterial populations.

This has been used successfully in the subtyping of *Vibrio* species – *Vibrio cholerae* O1 and O139 serogroups[61].

The method has proven very useful for detecting and investigating outbreaks, since it has the capacity to differentiate closely related strains. It also has comparable discriminatory power with PFGE. In addition, the combination of the two approaches (MVLA and PFGE) can further distinguish the strains from different sources and geographical regions of isolation[61]. The method is technically simple and inexpensive to perform.

Polymerase Chain Reaction- Restriction Fragment Length Polymorphism Analysis (PCR-RFLP)

This has proved a useful typing technique for a number of groups of organisms, and can be used to identify species within some genera. This method requires only PCR and one or two enzymes and therefore is technically less demanding than the majority of other molecular approaches. It is easier to use, less expensive and less equipment dependent than sequencing.

This has been used successfully for the detection of virulence factors of *Aeromonas* species[62].

Whole Genome Sequencing (WGS)

This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology, IIlumina sequencing and Ion Torrent sequencing. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

This has been used successfully to explore the genome of *V. cholerae*. The *Vibrio cholerae* genome sequence provides a new starting point for the study of this organism's environmental and pathobiological characteristics. The genome sequence may also hopefully provide important clues to understanding the metabolic and regulatory networks that link genes on the two chromosomes[63].

This has also been used to explore the genome sequence of *Aeromonas* species such as *A. taiwanensis,* which carries several genes encoding virulence determinants. However the knowledge of their sequence opens new avenues for further exploring important virulence determinants[64].

8.6 Storage and Referral

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

9 Reporting

9.1 Presumptive identification

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

9.2 Confirmation of identification

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

9.3 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 which suggests infection with *V. cholerae* or other *Vibrio* species.

According to local protocols, the medical microbiologist should also be informed of presumptive or confirmed *Vibrio* species in association with:

- suspected food poisoning (especially cases involving consumption of seafood)

Follow local protocols for reporting to clinician.

9.4 CCDC

Refer to local Memorandum of Understanding.

9.5 UK Health Security Agency[65]

Refer to current guidelines on CIDSC and COSURV reporting.

As Cholera is a notifiable disease in the UK, for public health management of cases, contacts and outbreaks, all suspected cases should be notified.

9.6 Infection Prevention and Control team

The infection prevention and control team should also be informed of presumptive and confirmed isolates of *Vibrio* species according to local protocols.

10 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see user manuals and request forms

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

England

Wales

Scotland

Northern Ireland

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

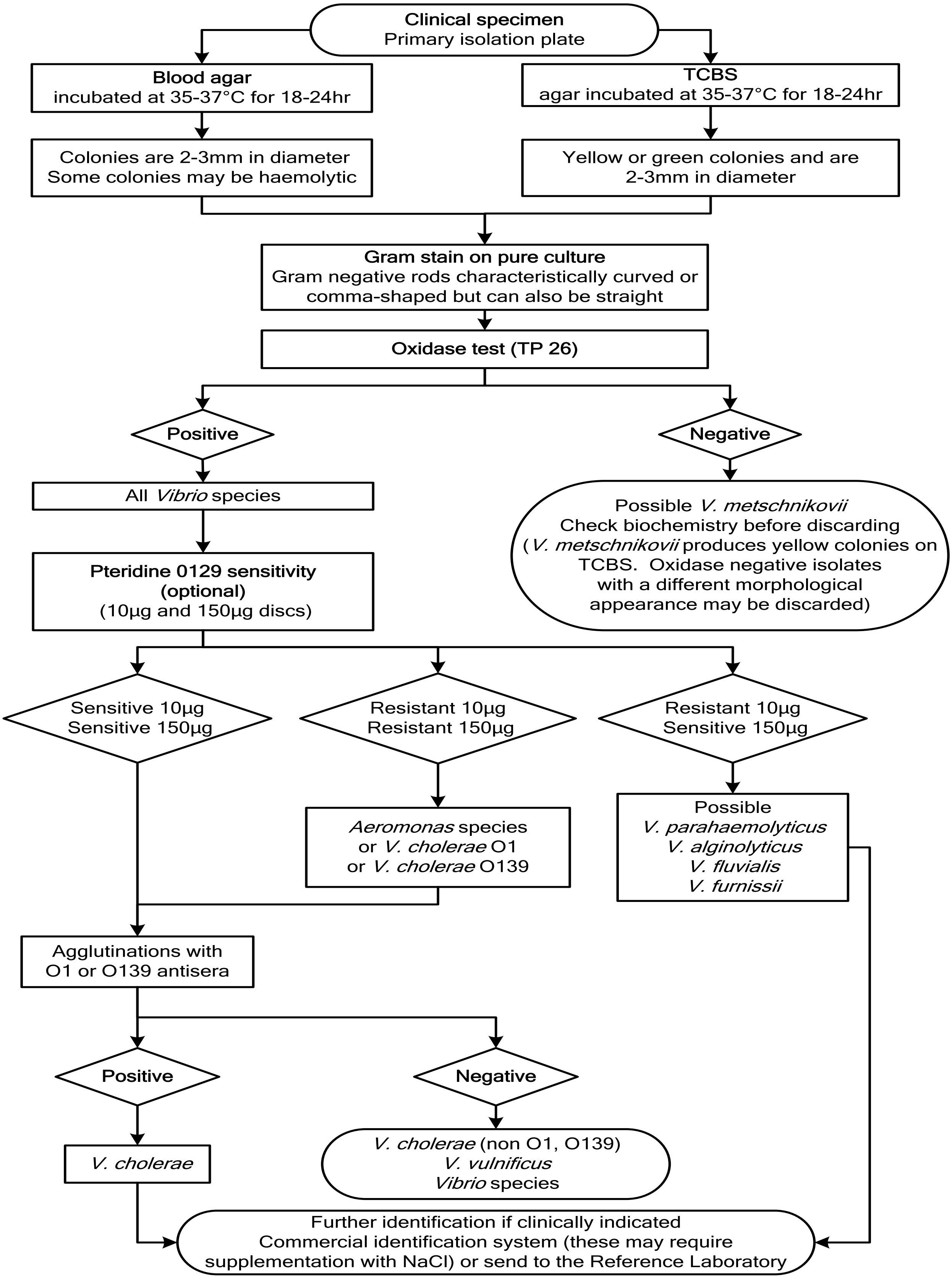
11 Public Health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral, to further characterise the organism, as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance, forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

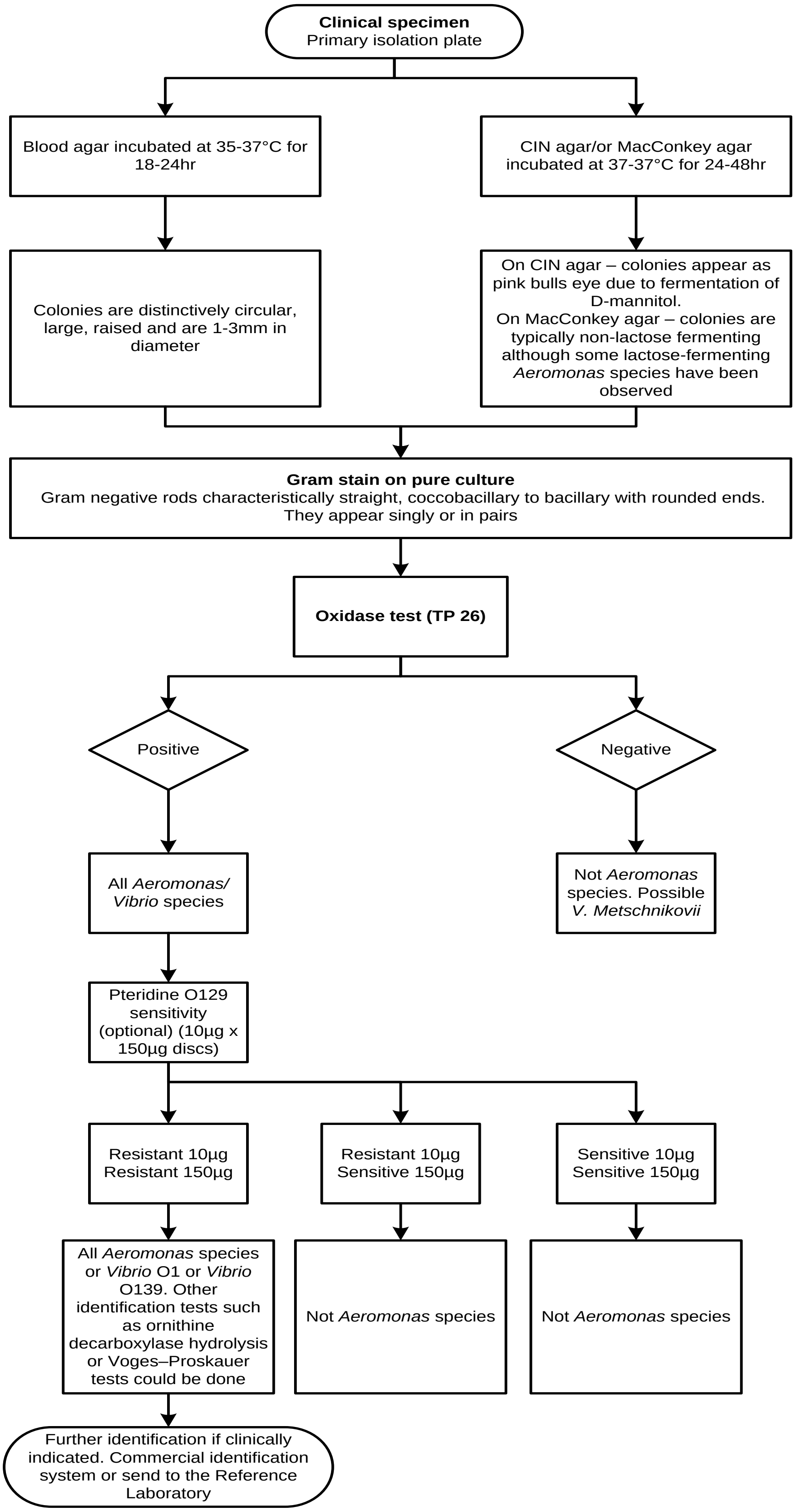
Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Algorithm: Identification of *Vibrio* species



The flowchart is for guidance only

Algorithm: Identification of *Aeromonas* species



The flowchart is for guidance only

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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