

NATIONAL STANDARD METHOD

UNDER CONSULTATION
**IDENTIFICATION OF
ENTEROBACTERIACEAE**

BSOP ID 16

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







IDENTIFICATION OF ENTEROBACTERIACEAE

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

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The reader is informed that all taxonomy in this document was correct at time of issue.

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

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AMENDMENT PROCEDURE

Controlled document reference	BSOP ID 16
Controlled document title	Identification of Enterobacteriaceae

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

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IDENTIFICATION OF ENTEROBACTERIACEAE

SCOPE OF DOCUMENT

This National Standards Method (NSM) describes the identification of members of the family Enterobacteriaceae. There are a large number of species included in the family. In routine clinical microbiology laboratories it is usual to attempt identification by use of biochemical tests. The level of identification depends on the site of infection, the immune status of the host and the need for epidemiological surveillance.

Because of the large number of species involved, this NSM will concentrate on the most common genera and species isolated from clinical specimens. The identification of Enterobacteriaceae can be simplified by taking advantage of the fact that three species comprise 80 to 95% of all isolates in the clinical setting. These are *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*². The other species can be easily identified using biochemical tests.

INTRODUCTION

Taxonomy

The nomenclature of the Enterobacteriaceae is complicated and has been based on biochemical and antigenic characteristics³. Recently, the application of new technologies such as DNA hybridisation has resulted in numerous changes in classification of the Enterobacteriaceae. In 1972 there were 26 recognised species, now there are in excess of 170.

Characteristics of Enterobacteriaceae

Members of the Enterobacteriaceae are Gram-negative, straight rods, some of which are motile. Most species grow well at 37°C, although some species grow better at 25 - 30°C. They are facultatively anaerobic, oxidase-negative and catalase-positive (except *Shigella dysenteriae* type 1). They are distributed worldwide and may be found in soil, water, plants and animals.

Common genera of the family Enterobacteriaceae

Citrobacter species

There are eleven species of which nine have been recovered from clinical material. They may be found in the faeces of humans and animals as part of the normal flora and grow readily on ordinary media. Colonies are generally smooth and moist although mucoid or rough strains occur. Some strains of *Citrobacter* resemble *Salmonella* species biochemically and agglutinate with *Salmonella* polyvalent antisera, which may lead to misidentification.

Enterobacter species

There are eleven species, but only eight have been isolated from clinical material (see section 2). They grow readily on ordinary agar, ferment glucose with the production of acid and gas, and are motile by peritrichous flagella. Some strains with a K antigen possess a capsule.

Escherichia species

There are six species, of which four are known to cause human disease (see section 2). The most commonly isolated is *Escherichia coli*, which contains numerous serotypes, some of which are associated with specific diseases.

A number of strains of *E. coli* may produce enterotoxins or other virulence factors, including those associated with invasiveness. Some strains are capsulated with a K antigen.

For more information on the identification of *E. coli* O157 see [BSOPID 22 - Identification of Escherichia coli O157](#).

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Hafnia alvei

The genus *Hafnia* contains a single species, *H. alvei*. It grows readily on ordinary media and is generally motile. Motility is more pronounced at 30°C than 37°C⁴. *H. alvei* can resemble non-motile, salmonella biochemically and can agglutinate in polyvalent salmonella antisera.

***Klebsiella* species**

The genus *Klebsiella* contains five species and four subspecies. Four species, previously named *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis* and *Klebsiella aerogenes* are now classed as subspecies of *K. pneumoniae*. *K. pneumoniae* subspecies *aerogenes* is the most frequently isolated species. All grow readily on ordinary media, are non-motile and are capsulated.

Morganella morganii

The genus *Morganella* contains a single species, *Morganella morganii*, which is divided into two sub species. It is motile with peritrichous flagella, but some strains do not form flagella above 30°C. *M. morganii* can resemble non-motile, salmonella biochemically and can agglutinate in polyvalent salmonella antisera.

***Proteus* species**

There are four species of *Proteus*, of which three cause disease (see section 2). All strains are urease positive and motile. They may swarm on blood agar, producing concentric zones or an even film. They are resistant to polymyxin B and colistin. *Proteus* species can resemble non-motile salmonella biochemically and can agglutinate in polyvalent salmonella antisera.

***Providencia* species**

The genus *Providencia* was originally established for organisms similar to *Proteus* species that were urease negative. There are five species within the genus, of which three cause disease (see section 2). All are motile but do not swarm. They are resistant to polymyxin B and colistin.

***Salmonella* species**

Serotypes of *Salmonella* and *Arizona* are now considered to belong to two species – *Salmonella* Bongori, (formerly subspecies V) and *Salmonella* Enterica, which comprises six subspecies: I = *enterica*, II = *salamae*, IIIa = *arizonae*, IIIb = *diarizonae*, IV = *houtenae*, and VI = *indica*. Most serotypes are motile; all except *Salmonella* Typhi produce gas from glucose. Most produce hydrogen sulphide except *S. Typhi* and *Salmonella* Paratyphi A (which is a weak producer).

For more information on serotyping of *Salmonella* species, see [BSOPID 24 - Identification of Salmonella Species](#)

***Serratia* species**

The genus *Serratia* contains ten species (but only two are commonly isolated from clinical material) and two sub species. They are *Serratia liquefaciens* and *Serratia marcescens*, the latter often producing a red pigment when grown at 20°C. Most of the species are motile. Members of the genus characteristically produce three enzymes - lipase, DNase and gelatinase. They are also resistant to polymyxin B and colistin, and this resistance may be heterogeneous, leading to a target-zone appearance.

***Shigella* species**

There are four species, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*. All are non-motile. *Shigella* species are highly infective, particularly *S. dysenteriae*⁵.

For more information on the identification of *Shigella* species, see [BSOPID 20 - Identification of Shigella Species](#)

***Yersinia* species**

The genus *Yersinia* contains eleven species, three of which (*Yersinia pestis*, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*) are known pathogens of man and animals⁶. All members of the genus grow readily on ordinary media.

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Y. pestis is not fastidious but, after incubation for 24 hours on blood agar, colonies are usually much smaller than those of other Enterobacteriaceae. *Y. pestis* is always non-motile. The other species are non-motile at 37°C but motile at 30°C.

For more information on the identification of *Yersinia* species see [BSOPID 21 - Identification of Yersinia Species from Faeces](#)

Other genera of the family Enterobacteriaceae⁷⁻¹⁰

Other genera of the family reported to have caused infection are listed in section 2.

Principles of identification

Colonial morphology, Gram's stain, oxidase and the use of several biochemical tests identify isolates from clinical material. Enteric pathogens such as *Salmonella* species should be identified biochemically and typed serologically. *Hafnia*, *Morganella* and *Proteus* species can resemble non-motile salmonella biochemically and can agglutinate in polyvalent salmonella antisera. Because of the diversity of biochemical activities, all the reactions of every species are not described in this NSM. Therefore only a few screening tests are included together with results for the more common genera and species.

If further identification or confirmation is required, isolates should be sent to the Reference Laboratory.

Careful consideration should be given to isolates that give an unusual identification.

TECHNICAL INFORMATION

N/A

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1 SAFETY CONSIDERATIONS¹¹⁻²³

All *S. Typhi*, *S. Paratyphi A*, *B* and *C*, *S. dysenteriae* type 1, *E. coli* O157, *Salmonella sendai* and *Salmonella cholera-suis*, and *Yersinia pestis* are Hazard Group 3 organisms and suspected isolates must be handled in a containment level 3 room.

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.

Shigella species and *E. coli* O157 are highly infective, and as few as 10 organisms are required for an infective dose. They have been reported as a cause of laboratory acquired infection.

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

Compliance with postal and transport regulations is essential.

2 TARGET ORGANISMS

Enterobacteriaceae reported to have caused human infections^{7,24,25}

<i>Cedecea</i>	<i>davisae</i> , <i>lapagei</i> , <i>neteri</i> , <i>sp 3</i> , <i>sp 5</i>
<i>Citrobacter</i>	<i>amalonaticus</i> , <i>braakii</i> , <i>farmeri</i> , <i>freundii</i> , <i>koseri</i> , <i>rodentium</i> , <i>sedlakii</i> , <i>werkmanii</i> , <i>youngae</i>
<i>Edwardsiella</i>	<i>hoshinae</i> , <i>ictaluri</i> , <i>tarda</i>
<i>Enterobacter</i>	<i>aerogenes</i> , <i>amnigenus</i> , <i>asburiae</i> , <i>cloacae</i> , <i>gergoviae</i> , <i>hormaechei</i> , <i>sakazakii</i> , <i>taylorae</i>
<i>Escherichia</i>	<i>coli</i> , <i>fergusonii</i> , <i>hermanii</i> , <i>vulneris</i>
<i>Ewingella</i>	<i>americana</i>
<i>Hafnia</i>	<i>alvei</i>
<i>Klebsiella</i>	<i>oxytoca</i> , <i>pneumoniae</i> subspecies <i>aerogenes</i> , <i>ozaenae</i> , <i>pneumoniae</i> , and <i>rhinoscleromatis</i>
<i>Kluyvera</i>	<i>ascorbata</i> , <i>cryocrescens</i> , <i>georgiana</i>
<i>Leclercia</i>	<i>adecarboxylata</i>
<i>Morganella</i>	<i>morganii</i>
<i>Pantoea</i>	<i>agglomerans</i> , <i>dispersa</i>
<i>Photobacterium</i>	<i>luminescens</i>
<i>Proteus</i>	<i>mirabilis</i> , <i>penneri</i> , <i>vulgaris</i>
<i>Providencia</i>	<i>alcalifaciens</i> , <i>rettgeri</i> , <i>stuartii</i>
<i>Rahnella</i>	<i>aquatilis</i>
<i>Salmonella</i>	<i>enterica</i> (>2000 serotypes)
<i>Serratia</i>	<i>fonticola</i> , <i>grimesii</i> , <i>liquefaciens</i> , <i>marcescens</i> , <i>odorifera</i> , <i>plymuthica</i> , <i>proteamaculans</i> , <i>rubidaea</i>
<i>Shigella</i>	<i>boydii</i> , <i>dysenteriae</i> , <i>flexneri</i> , <i>sonnei</i>
<i>Tatumella</i>	<i>ptyseos</i>
<i>Yersinia</i>	<i>aldovae</i> , <i>bercovieri</i> , <i>enterocolitica</i> , <i>intermedia</i> , <i>frederiksenii</i> , <i>kristensenii</i> , <i>mollaretti</i> , <i>pestis</i> , <i>pseudotuberculosis</i> , <i>rohdei</i>
<i>Yokenella</i>	<i>regensburgei</i>

Other genera and species of the Enterobacteriaceae may rarely be associated with human disease.

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3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

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

Gram-negative rods, some may show bipolar staining (eg *Yersinia* species)

3.2 PRIMARY ISOLATION MEDIA

Blood agar (BA): 16 – 24 h incubation in 5 - 10% CO₂ at 35°C - 37°C

MacConkey (MAC) agar: 16 – 24 h incubation in air at 35°C - 37°C

Cystine-lactose-electrolyte deficient (CLED) agar with bromothymol blue (CLED B) or Andrade's indicator (CLED A): 16 – 24 h incubation in air at 35°C - 37°C

Selective enteric media, incubation in air at 35°C - 37°C for 16 – 24 h:

Desoxycholate citrate agar (DCA)

Xylose-lysine-desoxycholate agar (XLD)

Cefixime-tellurite-sorbitol-MacConkey (CT-SMAC) agar

Thiosulphate-citrate-bile salt (TCBS) agar

Cefsulodin-Irgasan (triclosan)-novobiocin (CIN) agar incubated in air at 32°C for 24 – 48 h

Chromogenic media incubated in air at 35°C - 37°C for 16 - 24 h

3.3 COLONIAL APPEARANCE

BA - Gram-negative rods 2 - 3 mm diameter, low, convex, grey, smooth or mucoid, may be haemolytic or swarming.

MAC - Gram-negative rods may appear pink (lactose fermenting) or colourless (lactose non-fermenting) size and shape vary with individual species.

CLED B - Gram-negative rods may appear yellow (lactose fermenting) or blue (lactose non-fermenting) size and shape vary with individual species.

CLED A - Gram-negative rods may appear pink (lactose fermenting) or green translucent (lactose non-fermenting) size and shape vary with individual species.

DCA - Gram-negative rods may appear pink (lactose fermenting) or colourless (lactose non-fermenting) and may have black centre (H₂S producers).

XLD - Gram-negative rods may appear yellow (xylose, lactose or sucrose fermenting) or pink (non-fermenting) and may have black centre (H₂S producers).

CT-SMAC - Gram-negative rods may appear pink (sorbitol-fermenting) or colourless (sorbitol non-fermenting).

TCBS - Gram-negative rods may appear yellow (sucrose fermenting) or blue-green (sucrose non-fermenting).

CIN - Gram-negative rods, colonies may have deep red centres (mannitol fermenting) surrounded by a translucent border giving the appearance of a 'bull's eye'.

NOTE: Colonies of *Yersinia* species may be smaller than those of other Enterobacteriaceae.

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3.4 TEST PROCEDURES

Oxidase (see [BSOPTP 26 - Oxidase Test](#)) .

All Enterobacteriaceae are oxidase-negative.
Lactose fermentation exhibits variable results depending on the genus and species.

3.5 FURTHER IDENTIFICATION

Commercial identification kit

Serotyping.

3.6 STORAGE AND REFERRAL

Save the pure isolate on a nutrient agar slope for referral to the Reference Laboratory.

UNDER CONSULTATION

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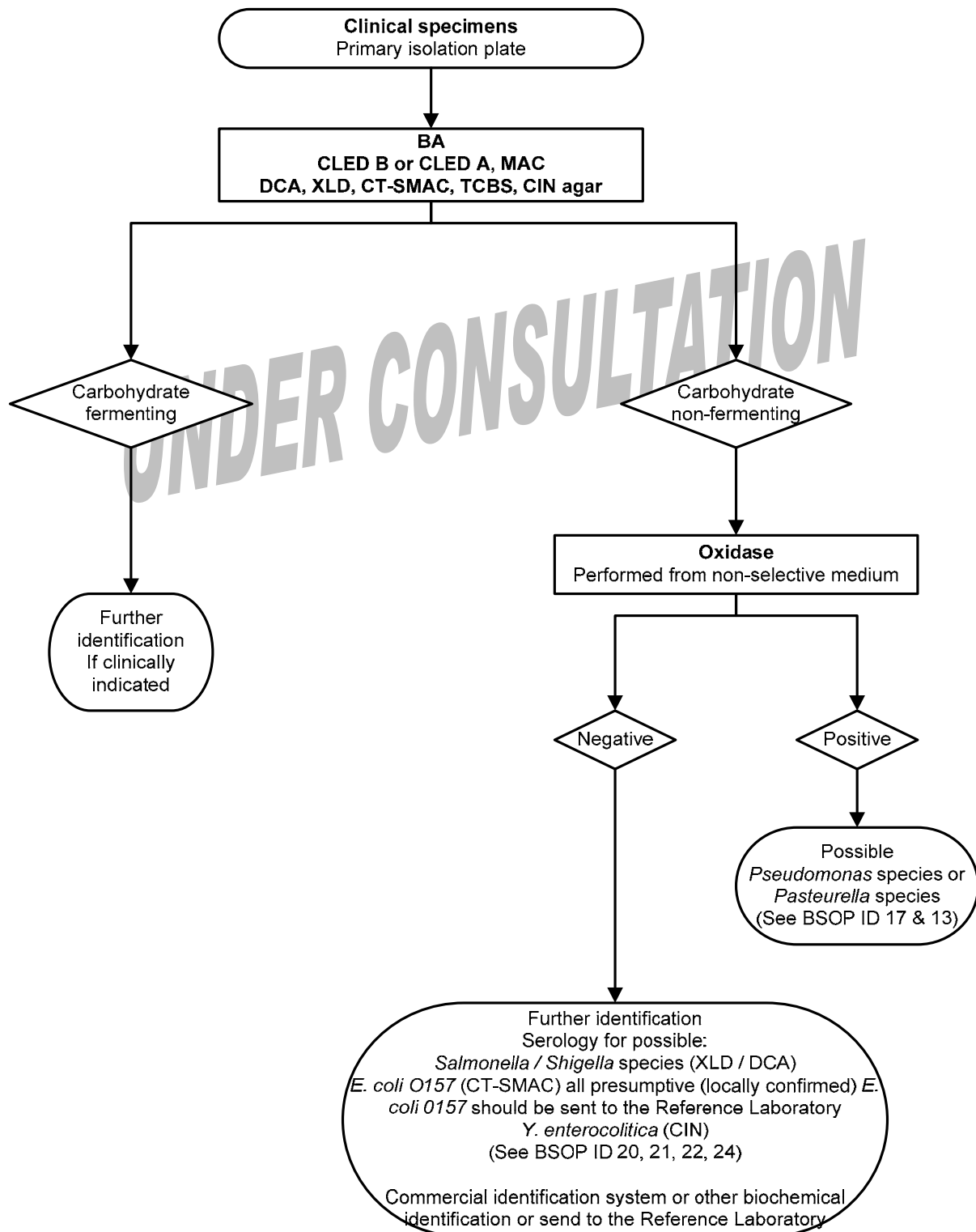
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4 IDENTIFICATION OF ENTEROBACTERIACEAE – FLOW CHART



The flowchart is for guidance only

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5 REPORTING

5.1 PRESUMPTIVE IDENTIFICATION

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

5.2 CONFIRMATION OF IDENTIFICATION

Following commercial identification kit or other biochemical identification results or send to the Reference Laboratory.

5.3 MEDICAL MICROBIOLOGIST

Inform the medical microbiologist of presumptive and confirmed *Y. pestis*, *S. Typhi*, *S. Paratyphi*, *Shigella* species, *E. coli* O157 and *Salmonella* species (according to local procedures).

The medical microbiologist should also be informed if the request card bears information relating to infection with *Y. pestis* eg

- ulceroglandular/pneumonic syndrome
- septicaemia
- travelling, hunting, farming, or veterinary work overseas

Information relating to cases of:

- enterocolitis
- dysentery
- septicaemia
- haemolytic-uraemic syndrome
- neurological dysfunction or confusional states
- (non-blanching) rash

Presumptive or confirmed agents of enteric fever, dysentery, and enterocolitis should also be relayed to the medical microbiologist, especially if the patient has a history of:

- recent foreign travel
- farming (or visits to farms)
- veterinary or laboratory work
- alcoholism, substance abuse, immunodeficiency or other serious underlying disorder such as cancer

Presumptive and confirmed isolates of Enterobacteriaceae from cases of food poisoning and from investigations of outbreak situations should additionally be reported to the medical microbiologist.

Follow local protocols for reporting to clinician.

5.4 NOTIFICATION TO THE HPA^{26,27}

Diagnostic laboratories are required to notify the HPA when specified causative agents of infectious disease are identified (Health Protection (Notification) Regulations 2010). This notification will usually be in a written format using Cosurv, to the local Health Protection Unit

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Notify all isolates of the following:

E. coli (presumptive [locally-confirmed] VTEC O157 and other possible VTEC strains)

Salmonella species

Shigella species

Yersinia pestis

Urgent oral notification to the Health Protection Unit within 24 hours of identification is likely to be necessary to protect human health when presumptive identification is made of the following:

S. Typhi or *S. Paratyphi*

Salmonella species if a suspected outbreak or a case in a food handler or closed community such as a care home

Shigella species other than *S sonnei*

S. sonnei if a suspected outbreak or a case in a food handler or closed community such as a care home

E coli O157 when presumptive (locally confirmed) at the diagnostic laboratory

Other verocytotoxigenic *E. coli* O157

Yersinia pestis

Confirmatory and typing results should be forwarded to the Health Protection Unit as soon as they are available to expedite appropriate health protection interventions.

5.5 INFECTION CONTROL STAFF

Inform the infection control team of presumptive and confirmed isolates of *E. coli* O157, *Yersinia*, *Salmonella* and *Shigella* species

6 REFERRALS

6.1 REFERENCE LABORATORY

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to: <http://www.hpa.org.uk/Centre for Infections/lep/default.htm>

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7 ACKNOWLEDGEMENTS AND CONTACTS

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