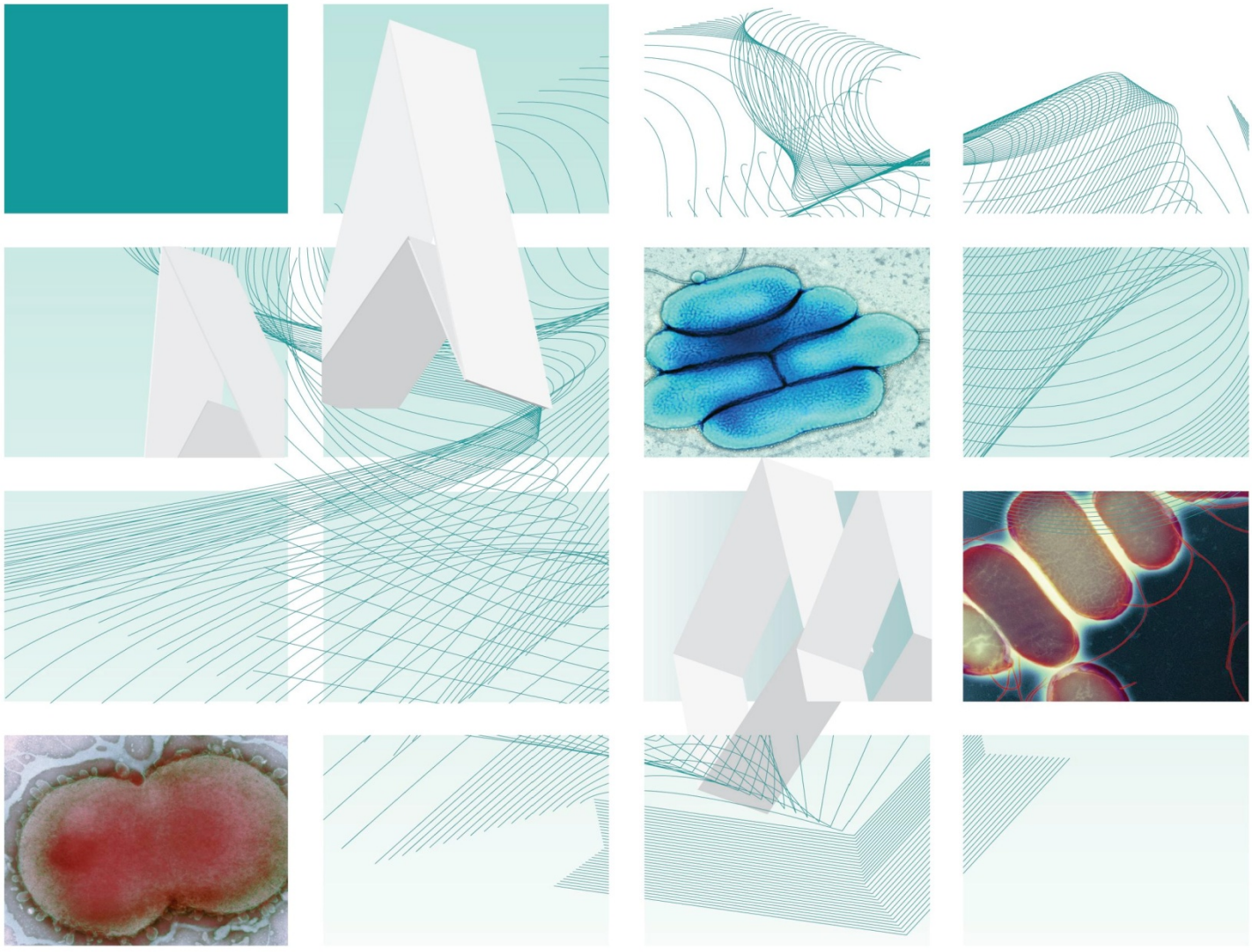
Public Health England

NHS

UK Standards for Microbiology Investigations

Identification of Enterobacteriaceae



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Bacteriology – Identification | ID 16 | Issue no: 3.2 | Issue date: 11.03.14 | Page: 1 of 20

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:

Contents

Amendment Table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	5/11.03.14
Issue no. discarded.	3.1
Insert Issue no.	3.2
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.	4/21.10.11
Issue no. discarded.	3
Insert Issue no.	3.1
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 Enterobacteriaceae. UK Standards for Microbiology Investigations. ID 16 Issue 3.2. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

This SMI describes the identification of members of the family Enterobacteriaceae. There are a large number of species included in the family. In diagnostic 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 SMI 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 80-95% of all isolates in the clinical setting. These are *Esherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*[1]. The other species can be easily identified using biochemical tests.

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The nomenclature of the Enterobacteriaceae is complicated and has been based on biochemical and antigenic characteristics. 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[2].

Characteristics

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 11 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, refer to ID 22 - Identification of *Escherichia coli* O157.

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°C[3]. *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. However, *Salmonella Paratyphi* A is normally hydrogen sulphide negative and *S. Typhi* is a weak producer.

For more information on serotyping of *Salmonella* species, refer to ID 24 - Identification of *Salmonella* species.

Serratia species

The genus *Serratia* contains ten species (but only two are commonly isolated from clinical material) and two subspecies. 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*[4,5].

For more information on the identification of *Shigella* species, refer to ID 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 animals[6]. All members of the genus grow readily on ordinary media.

Y. pestis is not fastidious but, after incubation for 24hr 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, refer to ID 21 - Identification of *Yersinia* Species from Faeces.

Other genera of the family Enterobacteriaceae[7-10]

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

Principles of Identification

Colonial morphology, Gram 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 SMI. 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. All evidence including growth characteristics, cultural morphology and serology should be considered before accepting commercial identification system results.

Technical Information/Limitations

N/A

1 Safety Considerations[11-27]

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 SMI.

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 ten 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[7-9]

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

Yersinia	*aldovae, bercovieri, enterocolitica, intermedia, frederiksenii, kristensenii, mollaretti, pestis, pseudotuberculosis, rohdei*
Yokenella	*regensburgei*

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

3 Identification

3.1 Microscopic Appearance

Gram stain (TP 39 - Staining Procedures)

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

3.2 Primary Isolation Media

Blood agar (BA): 16-24hr incubation in 5-10% CO_2 at 35-37°C.

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

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

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

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 24–48hr.

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

3.3 Colonial Appearance

BA–Gram negative rods 2-3mm 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.

3.4 Test Procedures

Oxidase Test (TP 26 - Oxidase Test)

All Enterobacteriaceae are oxidase negative.

Lactose fermentation exhibits variable results depending on the genus and species.

3.5 Further Identification

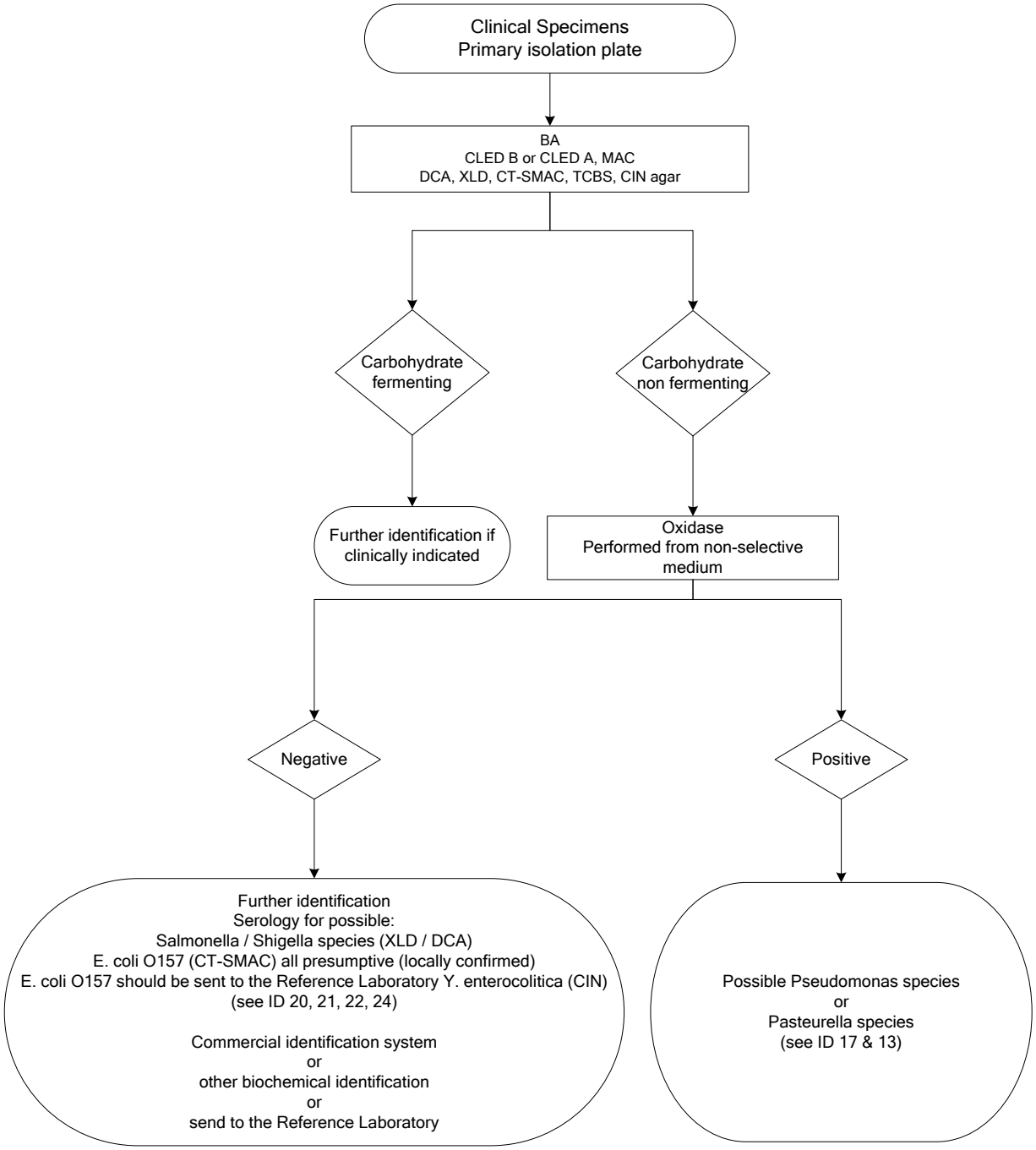
Commercial identification kit

Serotyping

3.6 Storage and Referral

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

4 Identification of Enterobacteriaceae



The flowchart is for guidance only.

5 Reporting

5.1 Presumptive Identification

If appropriate growth characteristics, colonial appearance, Gram stain of pure culture, oxidase and serological 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 presumed 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

Presumed 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

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

5.4 CCDC

N/A

5.5 Public Health England[28]

Refer to current guidelines on CDSC and COSURV reporting.

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 24hr 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 presumed (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.

5.6 Infection Control Team

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

6 Referrals

6.1 Reference Laboratory

Contact appropriate devolved nation reference laboratory for information on the tests available, turn around 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/Centre for Infections/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[28,29] or Equivalent in the Devolved Administrations[30-33]

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[30,31], Wales[32] and Northern Ireland[33].

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