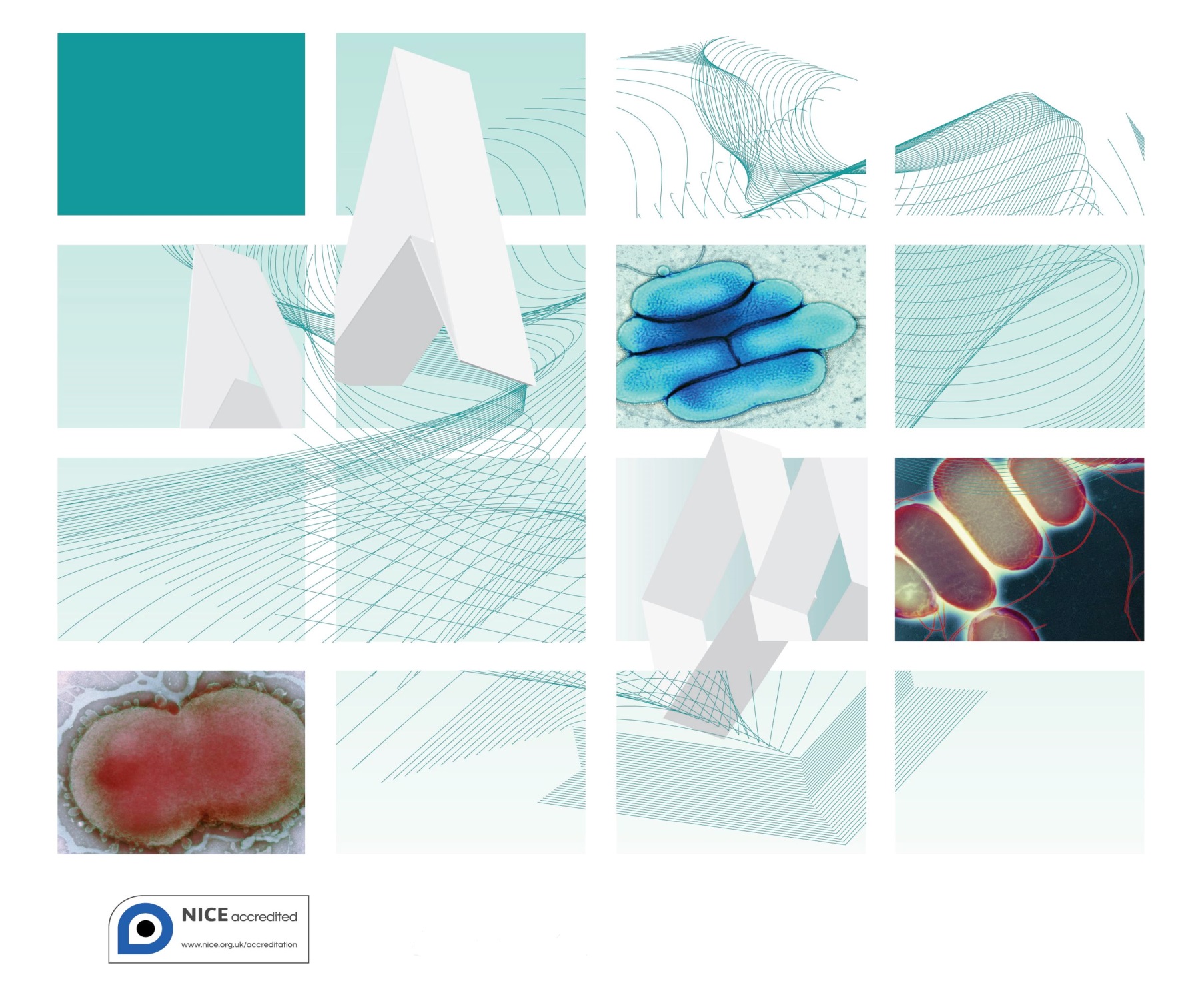
UK Standards for Microbiology Investigations

Identification of *Shigella* species



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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Microbiology Services

Public Health England

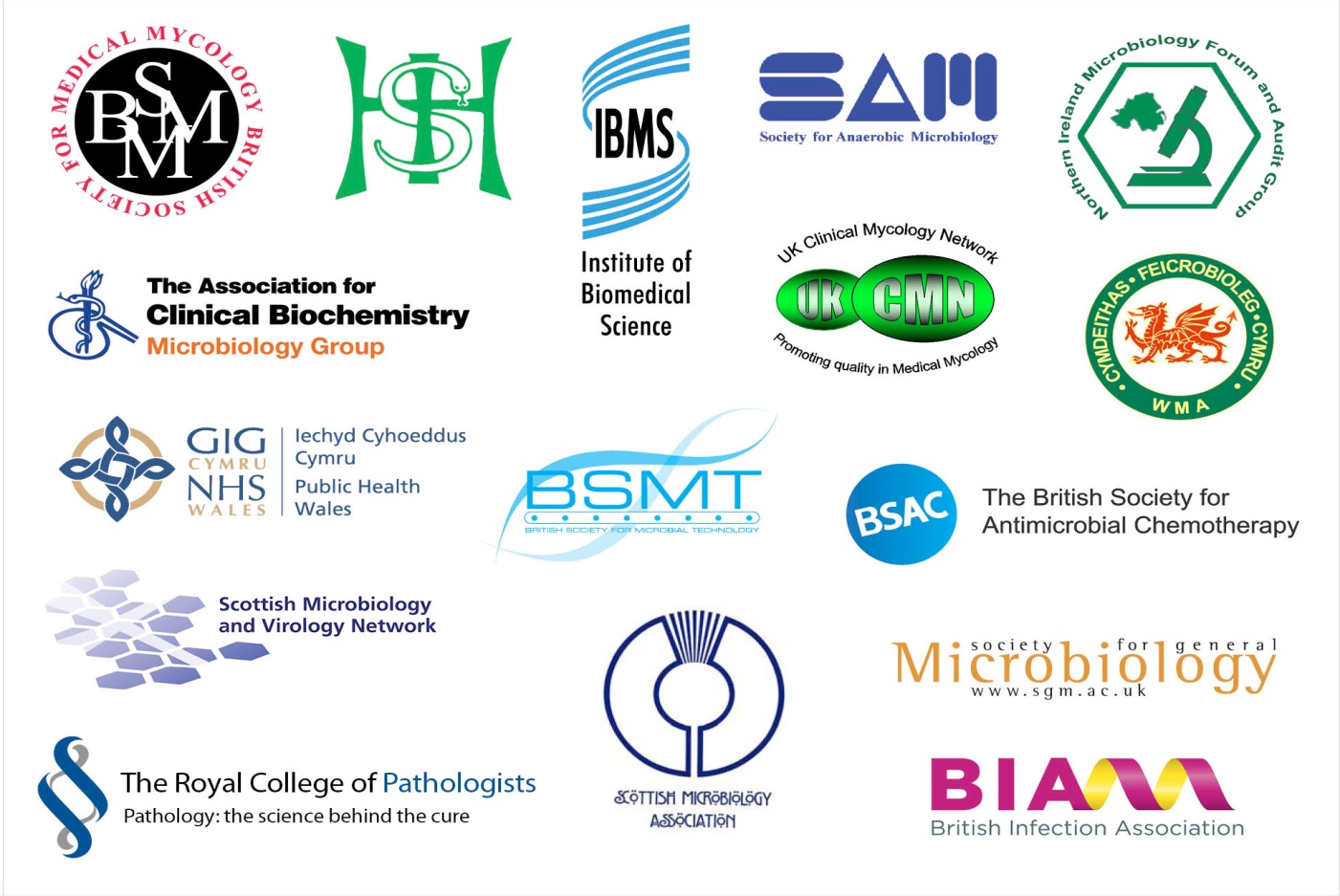
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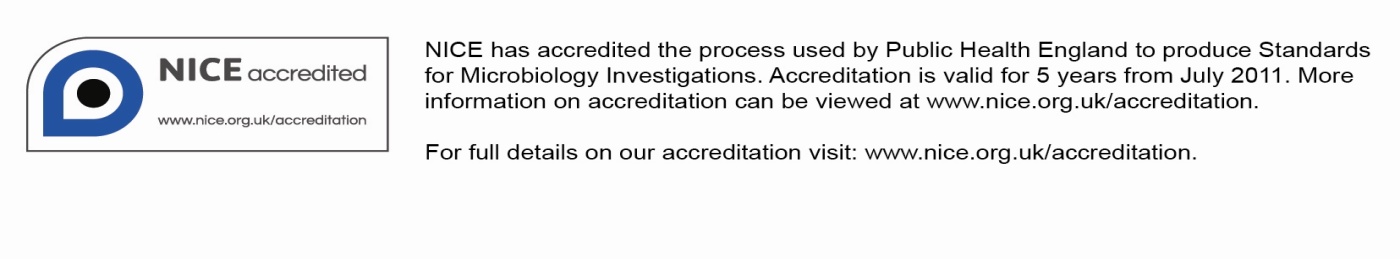
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Amendment Table

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

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

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| Amendment No/Date. | 4/ |
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| **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 references have been reviewed and updated. – remove if document does not contain safety references.  Scientific content remains unchanged. |

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| **Section(s) involved** | **Amendment** |
| Whole document. | Document presented in a new format. |
| References. | Some references updated. |

UK Standards for Microbiology Investigations[[1]](#footnote-1)#: 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.

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.

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Suggested Citation for this Document

Public Health England. (). Identification of *Shigella* species. UK Standards for Microbiology Investigations. ID Issue di+. <http://www.hpa.org.uk/SMI/pdf>.

Scope of Document

This SMI describes the identification of *Shigella* species with particular reference to isolation from faeces.

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The genus *Shigella* belongs to the family *Enterobacteriaceae* and consists of four species; *Shigella dysenteriae, Shigella flexneri,* *Shigella boydii,* and *Shigella sonnei*1*.* Each of the species, with the exception of *S. sonnei*, is subdivided by serotype.

Characteristics

*Shigella* species are small Gram negative rods, 0.3 - 1 µm in diameter and 1 - 6 μm in length, appearing singly, in pairs and in chains. *Shigella* species are facultative anaerobes and are non-spore formers. Unlike *Salmonella, Shigella* species do not possess flagella and hence are non-motile. *Shigellae* are differentiated into four subgroups on the basis of their O (somatic) antigens and further differentiated into serotypes;

- *S. dysenteriae* (Group A) contains 15 distinct antigenic serotypes.

- *S. flexneri* (Group B) contains 6 serotypes (1-6) that can be further divided into sub-serotypes based on their possession of group factors designated 3,4; 4; 6; 7; and 7,8

- *S. boydii (*Group C) contains 20 distinct antigenic serotypes.

- *S. sonnei* (Group D) contains only 1 serotype that may occur in two forms, form I (smooth) and form II (rough).

On XLD agar, *Shigella* species produce 1-2 mm diameter pink red colonies with no black centre. Some strains may have a pink or yellow periphery on XLD agar. Colonies on DCA agar are colourless (*S. sonnei* may form pale pink colonies because of late lactose fermentation). They are non- lactose fermenters (however *S. sonnei* can ferment lactose after prolonged incubation) and do not produce gas from carbohydrates. They all ferment glucose. They also tend to be overall biochemically inert. They are negative for urease, oxidase, do not decarboxylate lysine and give variable results for indole (with the exception of *S. sonnei* that is always indole negative) and positive for catalase with the exception of *S. dysenteriae* Type 12,3.

Differentiation of *Shigella* strains from *E. coli* is one of the problems faced by a diagnostic microbiology laboratories and may reflect the fact that *E. coli* and all four *Shigella* species are very closely on the basis of the DNA-DNA relationship. However, biochemical reactions are of considerable value in the differentiation of members of the genus *Shigella* from *Escherichia*3.

The *Shigella* species may be further differentiated by biochemical tests and serology of their lipopolysaccharides3. The majority of *Shigella* species, except *S. flexneri* 6, and *S. boydii* 13 and 14, ferment sugars without gas production. *S. boydii, S. flexneri* and *S. sonnei*, with a few exceptions, ferment mannitol; *S. dysenteriae* does not. *S. sonnei,* and *S. dysenteriae* type 1 are the only species that are ONPG positive. *S. boydii* 13 are ornithine positive, and some may be ONPG positive.

They have been isolated from faeces and rarely in blood samples4.

The type species is *Shigella dysenteriae*.

Principles of Identification

Isolates from primary culture are identified by colonial appearance, biochemical tests and serology (agglutination with specific antisera). *Plesiomonas shigelloides* cross reacts with *S. sonnei* antisera. 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.

Technical Information/Limitations

Quality control

Each new lot or shipment of antisera/commercial identification systems should be tested and validated for positive and negative reactivity using known control strains; ensuring it is fit for purpose. Laboratories must follow manufacturer’s instructions when using these products.

Serotyping

Rough strains:

Serotyping should not be attempted on *Shigella* strains which autoagglutinate saline. Rough isolates seldom revert to smooth forms. However, if autoagglutination in saline is observed, an attempt to recover smooth colonies may be made by performing 2- 4 serial sub-cultures on enriched media, such as blood agar3.

Capsular antigens:

Occasionally, the presence of capsular antigens may prevent some isolates of *Shigella* species from reacting with polyvalent antisera. The presence of capsular antigens should be considered when isolates which are biochemically typical of *Shigella* sp., fail to agglutinate (or agglutinate poorly) with *Shigella* polyvalent antisera. In these situations, an attempt should be made to remove the capsular antigen (by boiling at 100°C for 45 minutes) and then repeating the procedure.

*Shigella sonnei* polyvalent antisera can produce cross-reactions with some cultures of *Shigella boydii* type 6 due to the presence of conserved antigens. In these situations, biochemical profile and the use of *Shigella boydii* type 6 monovalent antiserum will be necessary to confirm the identification3.

Some *Plesiomonas shigelloides* strains share antigens with *Shigella sonnei*, and cross-reactions with *Shigella* antisera occur 5.

Oxidase Test

The test should not be performed on cultures from media containing tellurite and fermentable carbohydrates as these may prevent the reaction from occurring6.

Organisms grown on media containing dyes may give aberrant results7.

1 Safety Considerations8-24

All *Shigella dysenteriae* Type 1 are Hazard Group 3 organisms and suspected isolates must be handled in a containment level 3 room.

Most *Shigella* species are in Hazard Group 2. An important exception is *Shigella dysenteriae* Type 1. All work on *Shigella dysenteriae* type 1 must be performed under Containment level 3 conditions.

*Shigella species* are highly infective, particularly *S. dysenteriae* considered the most virulent, and can produce a potent cytotoxin known as “Shigatoxin” 15,25. *Shigella dysenteriae* type 1 causes severe and sometimes fatal disease.

*Shigella* species have been recently identified to be the most frequently identified agent of laboratory-acquired infections because of their high virulence and low infectious dose 26. A large number of laboratory acquired infections have been reported. Infection may be acquired through ingestion or accidental parenteral inoculation 27,28.

Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this SMI.

Appropriate personal protective equipment (PPE) and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times.

The most effective method for preventing laboratory-acquired infections is the adoption of safe working practices.

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

*Shigella* species reported to have caused human infections 4,29

All species cause human infections. *Shigella dysenteriae* (15 serotypes), *Shigella boydii* (20 serotypes), *Shigella flexneri* (6 serotypes which can be sub-divided into sub-serotypes), *Shigella sonnei* (1 serotype, 2 variants - rough and smooth).

3 Identification

3.1 Microscopic Appearance

Gram negative rods, 0.3 - 1 µm in diameter and 1 - 6 µm in length, arranged singly, in pairs and in chains.

3.2 Primary Isolation Media

Xylose-lysine-desoxycholate agar (XLD) agar incubated in air at 35-37°C for 16 - 24hrs.

Desoxycholate citrate agar (DCA) agar incubated in air at 35-37°C for 16 - 24hrs.

**NOTE:** For purity plate, Blood agar (BA) plate incubated in 5-10% CO2 at 35 37°C for 16 – 24hrs is recommended.

3.3 Colonial Appearance

XLD – Colonies are pink red, 1- 2 mm diameter, and with no black centre. Some strains may have a pink or yellow periphery on XLD agar.

DCA **-** Colonies are colourless (*S. sonnei* may form pale pink colonies because of late lactose fermentation).

3.4 Test Procedures

3.4.1 Serotyping

Serotyping is subtyping method based on the immuno-reactivity of various antigens. *Shigella* species are by definition non-motile, as such, only the somatic (O) antigens are utilized for the determination of serotype. Flagellar (H) antigens are not expressed.

Serological identification is performed by slide agglutination with polyvalent, somatic (O) antigen grouping sera, followed by testing with monovalent antisera for specific serotype identification 30.

**NOTE:** The commercially available antisera may not able to cover all possible epitopes of the O antigen of *Shigella* species. There are probably a multitude of epitopes not covered by the typing scheme currently in use. New serotypes or sub-serotypes are being isolated from different parts of the world eg *Shigella boydii* serovar 20 isolated in Canada29.

3.4.2 Biochemical tests

**Urease Test** ([TP 36 - Urease Test](http://www.hpa.org.uk/SMI/pdf/Testprocedures))

*Shigella* species do not produce urease.

**Oxidase Test** (optional) ([TP 26 - Oxidase Test](http://www.hpa.org.uk/SMI/pdf/Testprocedures))

*Shigella* species are oxidase negative.

**Carbohydrates Fermentation Tests**

All *Shigella* species ferment mannitol except *Shigella dysenteriae* and some serotypes of *Shigella flexneri.*

**Commercial identification Systems**

Laboratories must follow manufacturer’s instructions and rapid tests and kits must be validated and be shown to be fit for purpose prior to use.

3.5 Further Identification

**Rapid Molecular Methods**

Molecular methods have had an enormous impact on the taxonomy of *Shigella*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Shigella* 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 identification and sensitivity methods have been developed for isolates from clinical samples; these include molecular techniques such as Real-time Polymerase Chain reaction (PCR), Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable-Number Tandem-Repeat Analysis (MVLA), SNP assays, Whole Genome Sequencing (WGS) and Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF) Mass Spectrometry. All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, these methods are difficult to implement for routine bacterial identification in a clinical laboratory.

**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 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 use31.

This technique has not been exceptionally useful for identifying *Shigella* species. One of the limitations is the current inability of MALDI-TOF MS to reliably discriminate pathogenic *E. coli* from *Shigella* species because of the close genetic relatedness of the organisms which is challenging32.

**Real-time Polymerase Chain reaction (RT-PCR)**

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.

A PCR assay based on the amplification of the invasion plasmid antigen H (*ipaH*) gene sequence is used for the diagnosis of Shigellosis (bacillary dysentery)*. IpaH* is carried by all four *Shigella* species as well as by enteroinvasive *Escherichia coli* (EIEC). The *ipaH* PCR assay has been used to detect *Shigella* species and EIEC, indicating high specificity33,34.

**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 laboratories35,36.

PFGE has been employed successfully for strain discrimination of a variety of bacteria, including *S. sonnei* and *S. dysenteriae* type 1 isolates using restriction endonuclease *Not*1. This technique has been and is very useful for discriminating *Shigella* strains for investigation of outbreaks37. However, for *S. sonnei* isolates that are indistinguishable, inter-IS1 spacer typing (IST) provides greater subtyping information than PFGE and could investigate the genetic relationships among *S. sonnei* strains circulating over a longer time span38.

**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. 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 Internet39.

This has been used to identify Shiga-toxin producing *E. coli* and *Shigella* species 40.

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

**Multiple-Locus Variable-Number Tandem-Repeat Analysis (MVLA)**

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 to identify *Shigella* and *Escherichia* strains, suggesting that it could significantly contribute to epidemiological trace-back analysis of *Shigella* infections and pathogenic *Escherichia* outbreaks41.

**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, IIIumina sequencing, Ion Torrent sequencing, etc. 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 *Shigella* species and *E. coli O157:H7* to identify candidate genes responsible for pathogenesis, and to develop better methods of strain detection and to advance the understanding of the evolution of *E. coli*26,42.

**Other Specialised Tests**

**Phage Typing**

Phage typing has proven to be an important tool for strain characterisation and the results obtained have been used in surveillance, source attribution and outbreak investigations. Phage typing is, however, also a phenotypical method that depends very much on the experience of the individual laboratory and on support from the reference centre that coordinates the maintenance of phages and the updating of the system. Only when the phage typing method is harmonised and the performance in different laboratories is controlled, can the results be regarded as definitive and comparable between laboratories43.

However, it has been used successfully in the characterisation of *Shigella* species – *S. sonnei, S. boydii* 29.

3.6 Storage and Referral

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

4 Identification of *Shigella* speciesFlowchart



The flow chart is for guidance only

5 Reporting

5.1 Presumptive Identification

If appropriate growth characteristics, colonial appearance, urease and serology 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 presumptive or confirmed *Shigella dysenteriae* O1 isolates, according to local protocols.

The medical microbiologist should also be informed of a presumptive or confirmed *Shigella* species if the request card bears relevant information eg:

* enterocolitis, dysentery (especially if complicated by haemolytic-uraemic syndrome)
* neurological dysfunction or confusional states
* history of recent foreign travel or laboratory work
* food poisoning
* investigations of outbreak situations

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 Public Health England44-49

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

Inform the infection control team of presumptive or confirmed isolates of *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

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

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1. #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. [↑](#footnote-ref-1)