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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UK Standards for Microbiology Investigations are produced in association with:
Identification of *Shigella* species

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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.
# 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.

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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.

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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.
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/web/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.
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. Each of the species, with the exception of S. sonnei, is subdivided by serotype.

Characteristics

Shigella species are small Gram negative rods. They produce pink colonies on XLD medium and colourless colonies on DCA. Shigella species are facultative anaerobes, are non-motile, oxidase negative, urease negative, do not decarboxylate lysine, and all except S. dysenteriae type 1 are catalase positive. The species may be differentiated by biochemical tests and serology of their lipopolysaccharides. The majority of Shigella species, except S. flexneri 6a, 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.

Shigella species are highly infective. The infective dose is particularly low with S. dysenteriae, which may require as few as 10-100 organisms to cause infection.

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

N/A
1 Safety Considerations

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 dysenteriae type 1 causes severe and sometimes fatal disease. Laboratory acquired infections have been reported. Low numbers of Shigella species are required for an infective dose. Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this 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.

2 Target Organisms

Genus Shigella
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
N/A

3.2 Primary Isolation Media
XLD agar incubated in air at 35-37°C for 18-24hr
DCA incubated in air at 35-37°C for 18-24hr

3.3 Colonial Appearance
Shigella species on XLD agar produce 1-2mm diameter red colonies (no black centre). Colonies on DCA are colourless (S. sonnei may form pale pink colonies because of late lactose fermentation).
3.4 Test Procedures

3.4.1 Agglutination
Agglutination with shigella antiserum (not all the serotypes are contained in polyvalent antisera).

3.4.2 Biochemical tests
Urease Test (TP 36 - Urease Test)
*Shigella* species do not produce urease

Oxidase Test (optional) (TP 26 - Oxidase Test)
*Shigella* species are oxidase negative

Commercial identification kit
In house identification kit

3.5 Further Identification
N/A

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* species

Clinical specimen
Primary isolation plate

XLD agar-red colonies
DCA-colourless colonies

CLED purity plate

Oxidase test
An oxidase test will distinguish between the organisms
*S. sonnei* (oxidase neg) & *P. shigelloides* (oxidase pos)

Positive
Negative

Discard

Positive
Negative

General agglutinations

Urease
(37°C for up to 4 hr in air)

Positive
Negative

Pure culture

Biochemical tests

Mannitol negative
Mannitol positive

Pure culture
Specific agglutinations

*S. dysenteriae* Polyvalent
*S. sonnei*
*S. flexneri* Polyvalent
(1-6, x,y)
*Not all serotypes are contained in polyvalent antisera (1-6, 7-11, 12-15)*

*S. boydii* Polyvalent

Further identification if clinically indicated. Commercial identification kits or other biochemical identification or send to the Reference Laboratory. If required save the pure isolate on an agar slope.

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 presumed or confirmed *Shigella* species if the request card bears relevant information e.g.:

- 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 clinicians.

5.4 CCDC
Refer to local Memorandum of Understanding.

5.5 Public Health England
Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team
Inform the infection control team of presumed 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
Identification of *Shigella* species

London
NW9 5EQ
Contact PHE’s main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales

Scotland

Northern Ireland
http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm

7 Notification to PHE\(^{20,21}\) or Equivalent in the Devolved Administrations\(^{22-25}\)

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\(^{22,23}\), Wales\(^{24}\) and Northern Ireland\(^{25}\).
References


3. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.


