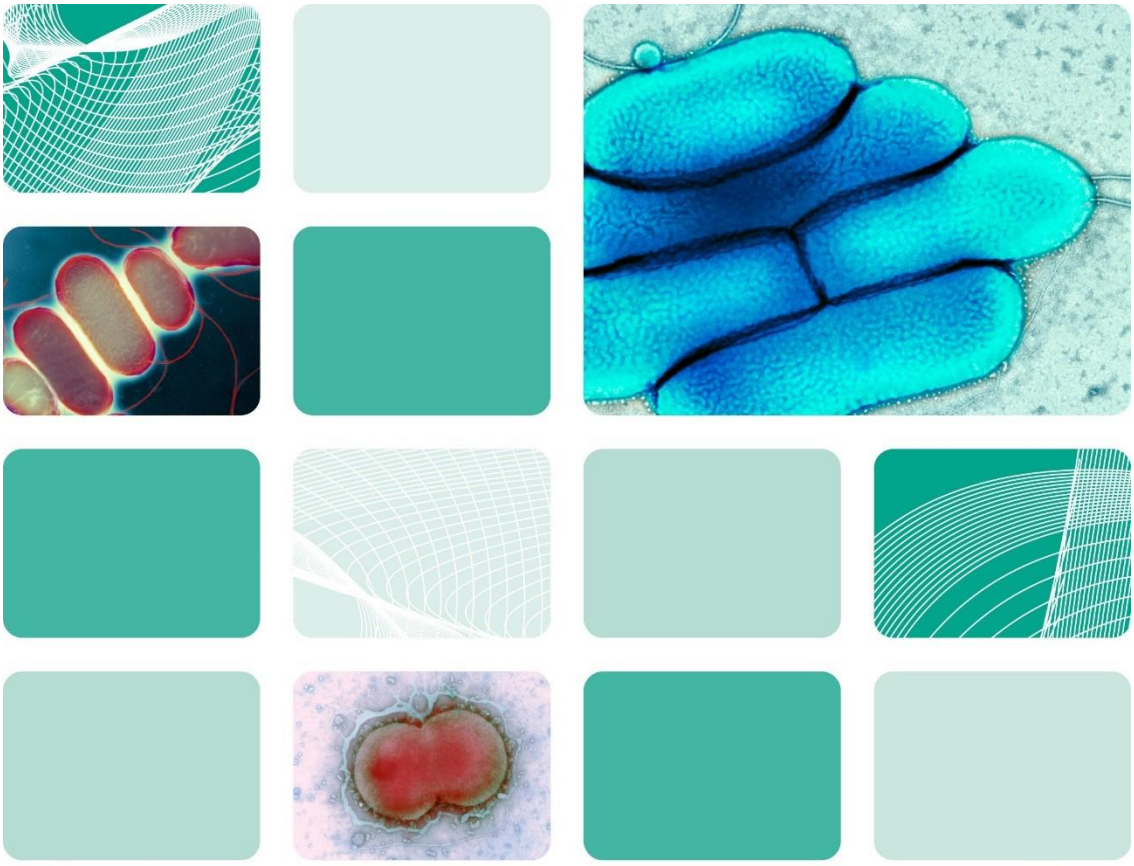
UK Health Security Agency

UK Standards for Microbiology Investigations

Identification of Shigella species



National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the **UK Health Security Agency** to produce **UK Standards for Microbiology Investigations (UK SMIs)**. The renewed accreditation is valid until **30 June 2026** and applies to guidance produced using the processes described in '**UK Standards for Microbiology Investigations Development Process' (2021)**. The original accreditation term began on 1 July 2011.

Issued by the Standards Unit, Specialised Microbiology and Laboratories, UKHSA

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see the Steering Committee page on GOV.UK).

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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@phe.gov.uk.

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

Amendment number/date	6 / 22 April 2022
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	22 April 2025
Section(s) involved	Amendment
Whole document	Document has been transferred to a new template and some text has been moved to the relevant sections
Section	Scope, introduction, safety considerations updated
8.2	Primary isolation media updated
8.5	Removal of some further identification tests
Appendix	Flowchart updated with MALDI-TOF, Whole genome sequencing and molecular tests
References	References reviewed and updated

*Reviews can be extended up to 5 years where appropriate

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 *Shigella* species. *Shigella species* are amongst the most important enteric pathogens and are transmitted via the faecal oral route, by person to person spread or by ingestion of contaminated food and water. Historically, shigellosis in the UK was most commonly associated with travellers' diarrhoea and outbreaks in institutional settings, mainly schools and nurseries. More recently, the highest burden of domestic acquisition of *Shigella* species in the UK has been linked to sexual transmission between men (1). The incubation period is from 1 to 7 days and symptoms include watery diarrhoea and dysentery. Individuals may shed viable bacteria for up to 4 weeks and remain infectious to other individuals even though they may not show symptoms.

This UK SMI includes routine culture and biochemical test methods for the identification of microorganisms. Some laboratories will perform biochemical tests for confirmation. However, many laboratories are implementing rapid techniques such as whole genome sequencing (WGS), matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS), nucleic acid amplification tests (NAATs) and molecular methods for the identification of *Shigella* species.

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

4 Introduction

4.1 Taxonomy and characteristics

The genus *Shigella* belongs to the family Enterobacterales and consists of 4 species: *Shigella dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei.* Each of these species, with the exception of *S. sonnei*, is subdivided by serotype.

Shigella species are differentiated into 4 subgroups on the basis of their O (somatic) antigens and further differentiated into serotypes (2):

- *S. dysenteriae* (Group A) contains 15 distinct antigenic serotypes
- *S. flexneri* (Group B) contains 8 serotypes and 9 subserotypes
- *S. boydii* (Group C) contains 20 distinct antigenic serotypes (3)
- *S. sonnei* (Group D) contains only 1 serotype that may occur in 2 forms, form I (smooth) and form II (rough)

The type species is *Shigella dysenteriae*.

These species are human pathogens and have no animal reservoir except for non-human primates. *Shigella* infection can occur from a low infectious dose of 10 to 100 organisms (4). *Shigella* species are facultative anaerobes with an optimum growth temperature of 37°C and they are non-spore formers. Unlike *Salmonella* species*, Shigella* species do not possess flagella and hence are non-motile. They reduce nitrate to nitrite, are oxidase negative and urease negative. *Shigella* species are catalase positive with the exception of *Shigella dysenteriae.* With a few exceptions, *Shigella* species ferment sugars without gas production*.* 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 and do not decarboxylate lysine and give variable results for indole (with the exception of *S. sonnei* that is always indole negative) (2,3),(5).

Phylogenetically, each *Shigella* species belongs to different clonal complexes within the genus *Escherichia coli*. Although the relatedness of DNA from *E. coli* and *Shigella* indicate that they constitute a single species, they are maintained as separate entities in the interests of epidemiology and clinical medicine. There is often a clinical or public health requirement to differentiate between *E. coli* and *Shigella* and to specify which species of *Shigella* has been isolated (6).

All 4 *Shigella* species can cause dysentery, however infection with *S. dysenteriae* type 1 may progress to haemolytic uremic syndrome (HUS) due to the production of Shiga toxin. HUS is a systemic condition characterised by kidney failure, cardiac and neurological complications that can be fatal.

In the UK *S. dysenteriae* is a cause of travellers' diarrhoea. In England *S. dysenteriae* is the least commonly isolated species (4%) compared to *S. sonnei* (49%), *S. flexneri* (39%) and *S. boydii* (7%) (7).

In the UK *S. flexneri* is associated with travellers' diarrhoea and gastrointestinal symptoms in men who have sex with other men (MSM), and maybe endemic in defined communities and populations. UK Health Security Agency (UKHSA), Gastrointestinal Bacteria Reference Unit (GBRU) for England and Wales reported 18,266 *Shigella* cases between 2004 and 2015 (8).

UKHSA has presented laboratory surveillance data in regard to sexual transmission of *Shigella* species among MSM in England. It was reported that between 2004 and 2019, there was an increase in the number of *Shigella* species diagnosed amongst adults. Due to

the COVID-19 pandemic there were fewer cases reported in 2020 compared to 2019. Before 2010 many cases have been travel associated (1).

Each year UKHSA, Second-Generation Surveillance System (SGSS) receives approximately 1,100 laboratory confirmed cases of *S. sonnei* reported by local hospital laboratories. Of these, approximately 800 isolates are from faecal specimens or community cases with symptoms of gastrointestinal disease which are sent for bacterial identification and typing (9).

It is estimated that globally there are 164.7 million cases of shigellosis each year. The majority of cases occur in low income countries and *S. flexneri* causes 60% of these cases (4).

5 Technical information and limitations

5.1 Quality control

If using commercially manufactured antisera, check suitability of use for all methods. Each new lot or shipment of antisera and 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.

5.2 Agglutination test

Slide agglutination tests are rapid and will presumptively identify *Shigella* species. However, agglutination with diagnostic antisera is time consuming and costly, requiring up to 10 separate agglutination tests using antisera for type antigens I, II, III, IV, V, and VI, antisera for group antigens 3 and 4, 7 and 8, and 6, and the monoclonal antibody MASF1c for serotype 1c (10).

6 Safety considerations

The section covers specific safety considerations (11-32) related to this UK SMI, and should be read in conjunction with the general safety considerations on GOV.UK.

Shigella species are Hazard Group 2 organisms with the exception of *Shigella dysenteriae* (Type 1) which is a Hazard Group 3 organism.

To minimise exposure to laboratory workers, appropriate personal protective equipment (PPE) such as gloves, must be worn when handling *Shigella* species. Techniques designed to minimise exposure should be adhered to at all times. It is also very important to

decontaminate the workspace where the samples are handled. Handwashing is important to minimise the spread of infection.

The most effective method for preventing laboratory acquired infections is the adoption of safe working practices including processing faecal samples and presumptive *Shigella* isolates 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 and should be verified. Currently there is no vaccine available to protect against infections caused by *Shigella* species. Production of a vaccine will contribute to enhanced safety and reduction of infections (33). Refer to immunisation against infectious disease: the green book for further guidance.

7 Target organisms

All species cause human infections. *Shigella dysenteriae* (group A consisting of 15 serotypes, 1 to 15), *Shigella flexneri* (group B consists of 6 serotypes 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 4c, 5a, 5b, and 6), *Shigella boydii* (group C consists of serotypes, 1 to 20) and *Shigella sonnei* (group D with 1 serotype) (3).

8 Identification

8.1 Microscopic appearance

Gram stain refer to TP 39 – Staining procedures

Shigella species are 1 to 3µm in length and 0.7 to 1.0µm in diameter and are identified as Gram negative straight rods (2).

8.2 Primary isolation media

Isolates from primary culture are identified by colony appearance on selective media, 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 a reference laboratory. Refer to section 8.7.

Isolation of *Shigella* species from food and water should involve an enrichment stage in *Shigella* broth, as the infecting dose is 10 to 100 organisms.

Faecal specimens should be sampled at the acute stage and examined as soon after collection as possible.

All identification tests should ideally be performed from non-selective agar. The following media supports the growth of *Shigella* species:

- MacConkey agar (MAC) is considered a differential between lactose fermenters and non-lactose fermenters, incubated in air at 35 to 37°C for 18 to 24 hour
- Xylose-lysine-desoxycholate agar (XLD) incubated in air at 35 to 37°C for 18 to 24hour
- Desoxycholate citrate agar (DCA) incubated in air at 35 to 37°C for 18 to 24 hour
- Hektoen enteric medium (HE) incubated in air at 35 to 37°C for 18 to 24 hour
- *Salmonella-Shigella* agar (SS) incubated in air at 35 to 37°C for 18 to 24 hour
- Blood agar incubated at 35°C for 16 to 24 hour

Non-inhibitory agar such as MAC should be used alongside the inhibitory agar such as XLD, DCA or HE agar as some strains of *Shigella* grow poorly on inhibitory media.

8.3 Colonial appearance

XLD – Colonies are red, 1 to 2mm diameter, and with no black centre.

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

MAC – contains lactose, neutral red, selective inhibitors crystal violet and bile salts. *Shigella* species are non-lactose fermenters oxidase negative and are transparent or colourless.

HE – contains lactose, sucrose, salicin, bromothymol blue, Andrade's pH indicator, ferric citrate and sodium desoxycholate. Based on bile salts that reduce growth of Gram positive organisms (3). Colonies appear blue green.

SS agar is similar to HE. Detection is based on fermentation of lactose and non-fermenters. Highly selective agar inhibits growth of some *Shigella* species. Colonies appear colourless.

Nutrient agar (NA) for pure cultures for slide agglutination. Colonies appear smooth, circular convex greyish or colorless, translucent often 2 to 3mm diameter.

Blood agar – large grey colonies often with a fringe or feathered edges and do not exhibit pigmentation (3).

8.4 Test procedures

8.4.1 Biochemical reactions

D-mannitol is especially important in classifying *Shigella.* Members of Group A cannot ferment D-mannitol, whilst members of Group B, C and D can ferment this sugar (2).

8.4.2 Serotyping

Serotyping is a subtyping method based on the immuno-reactivity of various antigens. *Shigella* species are by definition non-motile, and as such, only the somatic (O) antigens are utilised 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 (3).

Serotyping involves agglutination tests with diagnostic antisera: *Shigella sonnei* (1), *Shigella flexneri* (1 to 6, x,y) and *Shigella boydii* (1 to 6, 7 to11, 12 to 15). Not all serotypes are contained in polyvalent antisera. The results require visual assessment of the agglutination reaction (10).

Phenotypic serotyping is useful for the detection and investigation of local outbreaks but can be an expensive and labour intensive method. It is difficult to quality control and novel combinations of antigens can result in errors at the interpretation stage and unresolvable cross reactions (4).

Refer to manufacturer’s instructions.

8.5 Further identification

Culture based techniques have been used by laboratories as the gold standard method for identification, and involves multiple stages such as appropriate selective agar, slide agglutination and commercial biochemical identification kits. Laboratories are moving towards molecular techniques for rapid results. However these techniques require specialised laboratories, trained staff and expensive reagents which may not be available to all laboratories (34).

8.5.1 Rapid molecular methods

Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

8.5.2 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 to sequence an entire genome such as pyrosequencing, nanopore technology, IIIumina sequencing, Ion Torrent sequencing and others. This sequencing method gives rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reduction in infections, morbidity, and costs.

WGS is routinely used by UKHSA and has greatly improved surveillance capabilities and monitoring trends in antimicrobial resistance (7). WGS has replaced traditional phenotypic and PCR methods for routine surveillance.

The WGS data for *S. flexneri* and historical data provides a robust and highly discriminatory typing approach to outbreak detection and investigation. Analysis of WGS data also enables monitoring of the emergence of novel serotypes and the opportunity to examine the effects of prophage and plasmids on O-antigen expression and evolution (4).

8.6 Other specialised tests

8.6.1 MALDI-TOF MS

E. coli and *Shigella* species have similar gene sequences and protein expression making them difficult to classify and identify. *E. coli* and *Shigella* species cannot be reliably differentiated using MALDI TOF MS, so therefore traditional methods such as biochemical tests and agglutination are required (35).

8.6.2 NAATs

For reliable identification of *Shigella* species polymerase chain reaction (PCR) based methods have been used. Although PCR is a reliable method for the detection of *Shigella* species, and despite efforts on the specific detection, *Shigella* species, are still regarded as indistinguishable from enteroinvasive *E. coli* by PCR. Other methods, such as WGS can differentiate both species.

8.7 Storage and referral

The pure isolate is saved on a nutrient agar slope for referral to a reference laboratory.

As *Shigella* is a notifiable disease, for public health management of cases, all isolates of S. *flexneri*, *S. boydii* and *S*. *dysenteriae* should be referred to a reference laboratory for typing (27). Following the increase in sexual transmission of MDR *S*. *sonnei* it is recommended that all isolates of *S*. *sonnei* are sent to a reference laboratory for further testing.

9 Reporting

9.1 Infection Specialist

According to local protocols, inform the medical microbiologist of presumptive or confirmed *S. boydii, S. flexneri* and *S. dysenteriae* O1 isolates.

The medical microbiologist should also be informed of presumptive or confirmed *Shigella* species (*S. sonnei*, *S. dysenteriae*) if the request card bears relevant information such as:

- enterocolitis, dysentery (especially if complicated by haemolytic-uraemic syndrome)
- history of laboratory work
- investigations of outbreak situations

Follow local protocols for reporting to clinician

9.2 Preliminary identification

If appropriate growth characteristics, colonial appearance, urease and serology results are demonstrated

9.3 Confirmation of identification

Further biochemical tests, molecular methods and reference laboratory report.

For confirmation and identification please see Specialist and reference microbiology: laboratory tests and services page on GOV.UK for reference laboratory user manuals and request forms.

9.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

9.5 UK Health Security Agency

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (27).

9.6 Infection prevention and control team

Inform the infection prevention and control team of presumptive or confirmed isolates of *Shigella* species.

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

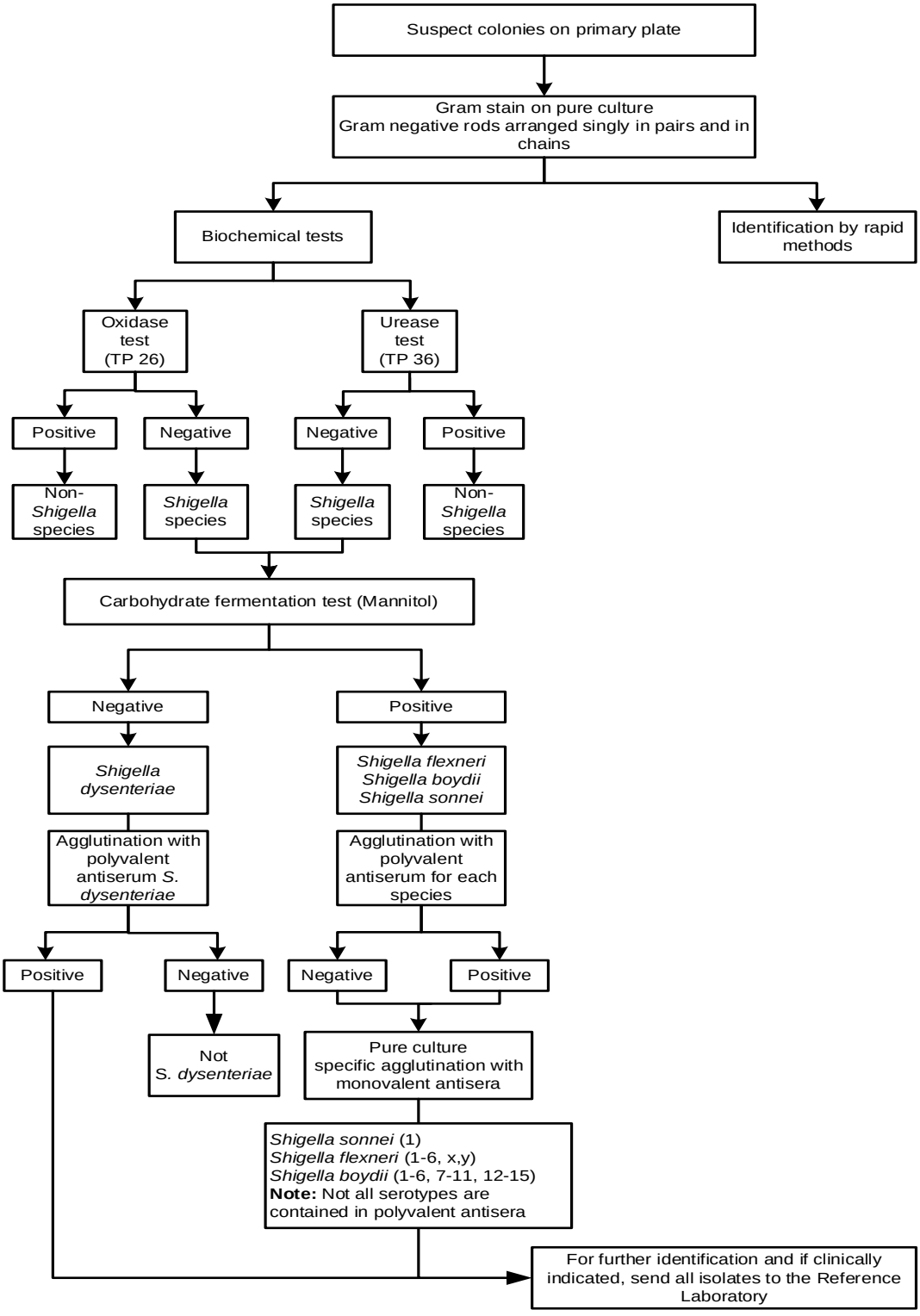
Contact the appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

- 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.

Algorithm: Identification of Shigella species

An accessible text description of this flowchart is provided with this document.



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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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