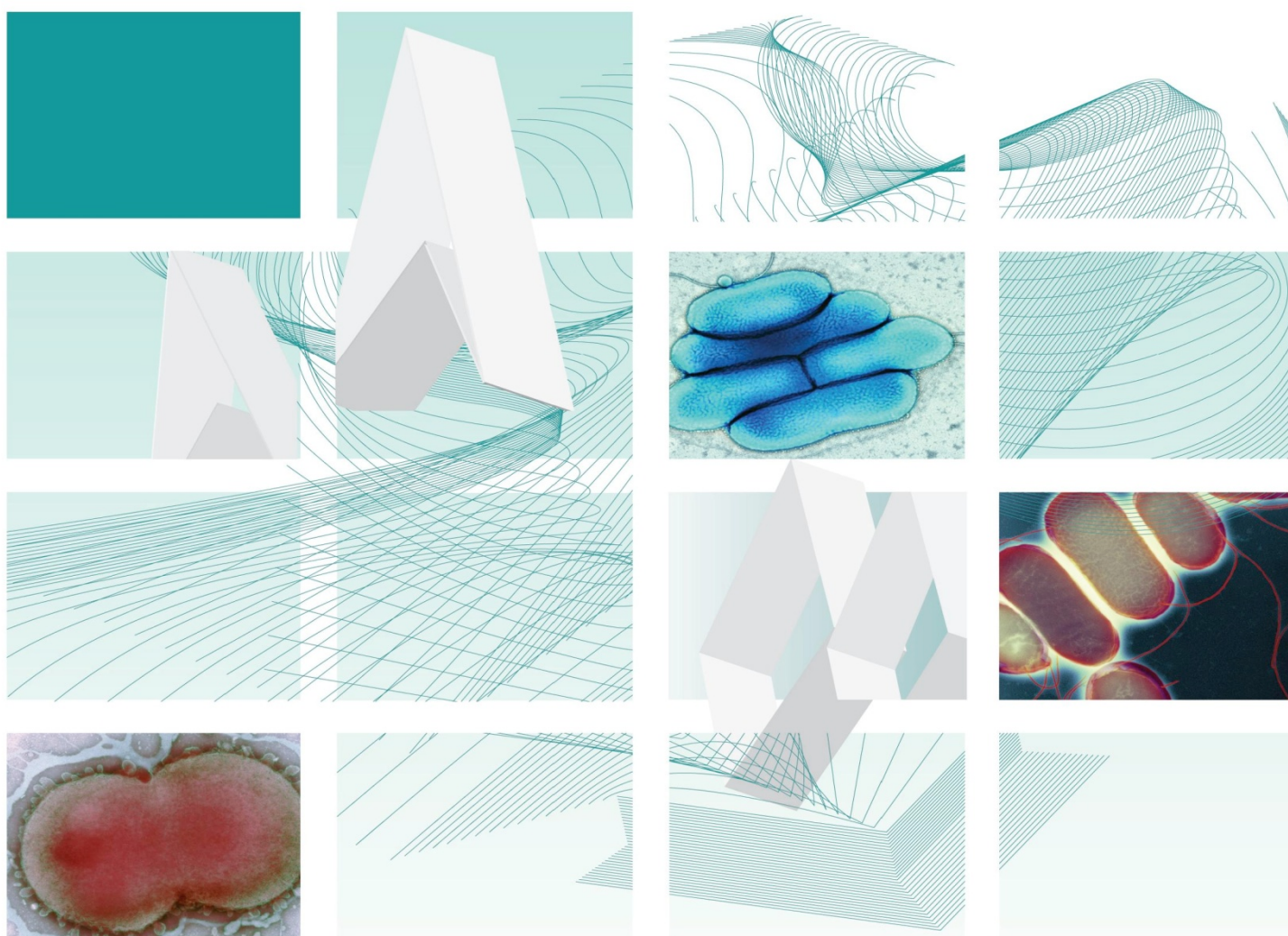




UK Standards for Microbiology Investigations

Identification of *Yersinia* 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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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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.

Amendment No/Date.	5/29.06.15
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope of document.	The scope has been updated to include all <i>Yersinia</i> species.
Introduction.	The taxonomy of <i>Yersinia</i> species has been updated. More information has been added to the Characteristics section. The medically important species of <i>Yersinia</i> are mentioned. Section on Principles of Identification has been updated to include the MALDI-TOF MS.
Technical information/limitations.	Addition of information regarding commercial identification systems and MALDI-TOF MS.
Safety considerations.	This section has been updated on the handling of <i>Yersinia</i> species.
Target organisms.	The section on the Target organisms has been updated and presented clearly.
Identification.	Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice. Section 3.4.1, 3.4.2 and 3.4.3 has been updated to include Commercial Identification Systems, MALDI-TOF MS and NAATs with references. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification flowchart.	Modification of flowchart for identification of <i>Yersinia</i> species has been done for easy

	guidance.
Reporting.	Subsections 5.3, 5.4 and 5.6 have been updated to reflect the information required on reporting practice.
Referral.	The addresses of the reference laboratories have been updated.
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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

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 <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. 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. (2015). Identification of *Yersinia* species. UK Standards for Microbiology Investigations. ID 21 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

Scope of document

This SMI describes the identification of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* isolated from faeces and *Yersinia pestis* isolated from other specimens such as blood, lymph nodes and abscesses.

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The genus *Yersinia* belongs to the family *Enterobacteriaceae* and comprises 17 recognised species and 2 subspecies¹. *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are associated with human and animal diseases².

Characteristics

Members of the genus *Yersinia* are Gram-negative, catalase-positive and oxidase-negative, facultatively anaerobic straight rods to coccobacilli³. Cells are 0.5 - 0.8µm by 1-3µm in size and show bipolar staining ("closed safety-pin appearance"). They have both respiratory and fermentative type of metabolism⁴.

All members of the genus grow readily on ordinary media. Their optimum growth temperature is 28-29°C. They are non-motile at 37°C, but motile with peritrichous flagella when grown below 30°C (except for *Yersinia pestis* which is always non-motile)⁵. Phenotypic characteristics are often temperature dependent and more are expressed by cultures at 22–29°C rather than 35-37°C⁶.

Yersinia species can be differentiated based on their biochemical reactions on tests such as motility, urease, lactose fermentation, indole and nitrate reduction tests.

They may be isolated from specimens such as bubo fluid, sputum, CSF, faeces, urine, blood, lymph nodes and abscesses³.

The type species is *Yersinia pestis*.

The medically important *Yersinia* species isolated are;

Yersinia enterocolitica

Cells are coccoid shaped, usually 0.5-0.8µm by 1-3µm in size. They are non-spore formers and are motile at room temperature (25°C) but non-motile at 37°C. They are facultative anaerobes. There are 6 biotypes (1A, 1B, 2, 3, 4 and 5 based on their genomic sequence) containing 50 different serogroups of *Yersinia enterocolitica*; however, only certain serogroups are pathogenic for humans and are categorized according to which O antigen they express. *Y. enterocolitica* biotypes 1B and 2-5 are considered pathogenic, whereas biotype 1A is in general considered non-virulent. *Y. enterocolitica* -like species, which can sometimes be misidentified as *Y. enterocolitica*, are considered non-virulent^{7,8}.

Typical *Y. enterocolitica* colonies on CIN (cefsulodin, Irgasan, novobiocin) agar will have a deep-red centre surrounded by a transparent border giving the appearance of a "bulls-eye". Strains of *Y. enterocolitica* usually are lactose negative, but lactose positive strains exist. They are urease positive and give variable indole results.

It has been found in faeces, blood or lymph node tissues³.

Yersinia pseudotuberculosis

Their cells are also coccoid shaped, usually 0.5-0.8µm by 1-3µm in size. They are non-spore formers and are motile at room temperature (25°C) but non-motile at 37°C. They are facultative anaerobes. Some freshly isolated strains may require subculturing before expressing their motility. There are 15 different O-serotypes (O:1–O:15) and 10 subtypes (O:1a–O:1c, O:2a–O:2c, O:4a–O:4b, O:5a–O:5b) based on variability in the lipopolysaccharide O-side chain (O-antigen)⁹.

On CIN agar, colonies of *Y. pseudotuberculosis* are smaller, deep red with a sharp border surrounded by a translucent zone. Strains of *Y. pseudotuberculosis* are lactose negative.

They are also urease positive and reduce nitrates whereas negative for indole.

It can be found in faeces, blood or lymph node tissues³.

Yersinia pestis

Cells are rod-ovoid shaped, 0.5-0.8µm in width and 1-3µm in length and show bipolar staining (safety pin appearance). They are facultatively intracellular and non-motile.

Y. pestis is not fastidious but, after incubation for 24hr on blood agar, colonies are grey-white, translucent, and usually much smaller than those of other *Enterobacteriaceae* or to be seen as individual colonies but on further incubation for another 24hr, colonies are about 1-2mm in diameter, grey-white to slightly yellow colour and opaque. Colonies have a raised, irregular “fried egg” morphology which becomes more prominent as the culture ages. Colonies can also be described as having a “hammered copper”, shiny surface with little or no haemolysis on blood agar. *Y. pestis* also grows well in nutrient-rich broth such as trypticase soy or nutrient broth. The cultures in broth can be described as suspended flocculent or crumbly clumps (“stalactites”) after 24- 48hr of incubation. These clumps are visible at the side and bottom of the tube with the rest of the medium remaining clear².

They are negative for indole, urease and lactose fermentation⁶.

It has been found in bubo fluid, blood, sputum, CSF, faeces and urine³.

Principles of identification

Isolates from primary faecal culture are identified by colonial appearance on selective media and biochemical tests. All identification tests should ideally be performed from non-selective agar. If confirmation of identification is required, isolates should be sent to the Reference Laboratory.

Full molecular identification using for example, MALDI-TOF MS and Real-time Polymerase Chain reaction (PCR) can be used to identify *Yersinia* isolates to species level.

Typing and differentiation between strains of *Yersinia* species can be achieved using a range of molecular techniques eg Multiple-Locus Variable-Number Tandem-Repeat Analysis, Pulsed Field Gel Electrophoresis (PFGE), Whole Genome sequencing, etc. For more information, see section 3.5 on further identification.

Technical information/limitations

Commercial identification systems

The commonly used commercial identification systems do not include all *Yersinia* species in their databases and as such there is a constant misidentification of certain *Yersinia* species, particularly *Y. bercovieri*, *Y. mollaretii*, *Y. rohdei*, and *Y. intermedia*, as *Y. enterocolitica*¹⁰.

MALDI-TOF MS

The limitation of this technique is the lack of an updated database that includes profiles of all *Yersinia* species and available databases which require optimisation³⁵.

1 Safety considerations¹¹⁻²⁷

All *Yersinia* species are in Hazard Group 2 with the important exception of *Yersinia pestis*. All work on *Yersinia pestis* must be performed under Containment level 3 conditions.

Yersinia enterocolitica and *Yersinia pseudotuberculosis* can cause gastroenteritis but no laboratory acquired infections have been reported to date. Their infectious dose is of 10^8 bacteria or more²⁸.

Yersinia pestis is the bacterial agent of plague. It is highly infective and the infectious dose is unknown. Laboratory acquired infections have been reported^{29,30}. Vaccination is recommended for laboratory personnel who are routinely exposed to live *Y. pestis*.

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 time.

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

***Yersinia* species isolated and reported to have caused human infections⁵**

Y. pseudotuberculosis, *Y. enterocolitica*, *Y. pestis*, *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*, *Y. bercovieri* (formerly called *Yersinia enterocolitica* biogroup 3B)*, *Y. mollaretii* (formerly called *Yersinia enterocolitica* biogroup 3A)*, *Y. rohdei**,

* There is no evidence of pathogenicity for humans but have been isolated in healthy and sick persons.

3 Identification

3.1 Microscopic appearance

Gram stain ([TP 39 - Staining procedures](#))

Gram negative rods that may show bipolar staining.

3.2 Primary isolation media

Cefsulodin, Irgasan, Novobiocin (CIN) agar incubated in air at 28-30°C for 24-48hr.

Blood (BA) agar incubated in air at 28-30°C for 24-48hr.

3.3 Colonial appearance

Typical *Y. enterocolitica* colonies on CIN agar will have a deep-red centre surrounded by a transparent border giving the appearance of a “bull’s-eye”.

Y. pseudotuberculosis colonies are smaller, deep red with a sharp border surrounded by a translucent zone.

On blood agar, *Y. pestis* colonies are grey-white, translucent, and usually much smaller than those of other Enterobacteriaceae after incubation for 24hr, or to be seen as individual colonies but on further incubation for another 24hr, colonies are about 1-2mm in diameter, grey-white to slightly yellow colour and opaque.

3.4 Test procedures

3.4.1 Commercial identification systems

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

3.4.2 Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry

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.

MALDI-TOF has the ability to accurately discriminate between the two clinically relevant and highly genetically similar organisms with identical 16S rRNA gene sequences, *Y. pestis* and *Y. pseudotuberculosis* as well as providing epidemiological information regarding *Y. pestis* biotypes³². The method of inactivation used for these pathogenic organisms does not have any influence on the MALDI-TOF MS spectra generated. This has also been used to identify and subtype *Y. enterocolitica* isolates^{33,34}.

One of the limitations is the lack of an updated database that includes profiles of all *Yersinia* species and available databases which require optimisation³⁵.

3.4.3 Nucleic acid amplification tests (NAATs)

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.

PCR has also been used to detect virulence genes of *Y. enterocolitica* and *Y. pseudotuberculosis* in human clinical isolates⁸. It has also been used to detect both *Y. pestis* and *Y. pseudotuberculosis* simultaneously in a multiplex assay³⁶.

3.5 Further identification

Rapid molecular methods

Molecular methods have had an enormous impact on the taxonomy of *Yersinia*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Yersinia* 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 typing methods have been developed for isolates from clinical samples; these include molecular techniques such as, Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable-Number Tandem-Repeat Analysis (MLVA) and Whole Genome Sequencing (WGS). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

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 laboratories^{37,38}.

This has been used successfully to discriminate between *Y. enterocolitica* strains and will still be useful for surveillance of the sources and transmission routes of sporadic *Y. enterocolitica* strains in future³⁹.

Multiple-locus variable number tandem repeat analysis (MLVA)

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 and discriminate between *Y. enterocolitica* strains and it has been found to be a more effective method than PFGE. This method is also less labour-intensive and the results from it are easier to analyse. This is also used in outbreak investigations³⁹. It has also been used to genotype *Y. pestis*⁴⁰.

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, Illumina sequencing, Ion Torrent

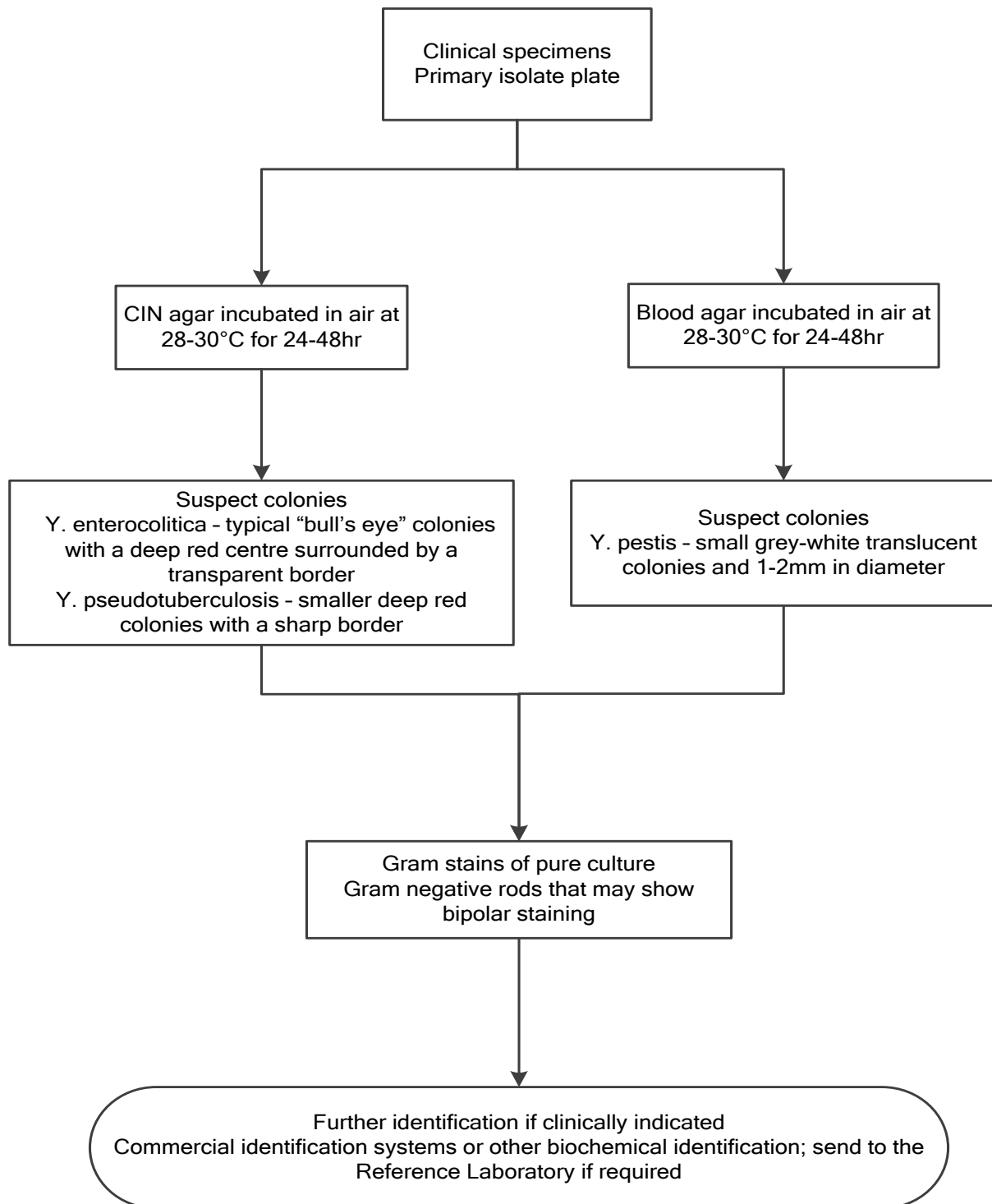
sequencing, etc. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

WGS has been used to provide important insights into the pathology of *Y. enterocolitica* and, more broadly, into the evolution of the genus and other human enteropathogens⁴¹. This rapid method has also been used successfully to identify the structural genes on the chromosome of *Y. pestis* and distinguish it from those of *Y. pseudotuberculosis*^{42,43}.

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 *Yersinia* species



The flowchart is for guidance only.

5 Reporting

5.1 Presumptive identification

If appropriate growth characteristics, colonial appearance and Gram stain of the culture 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 all positive cultures from normally sterile sites and of all presumptive and confirmed *Yersinia* species that are known to be pathogenic or potentially pathogenic.

According to local protocols, the medical microbiologist should be informed of a presumptive or confirmed *Y. enterocolitica* and *Y. pseudotuberculosis*, if the request bears relevant information eg:

- enterocolitis or mesenteric adenitis
- septicaemia
- immunologically-mediated epiphenomena (ie erythema nodosum or reactive arthritis)
- persons receiving blood or blood product transfusion, suffering from iron overload and/or receiving chelation therapy (eg haemoglobinopathy) with transfusion haemosiderosis or primary haemochromatosis
- cases associated with farming, veterinary or laboratory work
- food poisoning
- investigation of outbreaks
- plague (which may be Bubonic, Septicaemic or Pneumonic)
- travel to areas where *Y. pestis* is endemic

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding.

“Plague” is not a Notifiable disease but for public health management of cases, contacts and outbreaks, all suspected cases should be immediately notified to the local Public Health authorities or equivalent elsewhere.

All clinically significant isolates should be notified by the diagnostic laboratories to ensure urgent initiation of proper procedures.

5.5 Public Health England⁴⁴

Refer to current guidelines on CIDSC and COSURV reporting.

5.6 Infection prevention and control team

Inform the infection prevention and control team of presumptive and confirmed cases of *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis*.

6 Referrals

6.1 Reference laboratory

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

Gastrointestinal Bacteria Reference Unit
Bacteriology Reference Department
Public Health England
61 Colindale Avenue
London
NW9 5EQ

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm>

7 Notification to PHE^{44,45} or equivalent in the devolved administrations⁴⁶⁻⁴⁹

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’.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)^{46,47}, [Wales](#)⁴⁸ and [Northern Ireland](#)⁴⁹.

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