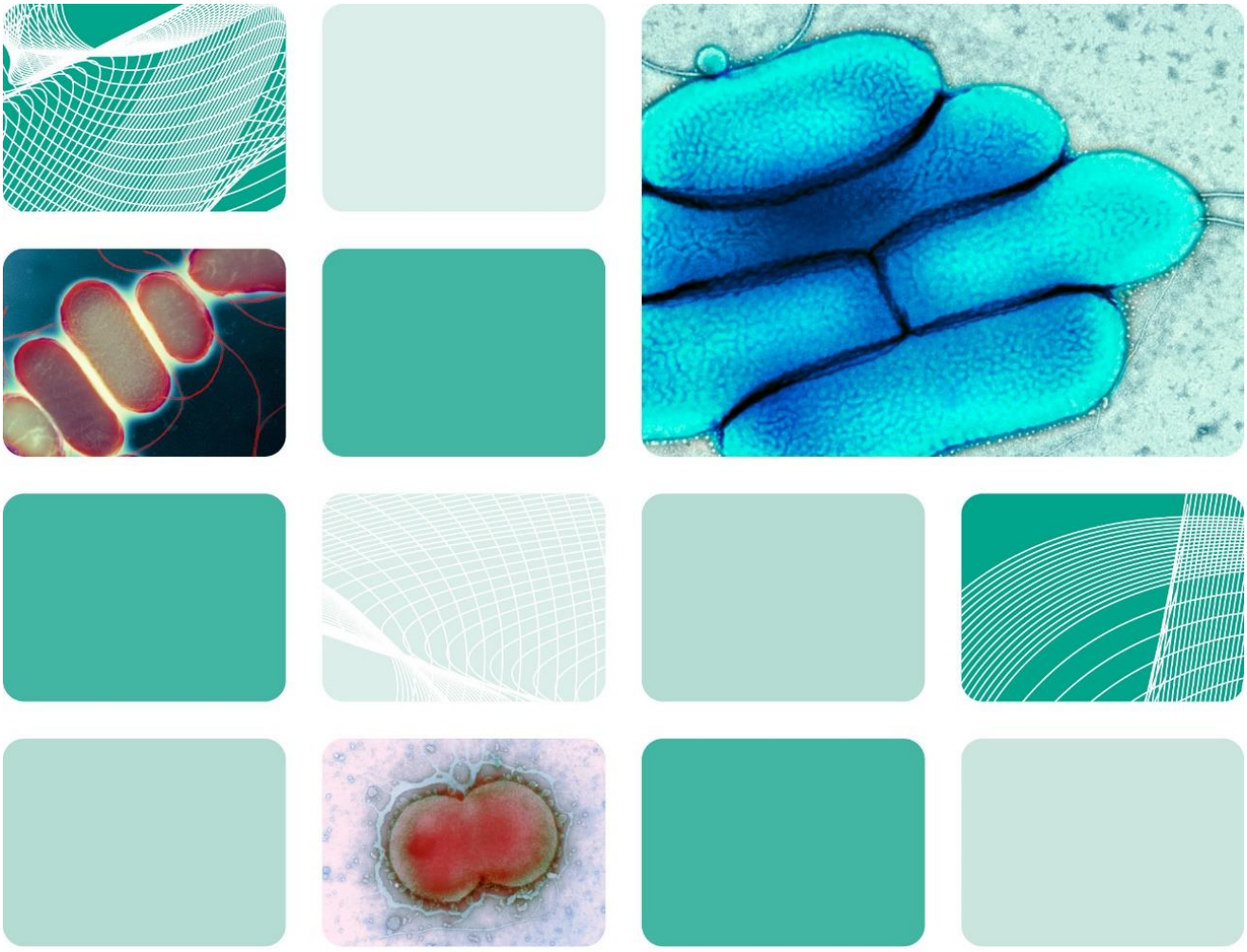
UK Health Security Agency

UK Standards for Microbiology Investigations

Identification of *Yersinia* species



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Acknowledgments

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UK SMIs are produced in association with:

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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@ukhsa.gov.uk.

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

Amendment number/date	6/31.07.25
Issue number discarded	3
Insert issue number	3.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 29/06/2015.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms. Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template. 'Public Health responsibilities of diagnostic laboratories' section added.

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 *Yersinia* species.
Introduction.	The taxonomy of *Yersinia* species has been updated. More information has been added to the Characteristics section. The medically important species of *Yersinia* 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 *Yersinia* 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 *Yersinia* 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.

*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 *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* isolated from faeces and *Yersinia pestis* isolated from other specimens such as blood, lymph nodes and abscesses.

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

4 Introduction

4.1 Taxonomy

The genus *Yersinia* belongs to the family *Enterobacteriaceae* and comprises 17 recognised species and 2 subspecies[1]. *Yersinia pestis, Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are associated with human and animal diseases[2].

4.2 Characteristics

Members of the genus *Yersinia* are Gram-negative, catalase-positive and oxidase-negative, facultatively anaerobic straight rods to coccobacilli[3]. 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 metabolism[4].

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°C[6].

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 abscesses[3].

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 tissues[3].

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 tissues[3].

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 clear[2].

They are negative for indole, urease and lactose fermentation[6].

It has been found in bubo fluid, blood, sputum, CSF, faeces and urine[3].

4.3 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 Generation sequencing, etc. For more information, see section 8.5 on further identification.

5 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 optimisation[35].

6 Safety considerations[11-27]

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 more[28].

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 UK 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.

7 Target organisms

***Yersinia* species isolated and reported to have caused human infections[5]**

*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.

8 Identification

8.1 Microscopic appearance

Gram stain

(UK SMI TP 39 - Staining procedures)

Gram negative rods that may show bipolar staining.

8.2 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.

8.3 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.

8.4 Test procedures

8.4.1 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.

8.4.2 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 MS 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 use[31].

MALDI-TOF MS 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* biotypes[32]. 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 optimisation[35].

8.4.3 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 isolates[8]. It has also been used to detect both *Y. pestis* and *Y. pseudotuberculosis* simultaneously in a multiplex assay[36].

8.5 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 (MVLA) 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 future[39].

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 investigations[39]. It has also been used to genotype *Y. pestis*[40].

Whole genome sequencing (WGS)

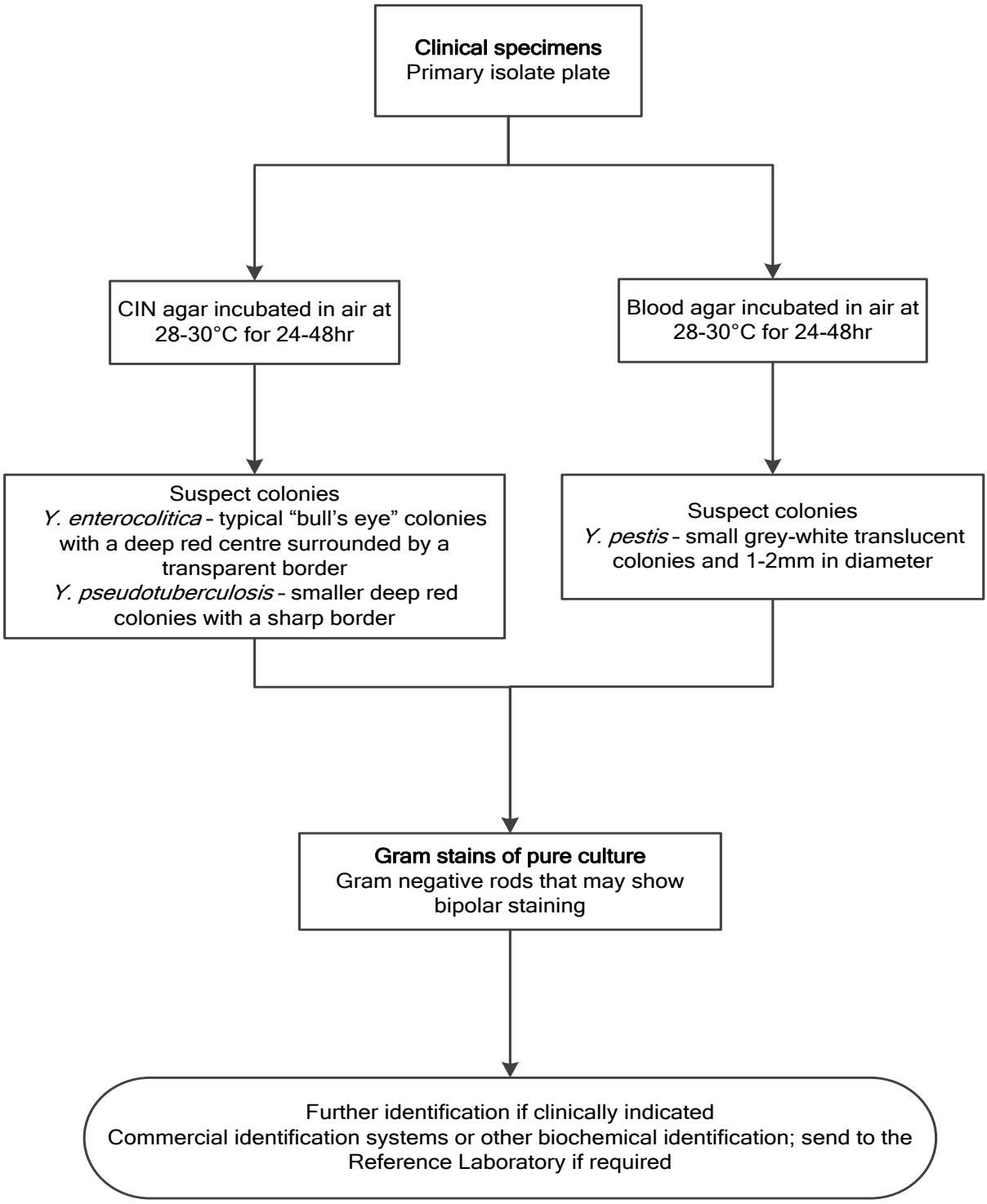
This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology, IIlumina 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 enteropathogens[41]. 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].

8.6 Storage and referral

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

Algorithm: Identification of *Yersinia* species



The flowchart is for guidance only.

9 Reporting

9.1 Presumptive identification

If appropriate growth characteristics, colonial appearance and Gram stain of the culture are demonstrated.

9.2 Confirmation of identification

Further biochemical tests and/or molecular methods and/or reference laboratory report.

9.3 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.

9.4 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.

9.5 UK Health Security Agency

Refer to current guidelines on CIDSC and COSURV reporting.

9.6 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*.

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 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.

11 Public Health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral, to further characterise the organism, as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance, forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

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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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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".

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