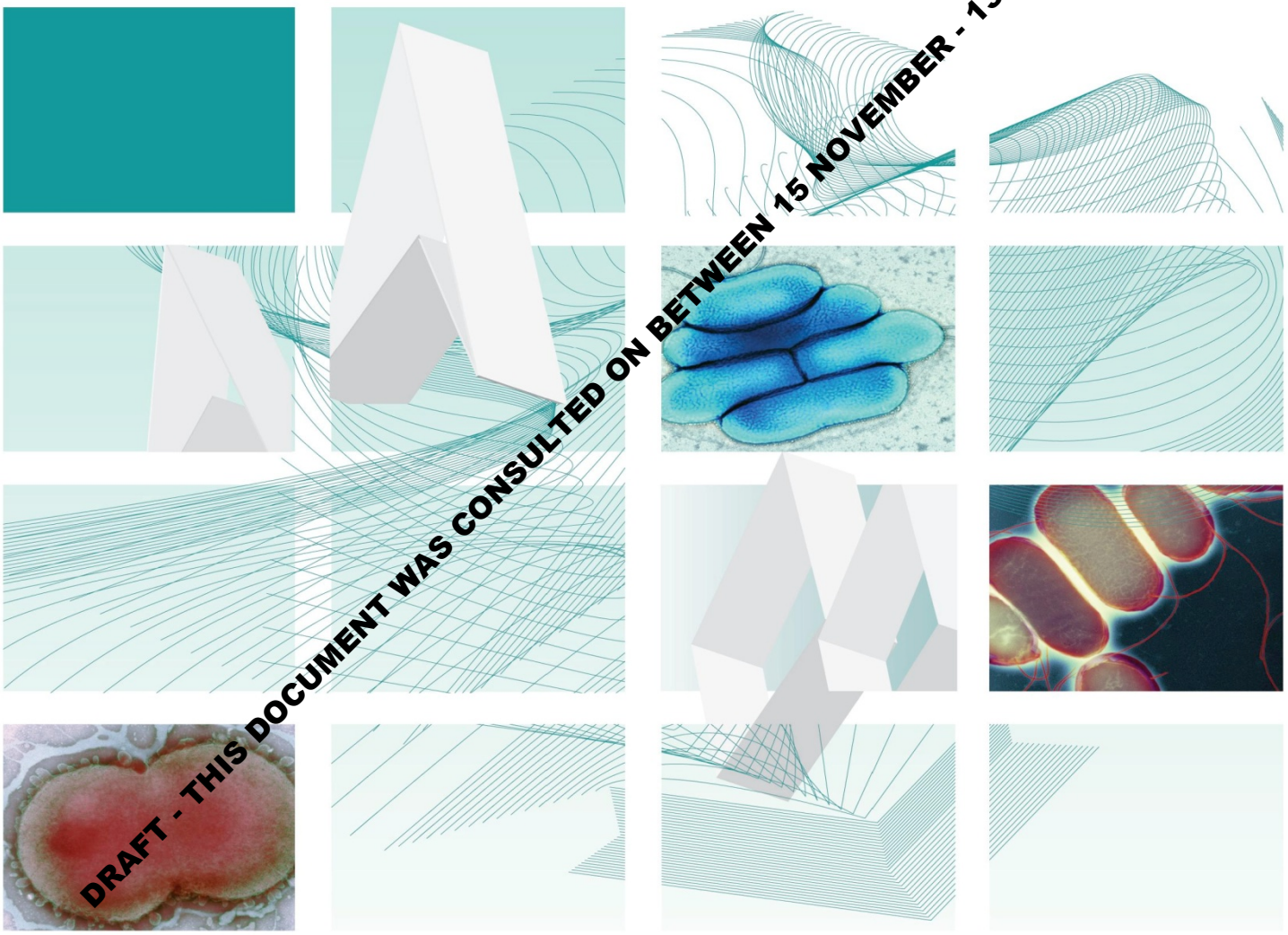
Public Health England

NHS

# UK Standards for Microbiology Investigations

## Identification of *Yersinia* species from Faeces





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# 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:

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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.

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/dd.mm.yy <tab+enter> |
| Issue no. discarded. | 2.1 |
| Insert Issue no. | #.# <tab+enter> |
| **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.<br>Front page has been redesigned.<br>Status page has been renamed as Scope and Purpose and updated as appropriate.<br>Professional body logos have been reviewed and updated.<br>Standard safety references have been reviewed and updated. – remove if document does not contain safety references.<br>Scientific content remains unchanged. |

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| Whole document. | Document presented in a new format. |
| 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 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.



[#]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.

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

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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

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## Suggested Citation for this Document

Public Health England. (YYYY <tab+enter>). Identification of *Yersinia* species from Faeces. UK Standards for Microbiology Investigations. ID 21 Issue df+. http://www.hpa.org.uk/SMI/pdf.

# Scope of Document

This SMI describes the identification of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* isolated from faeces. The organisms may also be 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 recognized species and 2 subspecies[1]. *Yersinia pestis, Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are associated with human and animal diseases[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 - 0.8 µm by 1-3 µm in size and show bipolar staining (“closed safety-pin appearance”).

All members of the genus grow readily on ordinary media. Their optimum growth temperature is 28-29°C. *Y. pestis* is not fastidious but, after incubation for 24hrs 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 24 hrs, colonies are about 1-2 mm in diameter, grey-white to slightly yellow colour and opaque. *Y. pestis* also grows well in nutrient-rich broth such as brain heart infusion (BHI), trypticase soy or nutrient broth. The cultures in broth can be described as suspended flocculent or crumbly clumps ("stalactites") after 24- 48 hrs of incubation. These clumps are visible at the side and bottom of the tube with the rest of the medium remaining clear[2].

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” whereas *Y. pseudotuberculosis* colonies are smaller, deep red with a sharp border surrounded by a translucent zone. Strains of *Y. enterocolitica* usually are lactose negative, but lactose positive strains exist.

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). Phenotypic characteristics are often temperature dependent and more are expressed by cultures at 22–29°C rather than 35-37°C[4]. They are also negative for urease, lactose fermentation, and indole tests and positive for 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 from faeces are;

***Yersinia enterocolitica***

Cells are coccoid shaped, usually 0.5-0.8 µm by 1-3 µm in size. 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[5].

They are non-spore formers and are motile at room temperature but non-motile at 37 °C. They are facultative anaerobes. 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.

It has been found in faeces, blood or lymph node tissues[3].

***Yersinia pseudotuberculosis***

Their characteristics are similar to that of *Y. enterocolitica.* On CIN agar, colonies of *Y. pseudotuberculosis* are smaller, deep red with a sharp border surrounded by a translucent zone.

## 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.

# Technical Information/Limitations

N/A

# 1 Safety Considerations[6-22]

## Hazard Group 2 organisms

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

## *Yersinia* species isolated from faeces reported to have caused human infection

*Yersinia pseudotuberculosis, Yersinia enterocolitica*

# 3 Identification

## 3.1 Microscopic Appearance

**Gram stain (TP 39 - Staining Procedures)**

Gram negative rods and they may show bipolar staining.

## 3.2 Primary Isolation Media

Cefsulodin, Irgasan, Novobiocin (CIN) 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 “bulls-eye”.

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

## 3.4 Test Procedures

Commercial identification Systems.

## 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 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), 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 remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

**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 use[23].

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*[24] as well as providing epidemiological information regarding *Y. pestis* biotypes. The methods of inactivation used for these pathogenic organisms does not have any influence on the on the MALDI-TOF MS spectra generated. This has also been used to identify and subtype *Yersinia enterocolitica* isolates[25,26].

One of the limitations is the lack of an updated database that includes profiles of all *Yersinia* species and available databases will need to be optimised as well[27].

**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.

PCR has also been used to detect virulence genes of *Yersinia enterocolitica* and *Y. pseudotuberculosis* in human clinical isolates[28].

**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[29,30]

This has been used successfully to discriminate between *Yersinia enterocolitica* strains and will still be useful for surveillance of the sources and transmission routes of sporadic *Yersinia enterocolitica* strains in future[31].

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

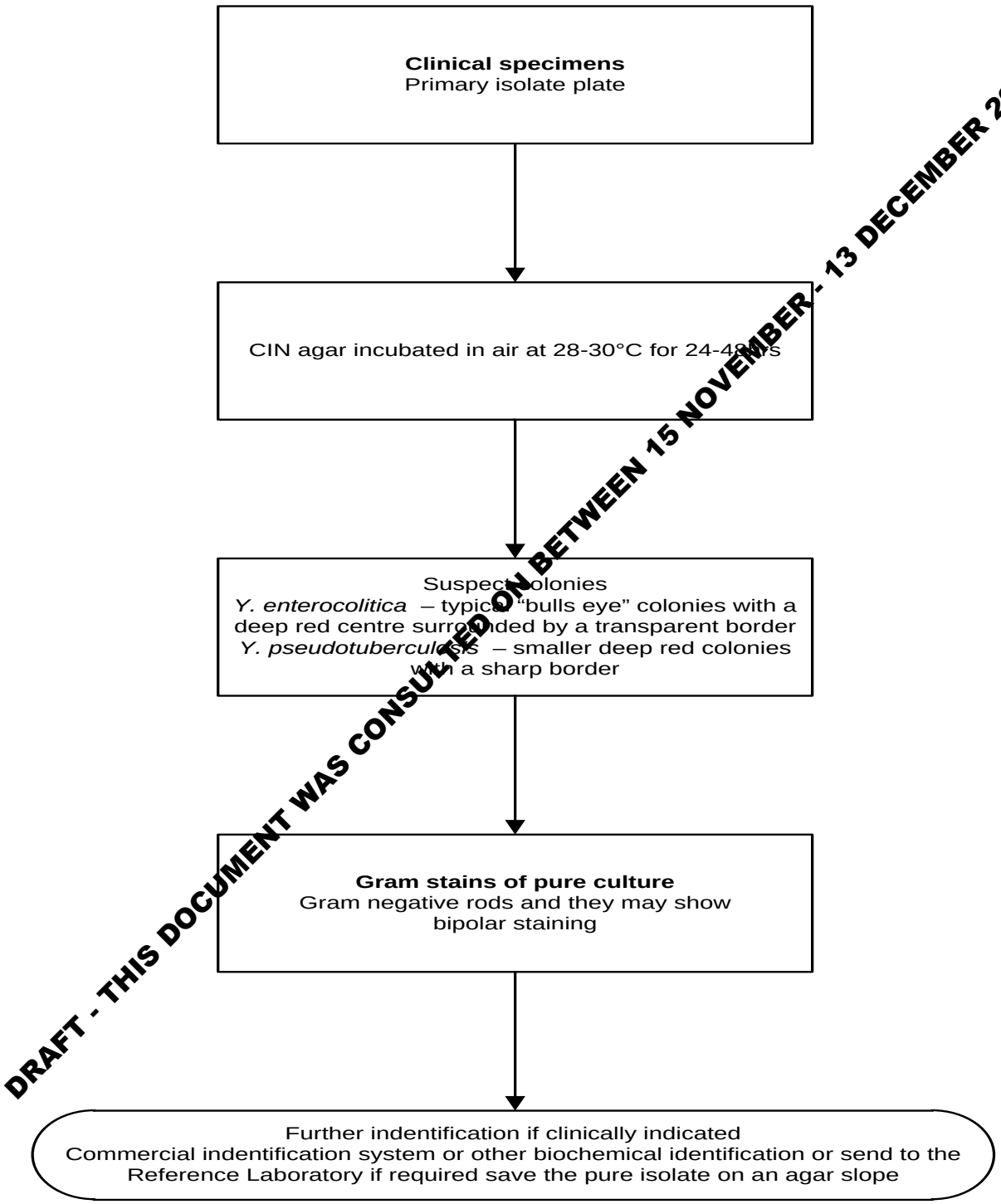
Multiple-Locus Variable number tandem repeat Analysis (MLVA) is a method used to perform molecular typing of particular microorganisms. It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in many different loci in the genome of a variety of organisms. The 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 and discriminate between *Yersinia 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[31].

## 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 from faeces Flowchart






The flowchart is for guidance only.

# 5 Reporting

## 5.1 Presumptive Identification

If appropriate growth characteristics, colonial appearance and Gram's 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 card bears relevant information eg.

- Enterocolitis or mesenteric adenitis
- Septicaemia
- Immunologically-mediated epiphenomena (i.e. erythema nodosum or reactive arthritis)
- Persons receiving blood or blood product transfusion, suffering from iron overload and/or receiving chelation therapy for same (e.g. haemoglobinopathy) with transfusion haemosiderosis or primary haemochromatosis
- Cases associated with farming, veterinary or laboratory work
- Food poisoning
- Investigation of outbreaks

Follow local protocols for reporting to clinician.

## 5.4 CCDC

Refer to local Memorandum of Understanding.

## 5.5 Public Health England[32-37]

Refer to current guidelines on CDSC and COSURV reporting.

## 5.6 Infection Control Team

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

# 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

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

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

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