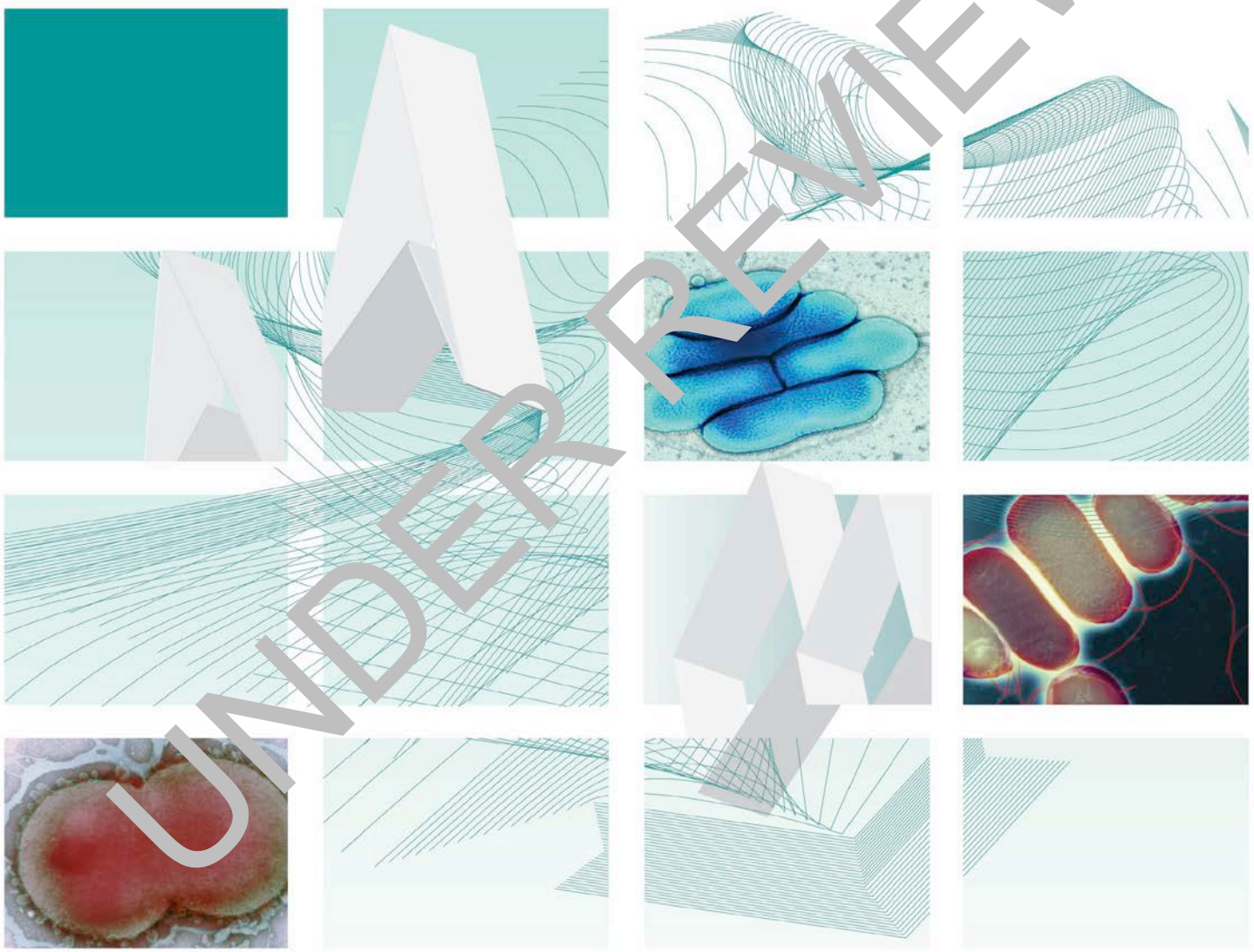
Public Health England

NHS

UK Standards for Microbiology Investigations

Identification of *Bacillus* species



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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:

Contents

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.	9/10.03.14
Issue no. discarded.	2.2
Insert Issue no.	2.3
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. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	8/14.10.11
Issue no. discarded.	2.1
Insert Issue no.	2.2
Section(s) involved	**Amendment**
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.

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 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

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2014). Identification of *Bacillus* species. UK Standards for Microbiology Investigations. ID 9 Issue 2.3. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

This SMI describes the identification of *Bacillus* species. The organisms described in this document are those which may be isolated from clinical material, although not all have been shown to cause human disease.

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The genus *Bacillus* currently comprises in excess of 60 species, commonly found in the environment and as laboratory contaminants[1].

Characteristics

Bacillus species are Gram positive rods often arranged in pairs or chains with rounded or square ends and usually have a single endospore. The endospores are generally oval and are very resistant to adverse conditions. Sporulation is not repressed by exposure to air[2]. *Bacillus* species can be broadly divided into three groups based on the morphology of the spore and sporangium[3]. The groups are:

Group 1 – Gram positive, produce central or terminal ellipsoidal or cylindrical spores that do not distend the sporangium.

Group 2 – Gram variable with ellipsoidal spores and swollen sporangia.

Group 3 – Gram variable, sporangia swollen with terminal or subterminal spores.

Virulent strains of *B. anthracis* produce a characteristic polypeptide capsule, which can be demonstrated by culture on a medium containing 0.7% bicarbonate and is incubated overnight in an atmosphere with a raised CO_2 concentration. Alternatively a small volume of sterile defibrinated horse blood may be inoculated and incubated for 6-18hr. Colonies of capsulate *B. anthracis* appear mucoid and the capsule can be seen by the use of McFadyean's polychrome methylene blue[4]. Avirulent strains may occur which do not produce a capsule or toxin, and these may be misidentified as *Bacillus cereus*.

Many *Bacillus* species are haemolytic, a useful characteristic in differentiating them from *B. anthracis* (which is non-haemolytic). They are aerobic or facultatively anaerobic and most species are motile (a notable exception is *Bacillus anthracis*) by peritrichous flagella. Most species are oxidase positive, which may lead to confusion with *Pseudomonas* species, especially if the *Bacillus* species are poorly stained. They are usually catalase positive and metabolise carbohydrates by fermentation. *B. anthracis* is almost invariably sensitive to penicillin whereas other species are generally resistant[5].

Principles of Identification

Isolates from primary culture on non-selective agar are identified by colonial appearance and the presence or absence of β-haemolysis. On selective agar such as Polymyxin egg yolk mannitol bromothymol blue agar (PEMBA) *B. cereus* (which is mannitol-negative and hydrolyses lecithin) produces characteristic blue colonies with a zone of precipitation. *Bacillus thuringiensis* produces a similar reaction. *B. cereus,*

unlike *B. thuringiensis,* does not produce cuboid or diamond shaped parasporal crystals in cultures on sporulation agar or nutrient agar incubated for at least two days. The crystals are demonstrated with phase contrast microscopy or staining with malachite green. Care must be taken to distinguish *B. cereus* from other organisms, such as *Staphylococcus aureus, Serratia marcescens,* and *Proteus vulgaris* which also grow on PEMBA. These colonies can be differentiated from *B. cereus* by colonial morphology and colour. They also produce an egg yolk clearing reaction in contrast to the precipitate produced by *B. cereus*. Identification is verified by Gram stain, lecithinase activity, motility, penicillin susceptibility and biochemistry. Significant isolates should be referred to the Reference Laboratory for confirmation of identity and toxin testing.

Species differentiation of the genus is complex and, in some instances in a routine laboratory, a combination of Gram stain and colonial appearance may be regarded as sufficient indication of a *Bacillus* species being present in a clinical specimen.

If *B. anthracis* is suspected, specimens should be referred directly to the Reference Laboratory. This organism is described on the HPA website.

Technical Information/Limitations

N/A

1 Safety Considerations[6-22]

Bacillus anthracis is a Hazard Group 3 organism - all work on suspected isolates must be performed in a microbiological safety cabinet in a Containment Level 3 room.

If *B. anthracis* is suspected clinically, refer specimens directly to the Reference Laboratory.

Refer to current guidance on the safe handling of all 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

Bacillus species Reported to Have Caused Human Infection[4]

Group 1 – Gram positive, produce central or terminal ellipsoidal or cylindrical spores which do not distend the sporangium.

Bacillus anthracis

Bacillus cereus

Bacillus megaterium

Bacillus mycoides

Bacillus thuringiensis

Group 2 – Gram variable with ellipsoidal, central or sub-terminal spores and swollen sporangia

Bacillus alvei

Bacillus brevis

Bacillus circulans

Bacillus coagulans

Bacillus licheniformis

Bacillus macerans

Bacillus pumilus

Bacillus subtilis

Group 3 – Gram Variable with Spherical, Terminal or Sub-Terminal Spores and Swollen Sporangia

Bacillus sphaericus.

Other species may rarely be associated with human infection.

3 Identification

3.1 Microscopic Appearance

(TP 39 - Staining Procedures)

Gram stain

Large Gram positive rods (which may have a single endospore). Some species may be Gram variable.

McFadyean stain

Use to stain the capsule of *B. anthracis.*

Giemsa stain

Use to stain the capsule of *B. anthracis*.

Note: capsules are only normally seen if *B anthracis* is growing in blood serum or is present in very fresh tissue samples.

3.2 Primary Isolation Media

Blood agar incubated in air/CO_2 at 35°C-37°C for 24–48hr.

Polymyxin, egg yolk, mannitol, bromothymol blue agar (PEMBA) – optional.

3.3 Colonial Appearance

Colonial appearance varies with species, and a brief description is given here:

Organism	**Haemolysis**	**Characteristics of growth on horse blood agar or PEMBA after incubation at 35°C–7°C for 18-24hr**
B. anthracis	non (may occasionally be weakly haemolytic	Blood agar - Colonies are flat and irregular, 2–5mm in diameter, grey/white in colour with a ground glass appearance.
B. cereus group	β	Blood agar - Colonial appearance is similar to that of *B. anthracis* although *B. cereus* colonies are cream to white and *B. mycoides* are rhizoid or hairy looking. PEMBA - Colonies are crenated, 5mm diameter, turquoise to peacock blue with a zone of egg yolk precipitation.
Other *Bacillus* species	β	Blood agar - Colonies are large (2-7mm) with a frosted-glass appearance, but may become opaque. Colour varies. Some species may produce mucoid or smooth colonies. PEMBA - *B. thuringiensis* forms similar colonies to *B. cereus*

3.4 Test Procedures

Lecithinase production (TP 22 - Nagler Test)

Inoculate an egg yolk agar plate and incubate at 35°C–37°C for 18-24hr, then examine for a zone of egg yolk precipitation. *B. anthracis, B. cereus*, *B. thuringiensis* and *B. mycoides* are positive.

Motility (TP 21 - Motility Test)

All *Bacillus* species are motile with the exception of *B. anthracis* and *B. mycoides.*

Penicillin susceptibility

All *Bacillus* species, with the exception of *B. anthracis,* are generally resistant to penicillin as determined by E-Test.

Crystal formation

Use to differentiate *B. cereus* from *B. thuringiensis.* After growth on sporulation agar or on nutrient agar for at least 48hr, *B. thuringiensis* produces cuboid or diamond shaped parasporal crystals. These are demonstrated with phase contrast microscopy or staining with malachite green.

Summary of test results

	Lecithinase	Motility	Penicillin susceptibility	Crystal formation
Bacillus anthracis	+*	-	S	-
Bacillus cereus	+	+	R	-
Bacillus megaterium	-	+	R	-
Bacillus mycoides	+	-	R	-
Bacillus thuringiensis	+	+	R	+
Bacillus alvei	-	+	R	-
Bacillus brevis	-	+	R	-
Bacillus circulans	-	+	R	-
Bacillus coagulans	-	+	R	-
Bacillus licheniformis	-	+	R	-
Bacillus macerans	-	+	R	-
Bacillus pumilus	-	+	R	-
Bacillus subtilis	-	+	R	-
Bacillus sphaericus	-	+	R	-

* *B. anthracis* may produce narrow lecithinase zones and colony may need to be scraped away to see reaction.

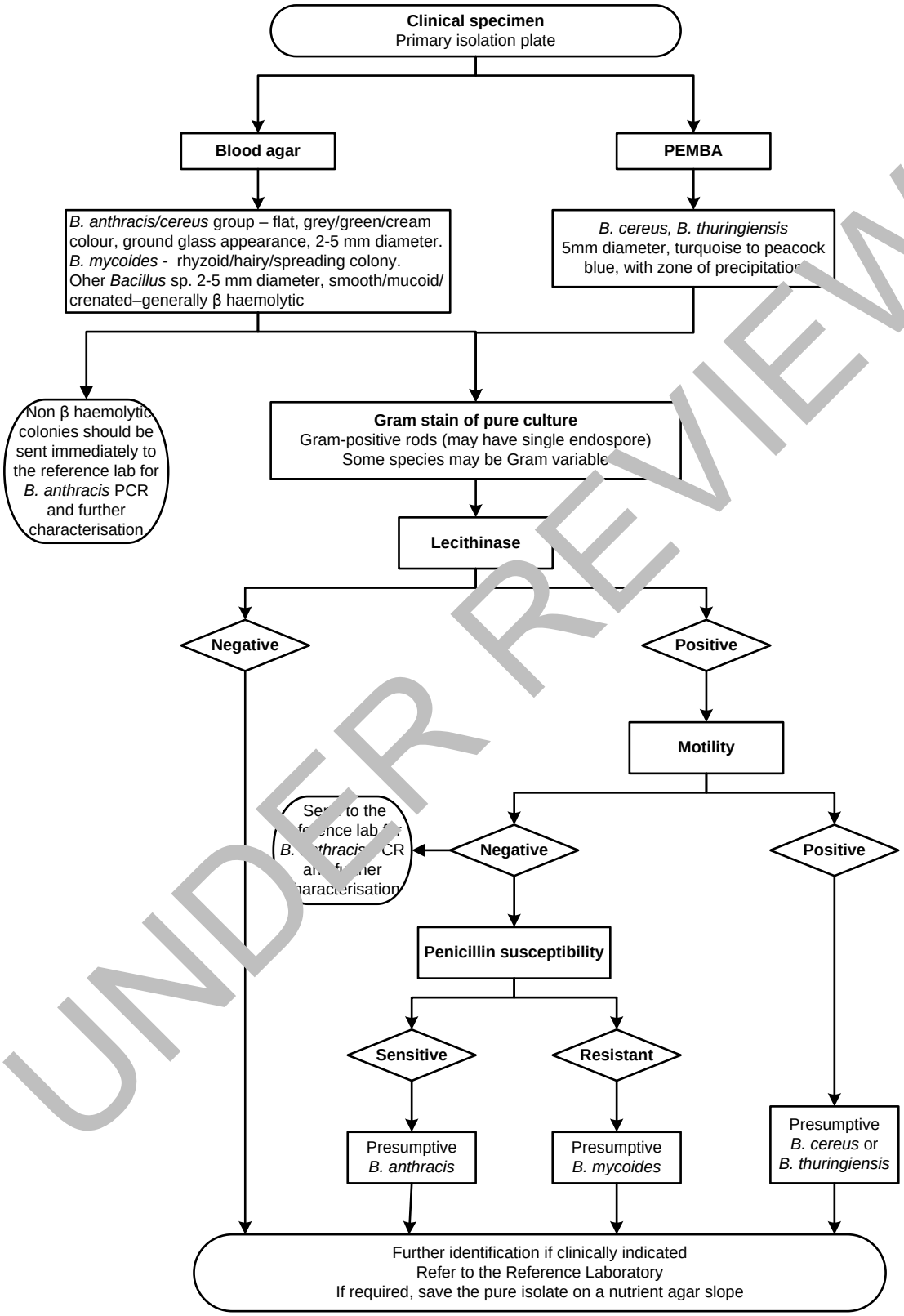
3.5 Further Identification

N/A

3.6 Storage and Referral

Save the pure isolate on a nutrient agar slope for referral to the Reference Laboratory.

4 Identification of *Bacillus* 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

Following lecithinase activity, motility, penicillin susceptibility and commercial identification kit results and/or the Reference Laboratory report.

5.3 Medical Microbiologist

Inform the medical microbiologist of all positive cultures from specimens from normally sterile sites, and of all isolates of presumed and confirmed *Bacillus anthracis.*

According to local protocols, the medical microbiologist should be informed when the request card bears relevant information which suggests anthrax among the differential diagnoses.

- Ulcerating skin lesions with a black eschar
- Fulminating pneumonia (especially with widening of the mediastinum on X-ray) and in outbreaks of the same
- Circumstances predisposing to infection with *B. anthracis,* eg farming, horticulture, veterinary, dockyard, tannery, woollen textile or medical laboratory work

The medical microbiologist should also be informed of other *Bacillus* species (other than *B. anthracis*), presumed or confirmed in accordance with local protocol, when the request form bears relevant additional information for example:

- Penetrating injury, compound fracture or retained foreign body
- Infection of an indwelling medical devices, such as prosthetic valves, pacemaker, CSF shunt or peritoneal or vascular catheter
- Food poisoning
- Investigation of a possible outbreaks

Follow local protocols for reporting to the patient's clinicians.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 Public Health England[23]

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

Inform the relevant infection control team of presumed or confirmed isolates of *B. anthracis.*

6 Referrals

6.1 Reference Laboratory

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to:

Bacillus anthracis

Rare and Imported Pathogens Laboratory
Public Health England
Porton Down
Salisbury
Wiltshire
SP4 OJG
United Kingdom SP5 OJG
Telephone +44 (0) 1980 612100

http://www.hpa.org.uk/cepr/specialpathogens/default.htm

***Bacillus cereus* and other *Bacillus* species**

Foodborne Pathogens Reference Section
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5EQ

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

http://www.hpa.org.uk/ProductsServices/InfectiousDiseases/LaboratoriesAndReferenceFacilities/LaboratoryOfGastrointestinalPathogens/FoodbornePathogensReferenceUnit/

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

England and Wales
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

7 Notification to PHE[23,24] or Equivalent in the Devolved Administrations[25-28]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt-Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland[25,26], Wales[27] and Northern Ireland[28].

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