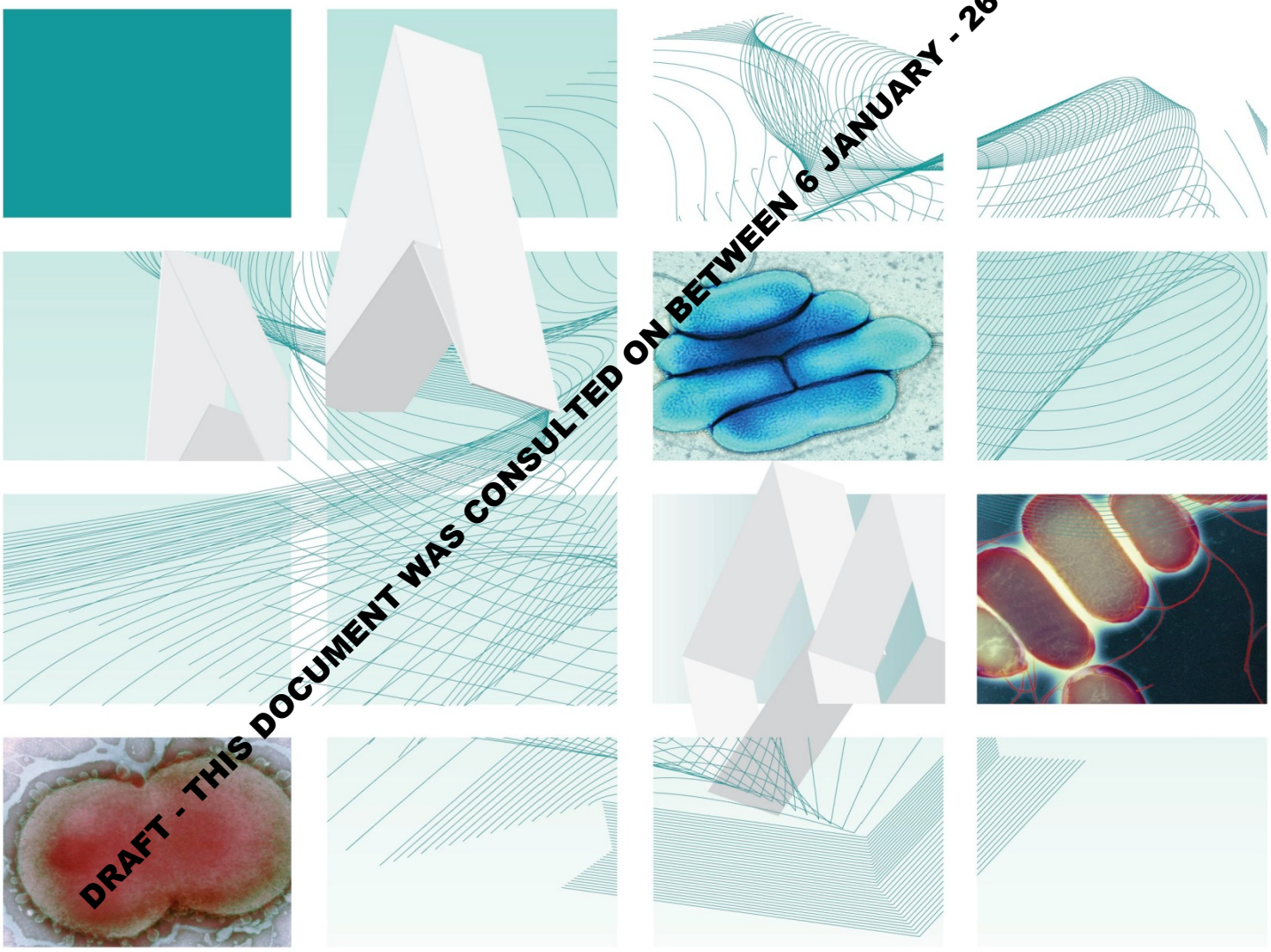
Public Health England

NHS

UK Standards for Microbiology Investigations

Investigation of Skin and Superficial Soft Tissue Infections



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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 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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UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

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.	8/dd.mm.yy <tab+enter>
Issue no. discarded.	5.1
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Section(s) involved	**Amendment**

Amendment No/Date.	7/03.07.12
Issue no. discarded.	5
Insert Issue no.	5.1
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. The term “CE marked leak proof container” is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to the Directive itself EC. Edited for clarity. Reorganisation of [some] text. Minor textual changes.
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also 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. The documents also 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.

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

[#] 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.

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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Scope of Document

Type of Specimen

Skin swab, swab from superficial, non-surgical and surgical wounds, pus

Scope

This SMI describes the processing and bacteriological investigation of skin, superficial, non-surgical and surgical wound swabs, and pus from superficial sites.

It should be noted that many conditions are best diagnosed by submission of a skin biopsy for culture and histopathological examination (refer to B17 Investigation of tissues and biopsies from deep-seated sites and organs). Viruses, such as herpes simplex and Varicella-zoster, as well as non-microbial agents, may also cause skin lesions but are outside the scope of this SMI.

This SMI should be used in conjunction with other SMIs.

Introduction

The skin is colonised by normally non-harmful flora. When the skin is broken as a result of trauma, burns, bites or surgical procedure colonisation with a range of bacteria may occur[1]. Infections of the skin and subcutaneous tissues are caused by a wide range of organisms, however the majority are caused by *Staphylococcus aureus* and β haemolytic streptococci groups A, C and G[2,3].

There is currently no standard classification system for SSTIs; particular organisms are often typically associated with specific clinical conditions, however overlaps in clinical presentation do occur[2,3]. Diagnosis is normally based on clinical presentation. Microbiological cultures may be undertaken to establish the causative organism enabling antibiotic sensitivity testing which is essential to ensure optimal treatment regimens.

Skin Infections[1,3]

Cellulitis and erysipelas[4,5]

Cellulitis and erysipelas are diffuse spreading skin infections excluding cutaneous abscesses, necrotizing fasciitis, septic arthritis and osteomyelitis[3]. Cellulitis involves the deeper layers of the skin and subcutaneous tissues, whereas erysipelas involves the upper dermis and superficial lymphatic system[3].

Cellulitis[6]

Cellulitis is commonly caused by[6,7]:

- β-haemolytic streptococci (including Streptococcus pyogenes)
- *S. aureus*

Wound infections may be caused by a broader range of organisms including:

- *Bacteroides* species
- Anaerobic cocci

- *Bacillus* cereus[8]

Blood culture are not usually done for skin infections (except in the more severe cases) as yields have been reported to be as low as 5-8%[9]. Superficial swabs in the absence of a skin break are often unrewarding; skin biopsies may produce better results but they are not frequently done. Recurrent cellulitis can occur following damage to local venous[10,11] or lymphatic drainage systems.

Haemophilus influenzae cellulitis, particularly of the orbit, occurs in children up to three years of age[12]. Invasive *H. influenzae* infections have become rare following the introduction of *H. influenzae* type B vaccine.

Facial cellulitis due to *Streptococcus pneumoniae* has also been described and occurs mainly in children. Cellulitis due to *S. pneumoniae* may also occur in patients with underlying conditions such as alcoholism, diabetes mellitus, intravenous drug abuse or systemic lupus erythematosus.

Ecthyma gangrenosum

Ecthyma gangrenosum is a focal skin lesion characterised by haemorrhage, necrosis and surrounding erythema. It is usually caused by:

- *P. aeruginosa*
- *Stenotrophomonas maltophilia*
- Haematogenous dissemination of fungal infection (eg *Candida* species and mucoraceous fungi)[13,14]

Similar lesions found in patients who are neutropenic may be due to infection with *Aspergillus* species or *Fusarium* species[15]. Diagnosis is usually based on clinical history and physical examination[7].

Impetigo

Impetigo is a superficial, intra-epidermal infection producing erythematous lesions that may be bullous or nonbullous.

Bullous impetigo is caused by[3,16]:

- *S. aureus*

Nonbullous impetigo is most frequently caused by:

- Lancefield Group A streptococci
- *S. aureus*

Nonbullous impetigo has occasionally been caused by streptococci of Lancefield Groups C and G[17].

Erysipelas

Erysipelas is a rare superficial infection of the skin. It primarily involves the dermis and the most superficial parts of the subcutaneous tissues, with prominent involvement of the superficial lymphatics. It presents as a painful, fiery red, oedematous area of skin, occasionally with small vesicles on the surface[3]. The margins have sharply demarcated, raised borders and the skin surface can appear orange peel like.

Erythrasma

Erythrasma is a common, chronic, superficial skin infection of the stratum corneum caused by *Corynebacterium minutissimum*. It presents with fine, scaly, reddish-brown plaques usually in the axillae and is often misdiagnosed as my mycotic infection[18]. Diagnosis is most often made on clinical grounds rather than by culture.

Superficial mycoses

Superficial mycoses are cutaneous fungal infections that involve the hair or nails or the keratinized layer of the stratum corneum (see B 39 - Investigation of Dermatological Specimens for Superficial Mycosis).

Causative organisms include[19]:

- Dermatophytes
- *Candida* species
- Lipophilic yeasts

Cutaneous *Cryptococcus neoformans* infections can be seen in HIV-infected patients. They present as widespread skin-coloured, dome-shaped, translucent papules. Skin scrapings are the specimens of choice.

Paronychia

Paronychia is a superficial infection of the nail fold occurring as either an acute or chronic condition. Common isolates include[20].

- *S. aureus*
- Lancefield Group A streptococci
- Yeasts
- Anaerobic bacteria
- *H. influenzae*

Folliculitis

Folliculitis is the infection and inflammation of a hair follicle[21,22]. Dome-shaped papules or pustules form. These are each pierced by a hair and surrounded by a rim of erythema.

The condition is usually caused by:

- *S. aureus*

Other possible causes include:

- *P. aeruginosa*
  (can follow exposure in swimming pools or whirlpools)[23-26]
- *Candida* species
  (in patients receiving prolonged antibiotic or corticosteroid treatment)
- *Malassezia furfur*
  (in patients with diabetes or granulocytopenia or receiving corticosteroid treatment)[27]

Necrotising skin and soft tissue infections[6,3,28]

The terminology used for necrotising soft tissue infections is not consistent. Terms may relate to the kind of pathogen, the tissues involved, or the presence or absence of gas in the tissues[29,30].

It is clinically important to recognise these conditions as surgical intervention as well, as antimicrobial therapy is essential. Appropriate specimens are blood, fluid from bullae, and tissue biopsies. Growth from swabs taken from the surface of a lesion tends to be misleading, often yielding mixed cultures of colonising organisms. Mortality rates are high, (30-60%)[30].

Gangrene

There are 4 main types:

Meleney's progressive synergistic gangrene presents as a burrowing lesion or chronic gangrene of the skin following abdominal operations, and results from mixed infections by organisms such as:

- *S. aureus*
- Streptococci
- Enterobacteriaceae
- Pseudomonads
- Anaerobic Gram negative bacilli[31]

Gas gangrene is a necrotising process associated with systemic signs of toxaemia and gas is present in the tissues. It often follows traumatic injuries such as penetrating wounds or crush injuries. Gas gangrene is caused by:

- *Clostridium* species
- *Clostridium perfringens*

These organisms may however colonise a wound without causing disease. Alternatively, they may cause a spreading cellulitis, or extend into the muscle causing myonecrosis[6]. Classical gas gangrene is associated with clinical shock, leakage of serosanguinous fluid, tissue necrosis and presence of gas in the tissues.

Fournier's gangrene applies to the non-sporing anaerobes. These are particularly important causes of infection in the pelvic and scrotal areas, and are common causes of gangrene in ischaemic and diabetic limbs. They often occur in infections mixed with:

- Enterobacteriaceae
- Streptococci
- *Clostridium* species[32]

Spontaneous gangrene occurs either with no apparent relation to trauma or following mild, non-penetrating trauma. It is most commonly seen in patients with colonic carcinoma, leukaemia or neutropaenia. The main causative organisms are[33]:

- *Clostridium perfringens*
- *Clostridium septicum*

Actinomycosis

Actinomycosis is a chronic suppurative infection characterised by abscess formation with the production of "sulphur granules" which mainly consist of micro-colonies of *Actinomyces* species[33]. Usual sites of infection are around the jaw, chest or abdomen. Material should be drained from these abscesses (B 14 - Investigation of Deep-Seated and Organ, Infections and Abscesses) and biopsies taken. Biopsies may reveal the presence of organisms. Most infections are due to *Actinomyces israelii*, Actinomycete-like-organisms and actinomycetes from IUCDs are commonly seen in cervical smears where the clinical significance is doubtful[34].

Necrotising fasciitis[35,36]

Necrotising fasciitis is a serious, infrequently occurring infection primarily affecting the subcutaneous fat and superficial fascia of muscles and often the overlying soft tissues. It is limited by the deep fascia. The infection spreads widely and rapidly due to the absence of internal barriers in the fascia. The infection can be fatal in a very short time. Some cases occur post-operatively or in patients with underlying clinical conditions such as malignancy. Some authorities consider that it exists as two types. Type I is due to infection by a polymicrobial mixture with aerobic and anaerobic organisms (group A streptococci, anaerobes, *S. aureus* and members of the Enterobacteriaceae). Type II (haemolytic streptococcal gangrene) is due to infection with group A streptococci[37].

Myositis [38]

Myositis is an inflammation of the muscle which may be caused by bacterial, fungal or parasitic infection as well as non-infective conditions such as autoimmune disease or genetic disorders etc. Localised infection is usually due to bacteria or fungi, whereas viral and parasitic infections tend to be more diffuse. Necrotising myositis rapidly involves the entire muscle bed and may spread to adjacent tissues. Both polymicrobial and unimicrobial forms may be seen.

Pyomyositis is a purulent infection of skeletal muscle and occurs more commonly in tropical countries. It usually presents as a single abscess but multiple abscesses do occur. Most patients have no underlying predisposing condition, previous trauma accounting for only 25% of cases. The majority of cases are due to *S. aureus*. More rarely, fungi and viruses may cause infection in patients who are immunocompromised.

Mycetoma[39-42]

Mycetoma occurs in people living in tropical and sub-tropical climates, usually following a puncture wound. The condition results from a chronic destructive process involving the skin, subcutaneous tissue, muscle and bone. Granulation tissue develops with chronic inflammation and fibrosis and is characterised by a draining sinus and the presence of granules. A mycetoma can form anywhere in the body, but is more common in the lower extremities. Formation in the foot is called "Madura foot".

Mycetomata are divided into two categories based on the aetiological agents involved; actinomycetoma caused by aerobic actinomycetes and eumycetoma caused by mould. There are at least twenty moulds that may cause this condition; the species involved are often associated with distinct geographical areas.

Ninety five percent of the cases are caused by:

Eumycetoma:

- *Acremonium* species
- *Leptosphaeria senegalensis*
- *Madurella grisea*
- *M. mycetomatis*
- *Scedosporium* (*Pseudallescheria*) *apiospermum.*
- *Pyrenochaeta romeroi*
- *Curvularia* species
- *Exophiala jeanselmei*
- *Phialophora verrucosa*

Actinomycetoma:

- *Actinomadura* species
- *Nocardia* species
- *Streptomyces* species
- *Maduralla* species

Organisms are found in tissue sinuses as aggregates of filaments. These are called granules but differ from the sulphur granules of actinomycosis in that they do not have the characteristic clubbed peripheral fringe. Granules obtained directly from tissue will ensure the best cultural recovery of the causative organism because granules found in sinus discharge contain only dead organisms. Surgical biopsy to obtain material for culture is important for diagnosis, especially if sinus discharge is culture-negative for aerobic actinomycetes or is contaminated by other bacteria.

Carbuncles, Furuncles, Cutaneous, Soft Tissue and other Abscesses[3]

Carbuncles are deep and extensive subcutaneous abscesses involving several hair follicles and sebaceous glands.

Furuncles are abscesses which begin in hair follicles as firm, tender, red nodules that become painful and fluctuant. Both carbuncles and foruncles are usually caused by:

- *S. aureus*

Cutaneous abscesses are usually painful, tender, fluctuant erythematous nodules often with a pustule on top. In some cases they are associated with extensive cellulitis, lymphangitis, lymphadenitis and fever. They are caused by a variety of organisms. The location of an abscess often determines the flora likely to be isolated. Thus *S. aureus* is most often isolated from cutaneous abscesses of the axillae, the extremities and the trunk, whereas cutaneous abscesses involving the vulva and buttocks may yield faecal or urogenital mucosal flora.

Burkholderia pseudomallei causes melioidosis, but is rare in the UK. The disease may present in a variety of forms with skin lesions and/or cellulitis. Diagnosis is

made by blood culture, serology or culture of pus (refer to B 37 – Investigation of Blood Culture (for Organisms other than *Mycobacterium* species)).

Abscesses in Intravenous Drug Users

Cutaneous abscesses frequently occur as a complication of injecting drug use. They commonly result from the use of non-sterile solutions in which the drug is dissolved or from lubrication of the needle using saliva.

Bacterial isolates include[43]:

- Oral streptococci
- *Streptococcus anginosus* group
- *Fusobacterium nucleatum*
- *Prevotella species*
- *Porphyromonas* species
- *Staphylococcus aureus*
- *Clostridium* species
- *Bacillus anthracis (this is a rare but severe infection that can occur by injecting heroin contaminated with anthrax)*

Scalp Abscess

Scalp abscesses are a recognised complication of electronic monitoring with fetal scalp electrodes during labour. A localised collection of pus surrounded by inflamed tissue forms where the electrodes are inserted. Anaerobes are most commonly isolated, probably as a result of contamination with vaginal organisms during delivery.

Polymicrobial infections also occur, involving[44]:

- Anaerobes
- β-haemolytic streptococci
- *S. aureus*
- Enterobacteriaceae
- Enterococci
- Coagulase-negative staphylococci

Kerion is a pustular folliculitis of adjacent hair follicles, creating dense inflamed areas of the scalp, and is caused by dermatophytes (refer to B 39 – Investigation of Dermatological Specimens for Superficial Mycoses). Secondary bacterial infection may occur.

Ulcers

A skin ulcer is a lesion of the skin with loss of the skin integrity, which can extend from the epidermis down to deeper layers. There are various types of ulcers with different etiology: pressure sores, diabetic foot ulcers, venous leg ulcers, arterial ulcers. All ulcers are invariably colonised by a polymicrobial flora and microbiology samples should be taken only if a clinical diagnosis of infection has been made[45,46]. When

swabs are taken from infected ulcers, they should be taken after cleansing and debridement: this aims at eliminating part of the superficial colonising flora[46]. Sometimes chronic ulcer swabs are taken to identify the cause of underlying bone infections: in this scenario invasive bone biopsy specimens would be preferable, but ulcer swabs (after cleansing and debridement) are often taken in real practice but the results need careful interpretation[47].

Genital ulcers can have a different etiology and are dealt with elsewhere (see B 28 - Investigation of Genital Tract and Associated Specimens).

Swabs from chronic non-healing ulcers or skin lesions with one of the following risk factors reported should be tested for *Corynebacterium* species:

- travel abroad to high risk area within the last 10 days
- contact with someone who has been to a high risk area within the last 10 days
- the patient works in a clinical microbiology laboratory, or similar occupation, where Corynebacterium species may be handled

Burns[48]

Patients suffering from severe burns are at a higher risk of both local and systemic infection; sepsis is an important cause of mortality in this group of patients[48].

Organisms encountered include[48-50]:

- *Staphylococcus aureus*
- β-haemolytic streptococci
- Pseudomonads, especially *Pseudomonas aeruginosa*
- *Acinetobacter* species
- *Bacillus* species
- Enterobacteriaceae
- Filamentous fungi eg: *Fusarium* species *and Aspergillus* species
- *Candida albicans,* non- *albicans Candida* species and other yeasts
- Coagulase-negative staphylococci

Gram negative organisms cause the most severe infections; fungal infections on the other hand can spread quickly, but are more easily treated, although a definitive diagnosis is or difficult to obtain[48].

Bite Wounds and Contact with Animals[3,51]

Bite wounds

Bite wounds can become contaminated by oral flora and normal human skin flora. Most bites are due to cats and dogs, but some are due to other pets (including reptiles, rodents and birds), domesticated animals (including horses, sheep etc) wild animals or other humans[3,51]. Organisms most commonly isolated include[3,52,53]:

- *Pasteurella multocida*
- *S. aureus*

- α-haemolytic streptococci
- Anaerobes (including *Bacteriodes* species and Fusobacteria)
- *Capnocytophaga canimorsus* (formerly known as DF-2)
- *Eikenella corrodens*
- *Haemophilus* species
- Coagulase negative staphylococci
- *Streptobacillus moniliformis*
- *S. intermedius*
- Anaerobes (including Fusobacterium, Porphyromonas, Prerevotella etc)

Capnocytophaga canimorsus is associated with dog bites and causes septicaemia, particularly in patients who are asplenia or underlying hepatic disease. This organism is usually isolated only from blood cultures.

Streptobacillus moniliformis is associated with rat bites and diagnosis is confirmed by culturing the organism from blood or joint fluid.

Other unusual organisms may be isolated including *Weeksella zoohelcum*, *Actinobacillus* species and *Neisseria canis*.

Insect bites are often associated with secondary Lancefield Group A streptococcus and *S. aureus* infection.

Contact with Animals

Erysipeloid

Erysipeloid is an uncommon nonsuppurative cellulitis due to Erysipelothrix rhusiopathiae[21]. It is an occupational disease of fishermen, fish handlers, butchers and abattoir workers. It affects the hands and fingers causing lesions which present as painful purplish areas of inflammation with erythematous advancing edges.

Aeromonas and non-cholera Vibrio species

Aeromonas and non-cholera *Vibrio* species are predominantly isolated from traumatic water-related wounds or lacerations received whilst swimming in fresh or salt water[54], from other environmentally contaminated wounds or from fishing or shellfish inflicted injuries[55,56]. Aeromonas infection may also follow the therapeutic use of leeches[57,58]. Water-related injuries can be polymicrobial involving environmental Gram negative organisms such as *Edwardsiella tarda* and pseudomonads[59].

Bacillus anthracis

Bacillus anthracis is the causative agent of anthrax which appears clinically in one of two forms, cutaneous (skin) anthrax or inhalation anthrax. Following the deliberate release of B. anthracis in the USA in 2001, there is an increased awareness of the release of this and other organisms which may pose a biological threat[60]. Cutaneous anthrax occurs through inoculation of spores to the skin or by contamination of abrasions. Skin lesions called malignant pustules develop, which are characteristic ulcers with a black centre[61]. They are rarely painful, but if untreated the infection can spread to cause septicaemia. If untreated, the disease can be fatal in 5% of cases, but with antibiotic treatment recovery is usual. Cutaneous infection with *B. anthracis* can

occur in industrial workers who use materials of animal origin eg wool, leather, bristles and fur or in the agricultural workplace eg farmers, husbandmen, butchers and vets. In rare cases *B. anthracis* has been transmitted via insect bites[62].

Other Skin Infections[3]

Skin infections may also be caused by the following:

- *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* can cause cutaneous diphtheria[18]. For more information refer to ID 2: Identification of *Corynebacterium* species)[63,64].
- *Leishmania* species can cause the skin disease oriental sores, chronic skin granulomas or ulcerating lesions[65]. Cutaneous leishmaniasis is most commonly seen in South America, the Far East and Ethiopia. Diagnosis is made by demonstrating the parasite in stained impression smears and tissue sections. Detection by nucleic acid amplification techniques and by culture is also available in reference centres[66]. Leishmania speciation is a guide to appropriate therapy and to prognosis.
- MRSA may colonise and or infect wounds and soft tissue[67]. Newly emerging community (mecIV) MRSA with virulence factors such as Panton-Valentine leukocidin (PVL) or Scalded Skin toxin (SST) are causing highly contagious infections (eg follicultis) in healthy children and young adults[68,69]. Infections are often spread through poor hygiene[70]. Panton-Valentine Leukocidin (PVL) is a toxin which is capable of destroying white blood cells[69]. Scalded skin syndrome (Lyell's syndrome in older children; Ritter's syndrome in infants) is caused by *S. aureus* phage types group II and 71[71].
- *Mycobacterium* species can cause cutaneous infections[72]. These may signify a disseminated systemic infection or may represent a local infection by a non-tuberculous *Mycobacterium* (see B 40 - Investigation of Specimens for *Mycobacterium* species).
- Rapid growing Mycobacterial strains such as *M. chelonae* and *M. fortuitum* have also been isolated from superficial skin infections[73]. *M. chelonae* has been shown to be associated with tattoo related infections.
- Sporothrix schenkii causes sporotrichosis[74]. Cutaneous sporotrichosis is acquired by contamination with soil, sphagnum moss or other vegetable matter and develops at the site of inoculation to form a primary lesion with lymphatic spread (see B 39 - Investigation of Dermatological Specimens for Superficial Mycoses). It is more common in warmer climates.
- Cutaneous salmonellosis and listeriosis may also occur in veterinarians and farmers, typically on the arms, following assisted delivery of farm animals, usually cattle infected in utero[75,76]. Cutaneous listeriosis in a patient with AIDS has also been reported[77].
- *Yersinia enterocolitica* can cause cutaneous infections[78].
- *Bacillus anthracis* infection had been associated with the production of drums using animal hides[79].

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedure

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers[80,81]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU In vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible 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”.

Anaerobic Plate Incubation

The recommended incubation time for anaerobic plates is 48 hours. However some anaerobic bacteria such as certain species of *Actinomyces* require longer incubation (7 days) and will not be detected if plates are examined sooner.

Rapid methods

To reduce turnaround times, rapid identification and sensitivity tests may be performed in conjunction with routine methods where appropriate. A variety of rapid identification and sensitivity methods have been evaluated; these include molecular techniques and the Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)[82,83]. It is important to ensure that fresh cultures of pure single isolates are tested to avoid reporting misleading results.

Laboratories should follow manufacturers’ instructions and all rapid tests must be validated and be shown to be fit for purpose prior to use.

1 Safety Considerations[80,81,84-98]

1.1 Specimen Collection[80,81]

Use aseptic technique.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[80,81,84-98]

Containment Level 2.

If infection with a Hazard Group 3 organism, e.g. *Bacillus anthracis* (cutaneous anthrax is rare but needs to be recognised as a possibility in certain settings such as exposure to animal hides, injection of contaminated heroin in IVDUs and bioterrorist events such as the dissemination of spores in letters that took place in the USA in 2001), all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[90].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen Collection

2.1 Type of Specimens

Skin swab, swab from superficial wound, pus

2.2 Optimal Time and Method of Collection[99]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[99].

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium[100-104].

Samples of pus/exudate, if present, are preferred to swabs (see B 14 – Investigation of Deep-Seated and Organ, Infections and Abscesses). If only a minute amount of pus or exudate is available it is preferable to send a pus/exudate swab in transport medium to minimise the risk of desiccation during transport.

Sample a representative part of the lesion. Swabbing dry crusted areas is unlikely to yield the causative pathogen.

If specimens are taken from ulcers, the debris on the ulcer should be removed and the ulcer should be cleaned with saline. A biopsy or, preferably, a needle aspiration of the edge of the wound should then be taken[105].

A less invasive irrigation-aspiration method may be preferred. Place the tip of a small needleless syringe under the ulcer margin and irrigate gently with at least 1mL sterile

0.85% NaCl without preservative. After massaging the ulcer margin, repeat the irrigation with a further 1mL sterile saline. Massage the ulcer margin again, aspirate approximately 0.25mL of the fluid and place in a CE marked leak proof container[106].

Fungal specimens for dermatophytes: See B 39 - Investigation of Dermatological Specimens for Superficial Mycoses.

2.3 Adequate Quantity and Appropriate Number of Specimens[99]

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen Transport and Storage[80,81]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[99].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[99].

4 Specimen Processing/Procedure[80,81]

4.1 Test Selection

N/A

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment

N/A

4.3.2 Specimen processing

See Q 5 - Inoculation of Culture Media for Bacteriology.

4.4 Microscopy

4.4.1 Standard

Gram stain is not normally required.

4.4.2 Supplementary

See B 40 - Investigation of Specimens for *Mycobacterium* species, and TP 39 - Staining Procedures.

4.5 Culture and Investigation

Inoculate each agar plate using a sterile pipette (Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
All conditions	Swabs Pus	Blood agar	35-37	5 -10% CO_2	40-48hr	daily	Any organism including: Lancefield Groups A, C and G streptococci *Pasteurella* species *S. aureus* *Vibrio* species *Aeromonas* species
	Pus	CLED/ MacConkey agar	35-37	Air	18-24hr	48hr	Any organism including Enterobacteriaceae Pseudomonads
		Selective anaerobe agar with metronidazole 5 µg disc	35-37	Anaerobic	5d	≥40hr and at 5 d	Anaerobes
		Fastidious anaerobic, cooked meat broth or equivalent Subculture to BA if evidence of growth (≥40hr), or at day 5	35-37 35-37	Air 5 -10% CO_2	5d 40-48hr	N/A daily	Any organism
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
All wound swabs eg chronic ulcers, traumatic wounds	Swabs	Selective anaerobe agar with metronidazole 5µg disc	35-37	Anaerobic	5 d	≥40hr+ and at 5 d	Anaerobes
Cellulitis in children Human bites	Swabs Pus	Chocolate agar †	35-37	5-10% CO_2	40-48hr	daily	Fastidious organisms *Haemophilus* species
Burns Swabs from	Swabs Pus	Sabouraud agar	28-30	Air	14 d	daily	Yeast Mould

dirty sites Patients who are Immunocompromised Diabetic patient Intertrigo Paronychia							
Suspected cutaneous diphtheria Foreign travel with <10 d Non-healing ulcers	Swabs Pus	Hoyle's tellurite agar	35-37	Air	40-48hr	daily	*C. diphtheriae* *C. ulcerans*
Clinical details/ conditions	**Specimen**	**Optional media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Diabetic wounds	Swabs Pus	MacConkey/ CLED	35-37	Air	18-24hr	18hr	Enterobacteriaceae Pseudomonads
Swabs from dirty sites	Swabs Pus	Staph /strep selective agar or MSA	35-37	Air	40-48hr	daily	*S. aureus* Lancefield Groups A, C and G streptococci
Other organisms for consideration: Dermatophytes (B 39 - Investigation of Dermatological Specimens for Superficial Mycosis) and *Mycobacterium* species (B 40 - Investigation of Specimens for *Mycobacterium* species)							
[+] Some anaerobic bacteria such as certain species of Actinomyces require longer incubation (7 days) and will not be detected if plates are examined sooner. † Either bacitracin 10 unit disc or bacitracin - containing agar may be used.							

4.5.2 Supplementary investigations

Toxigenicity testing of *C. diphtheriae*

See B 40 - Investigation of Specimens for *Mycobacterium* species.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Anaerobes	"anaerobes" level
Bacillus species	species level exclude anthrax
β-haemolytic streptococci	Lancefield Group level
Coagulase negative staphylococci	"coagulase negative" level
C. diphtheriae	species level and urgent (same-day) toxigenicity test
C. minutissimum	species level
C. ulcerans	species level

E. corrodens	species level
Enterobacteriaceae	"coliforms" level
E. rhusiopathiae	species level
Haemophilus	species level
Pasteurella	species level
Pseudomonads	“pseudomonads" level
S. aureus	species level (consider Panton-Valentine leukocidin (PVL) and toxin testing if appropriate clinical details)
S. pneumoniae	species level
Yeasts	"yeasts" level
Vibrio	species level
Aeromonas	species level
Dermatophytes	B 39 - Investigation of Dermatological Specimens for Superficial Mycosis

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: All work on suspected isolates of *C. diphtheriae* which is likely to generate aerosols must be performed in a safety cabinet[8].

A medical microbiologist must be informed of all suspected isolates of *C. diphtheriae* as soon as possible (same-day toxigenicity testing is available from the reference laboratory).

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

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

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.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting Procedure

5.1 Microscopy

Standard

Gram stain (not usually required)

Report on WBCs and organisms detected.

Supplementary

For the reporting of microscopy for *Mycobacterium* species refer to B 40 – Investigation of Specimens for *Mycobacterium* species.

5.1.1 Microscopy reporting time

Urgent microscopy should be released immediately, following local policy.

Written or computer generated reports should follow preliminary/verbal reports within 24-72hrs.

5.2 Culture

Following results should be reported:

- clinically significant organisms isolated
- other growth
- absence of growth

5.2.1 Culture reporting time

Clinically urgent results should be telephoned or sent electronically or according to local protocols

Final written or computer generated reports should follow preliminary/verbal reports on the same day as confirmation where possible, and within a 24 - 72hr.

5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[107,108] or Equivalent in the Devolved Administrations[109-112]

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.

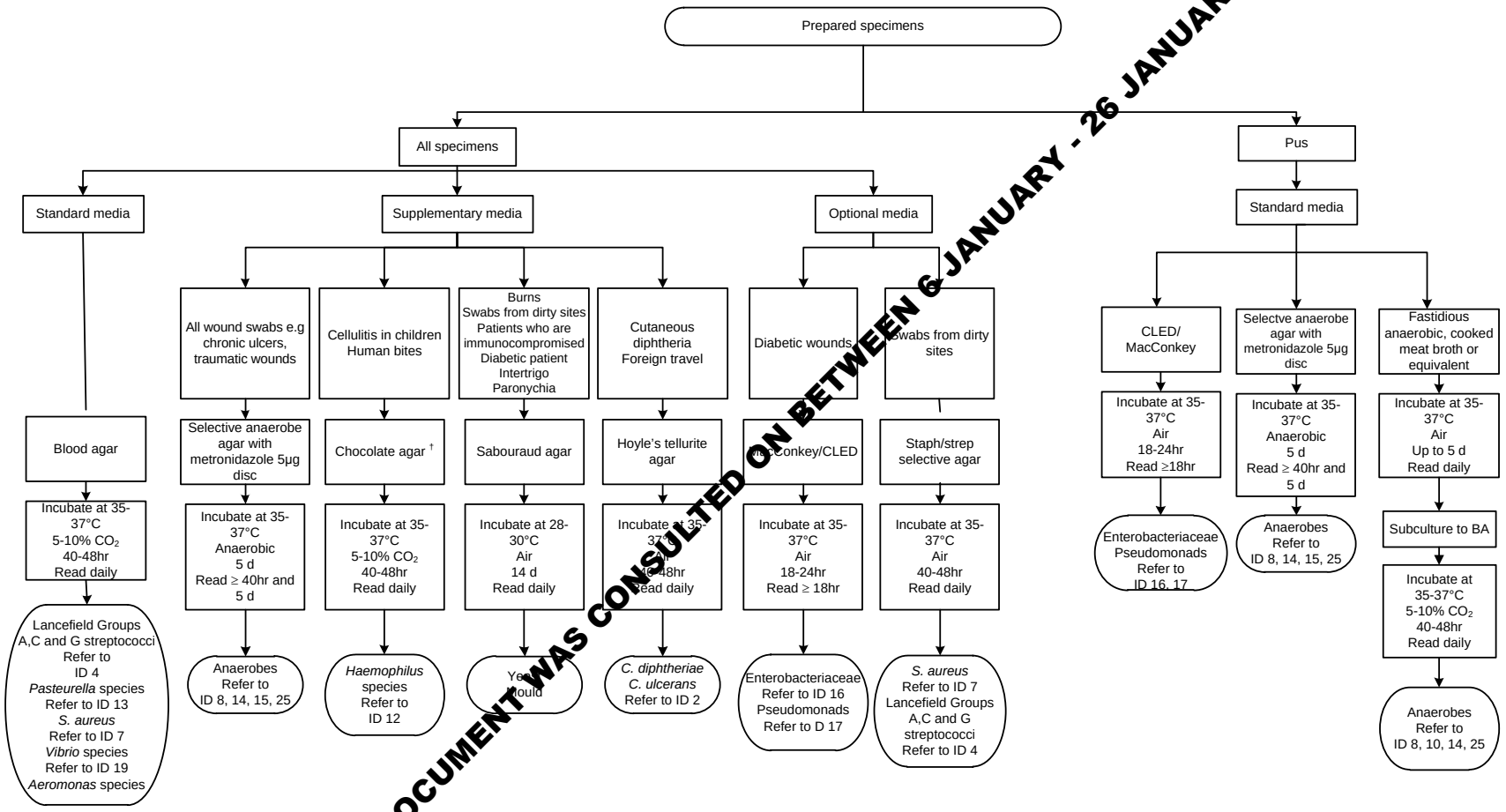
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local 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[109,110], Wales[111] and Northern Ireland[112].

Appendix: Investigation of Skin and Superficial Soft Tissue Infections



† Either bacitracin 10 unit disc or bacitracin - containing agar may be used

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