**SOP: INVESTIGATION OF SKIN, SUPERFICIAL AND NON-SURGICAL WOUND SWABS**

**Purpose of the Examination**

This SOP describes the processing and bacteriological investigation ofskin,superficial and non-surgical wound swabs. This SOP should be used in conjunction with other SOPs.

However, it should be noted that many conditions are best diagnosed by submission of a skin biopsy for culture and histopathological examination. 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 SOP.

Infections of the skin and subcutaneous tissues are caused by a wide range of organisms. Organisms isolated from a clinically infected wound may be clinically significant, but this decision needs to be made in conjunction with clinical details. Examination of biopsies might be more effective for diagnosis than swabs. Commonly isolated organisms include:

* *Staphylococcus aureus*
* Lancefield groups A, B, C and G streptococci
* *Bacteroides* species
* *Clostridium* species
* Anaerobic cocci
* Coagulase negative staphylococci
* *Corynebacterium* species
* Enterobacteriaceae
* Pseudomonads

Organisms isolated from a clinically infected wound may be clinically significant, although they must be carefully assessed for their true clinical significance. Particular organisms are often typically associated with specific clinical conditions, as described below.

**Cellulitis**

Cellulitis is a diffuse spreading infection involving the loose connective tissue of the deeper layers of the skin and subcutaneous tissues. Blood culture is the investigation of choice (see MB-BS-37 – Investigation of blood cultures for organisms other than *Mycobacterium* species) and superficial swabs in the absence of a skin break are unrewarding. Recurrent cellulitis can occur following damage to local venous or lymphatic drainage systems.

Cellulitis is characterised by local pain, tenderness, erythema and oedema. The margins of the infected areas are ill defined, being neither elevated nor sharply demarcated. The most common causative organisms are β-haemolytic streptococci and *Staphylococcus aureus*.

*Haemophilus influenzae* cellulitis, particularly of the orbit, occurs in children up to three years of age. 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.

Cellulitis around wound infections is commonly caused by:

* β-haemolytic streptococci
* *S. aureus*
* *Bacteroides* species
* Anaerobic cocci

**Bite Wounds**

Bite wounds in humans and animals can become contaminated by oral flora. Organisms most commonly isolated include:

* *Pasteurella multocida*
* *S. aureus*
* α-haemolytic streptococci
* Anaerobes
* DF-2 (*Capnocytophaga canimorsus*)
* *Eikenella corrodens*
* *Haemophilus* species
* Coagulase negative staphylococci
* *Streptobacillus moniliformis*
* *S.* *intermedius*

*Capnocytophaga canimorsus* is associated with dog bites and causes septicaemia, particularly in patients who are splenectomised. This organism is only usually isolated 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 *Bergeyella zoohelcum*, *Actinobacillus* species and *Neisseria canis*.

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

**Burns**

Burns sepsis is a potential cause of death in patients suffering from burns. Organisms encountered include:

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

**Impetigo**

Impetigo is a superficial, intra-epidermal infection producing erythematous lesions that may be bullous or non-bullous. Bullous impetigo is caused by *S. aureus*. Non-bullous impetigo is most frequently caused by Lancefield Group A streptococci or *S. aureus* or a combination of both. Lesions of bullous impetigo begin as vesicles and evolve into groups of superficial flaccid bullae with little or no surrounding erythema and they rupture easily. Lesions of non-bullous impetigo begin as small erythematous papules which form vesicles that develop into pustules and then rupture. Non-bullous impetigo has occasionally been caused by streptococci of Lancefield Groups C and G.

**Ulcers**

Ulcers of the skin are most often due to vascular insufficiency from venous or arterial disease, pressure (decubitus ulcers or bedsores), neuropathic changes or some combination of these. Ulcerated skin lesions may also result from the various collagen-vascular diseases, e.g. rheumatoid arthritis, and may complicate other disorders such as inflammatory bowel disease. In clinical practice the most common encountered type are chronic leg ulcers relating to venous insufficiency.

Precise diagnosis of the aetiology of any ulcer is important but may not be rigorously practised by attending health care workers. This may affect interpretation of the biomedical literature and of microbiological results, and may adversely affect management and outcome for the individual.

All breaches of the integument will regularly become colonised (or infected) with bacteria. Bacteria may be detected by culture, or by nucleic acid amplification techniques. However, the clinical significance of such findings depends heavily on the precise nature of the lesions, the clinical situation prevailing at the time of sampling (stability, chronicity, presence of local and systemic signs and symptoms of infection), and sampling methodology.

Swab cultures from stable, chronic venous leg ulcers, without signs or symptoms of infection, are of questionable clinical value as opposed to biopsies of material from the depth of the ulcer, or aspirates of the leading edge of any cellulitic reaction. In clinical practice superficial swabs are most likely to be received.

When infections are complicated by the involvement of soft tissue and bone, isolates from superficial swabs taken from ulcers may correlate poorly with cultures of specimens taken by other more invasive means. Such specimens are biopsies and excised tissues, surgically obtained curettage, and aspirates from abscesses (see MB-BS-14 – Investigation of abscesses and post-operative wound and deep-seated infections, and MB-BS 17 – Investigation of Tissues and Biopsies). Sampling by irrigation aspiration rather than biopsy has been recommended, and results correlate well with responses to clinical measures.

In common with other situations, it is likely that the role of anaerobic bacteria (especially anaerobic cocci and Gram negative rods) and of synergically pathogenic bacterial populations (featuring aerobic and anaerobic bacteria) may have been under-appreciated as causes of clinically-evident infection arising from ulcerated skin lesions. Anaerobic infection may be associated with foul smelling odour or discharge and evidence of tissue necrosis.

Tropical ulcers and chronic non-healing lesions may occur on previously healthy skin, often after trauma. Causative organisms include anaerobes.

Genital ulcers may result from trauma or from a sexually transmitted infection. Traumatic ulcers usually heal rapidly and are associated with genital tract commensal organisms or with *S. aureus* or β-haemolytic streptococci. Ulcers caused by sexually transmitted organisms tend to persist and may be caused by herpes simplex virus (usually type II) or by a variety of bacterial agents, notably *Treponema pallidum* in the UK.

Ulcerative skin lesions may be caused by parapoxviruses such as orf virus. These are acquired through skin abrasions after contact with infected domesticated animals, including sheep, goats and cattle.

Diabetic ulcers - foot infection in patients who are diabetic is both a common and potentially disastrous complication that can progress rapidly to irreversible septic gangrene necessitating amputation of the foot. *Corynebacterium* species, streptococci, methicillin susceptible and resistant staphylococci, pseudomonads, *Enterobacter aerogenes*, *Bacteroides fragilis*, *Fusobacterium* species and *Prevotella bivia* are among organisms that have been isolated from limb threatening diabetic foot ulcers.

**Principle and Method of the procedure used for examinations**

This process is principally a culture procedure used to isolate microbes. Isolates will be subjected to identification and, if deemed pathogenic, susceptibility testing.

**Performance characteristics**

As with all culture-based techniques performance will vary depending on sample collection. Samples should be collected before antimicrobial therapy is initiated and samples should be processed as soon as possible. Sample collection should minimize sample contamination as much as possible.

**Type of Sample**

* For safety considerations refer to Section 8.
* Skin swab, swab from superficial wound, and swab from non-surgical wound are the specimens of choice.
* Specimens should be collected before antimicrobial therapy where possible.
* Unless otherwise stated, swabs for bacterial and fungal culture should be received in appropriate transport medium.
* Samples of pus/exudate are preferred to swabs (see MB-BS 14 – Investigation of Abscesses and Deep Wound Infections). 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.
* If specimens are taken from ulcers, a biopsy or, preferably, a needle aspiration of the edge of the wound should then be taken.
* Fungal specimens for dermatophytes: See MB-BS-39 – Investigation of Dermatological Specimens for Superficial Mycosis.
* Numbers and frequency of specimen collection are dependent on clinical condition of patient.
* Specimens should be transported and processed as soon as possible.
* If processing is delayed, refrigeration is preferable to storage at ambient temperature.
* Biopsies should be received in a CE marked leak proof container in a sealed plastic bag with a small amount of sterile normal saline to prevent desiccation.

**Patient Preparation**

See *COLLECTION OF MICROBIOLOGICAL SPECIMENS POLICY* on the intranet for information on this topic.

**Type of container and additives**

Swabs are charcoal swabs in Amies transport medium.

If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48h are undesirable.

**Required Equipment and Reagents**

Standard microbiology laboratory equipment, media and other consumables are required. For media selections see below.

**Environmental and Safety Controls**

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| **COSHH ASSESSMENT** |
| COSHH Reference No. MI-COSHH-13 |
| Significant Risks: Low risk associated with contact with body fluids and reagentsIf a category 3 pathogen is suspected all manipulations must be carried out under Containment Level 3 |
| P.P.E.   |

* Use aseptic technique.
* Collect swabs in appropriate transport medium and transport in sealed plastic bags.
* Compliance with postal, transport and storage regulations is essential.
* Work carried out at containment Level 2.
* 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.

**Calibration procedures (metrological traceability)**

N/A

**Procedural steps**

**Microscopy**

Gram staining is not normally required on swabs. Any fluid/tissue must receive a gram stain.

**Culture and Investigation**

See MB-BS-54 – Inoculation of culture media.

**Culture media, conditions and organisms: for all specimens**

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| **Clinical details/****conditions** | **Standard media** |  **Incubation** | **Cultures read** | **Target organism(s)** |
|  |  | **Temp °C** | **Atmos** | **Time** |  |  |
| All swabs(except skin swabs and line sites) |  | Blood agar | 35–37 | 5–10% CO2 | 40–48 h | Daily | Lancefield Groups A, C and G streptococci*Pasteurella* species*S. aureus**Vibrio species**Aeromonas* species |
|  | CLED agar | 35–37 | Air | 16–24 h | Daily | EnterobacteriaceaePseudomonads |
|  | Staph/strep selective agar  | 35–37 | Air | 40–48 h | Daily | *S. aureus*Lancefield Groups A, C and G streptococci |
|  | Neomycin fastidious anaerobe agar with metronidazole 5 μg disc | 35–37 | Anaerobic | 40–48 h\* | ≥40 h | Anaerobes |
| Line sites | Blood agar | 35–37 | 5–10% CO2 | 40–48 h | Daily | Lancefield Groups A, C and G streptococci*S. aureus* |
| Skin swabs | Blood agar | 35–37 | 5–10% CO2 | 40–48 h | Daily | Lancefield Groups A, C and G streptococci*S. aureus* |
| Staph/strep selective agar | 35–37 | Air | 40–48 h | Daily | *S. aureus*Lancefield Groups A, C and G streptococci |
| For these situations, add the following: |
| **Clinical details/****conditions** | **Supplementary media** | **Incubation** | **Cultures read** | **Target organism(s)** |
|  |  | **Temp °C** | **Atmos** | **Time** |  |  |
| Cellulitis in childrenHuman bites | Chocolate agar† | 35–37 | 5–10% CO2 | 40–48 h | Daily | *Haemophilus* species |
| BurnsPatients who areImmunocompromised PEG siteDiabetic patientIntertrigoParonychia | Sabouraud agar | 35–37 | Air | 40–48 h\* | ≥40 h | Fungi |
| Cutaneous diphtheriaForeign travel | Hoyle's tellurite agar | 35–37 | Air | 40–48 h | Daily | *C. diphtheriae* *C. ulcerans* |
| Other organisms for consideration: Dermatophytes (MB-BS-39 – Investigation of dermatological specimens for superficial mycosis) and *Mycobacterium* species (MB-BS-40 – Investigation of specimens for *Mycobacterium* species) |
| \*Incubation may be extended to 5 d if clinically indicated; in such cases plates should be read at ≥40h and then left in the incubator/cabinet until day 5†Either bacitracin 10-unit disc or bacitracin containing agar may be used. |

**Identification**

Primary identification system is the MALDI-TOF which will identify to species level. Conventional identification procedures are described in the appropriate identification SOPs.

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| 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 |
| *S. pneumoniae* | species level |
| Yeasts | ‘yeasts’ level |
| *Vibrio* | species level |
| *Aeromonas* | species level |
| Dermatophytes  | MB-BS-39 – Investigation of Dermatological Specimens for Superficial Mycosis |
| Mycobacterium | MB-BS-37 – Investigation of blood cultures for organisms other than Mycobacterium species |

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

**Antimicrobial Susceptibility Testing**

See MB-BS-45 for antibiotic susceptibility testing. Antibiotics reported are built into Telepath.

**Referral to Reference Laboratories**

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.

**Quality Control Procedures**

Quality control is applied to all reagents used in the process, see relevant process. The laboratory also participates in external and internal quality assessment schemes.

**Interferences (e.g. lipaemia, haemolysis, bilirubinemia, drugs) and Cross Reactions**

As for all culture processes samples should be collected before antibiotic therapy is initiated and collection should aim to minimise any contamination of the sample with normal indigenous microflora. Samples should be processed as soon as possible.

**Reportable interval of examination results**

**Microscopy**

Urgent microscopy results should be telephoned or sent electronically as soon as possible.

Written report should be issued after 16–72 h.

**Culture**

Results should be reported as:

* No bacterial growth.
* Presence or absence of specifically named organisms e.g*. S. aureus* in the organism field.
* All other organisms are reported in the negative field.

Culture reporting time:

Clinically urgent culture results should be telephoned or sent electronically as soon as possible.

Written reports should be issued after 16–72 h stating, if appropriate, that a further report will be issued.

**Antimicrobial susceptibility testing**

Report susceptibility results as clinically indicated.

**Potential sources of variation**

The recommendations made in this SOP are based on evidence (e.g. 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.

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.

**References and related documents**

Public Health England. (2014). Investigation of Skin, Superficial and Non-Surgical Wound Swabs. UK Standards for Microbiology Investigations. B 11 Issue 5.2. http://www.hpa.org.uk/SMI/pdf

**REPORTING OF LEG ULCER AND SUPERFICIAL SKIN SWAB RESULTS**

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| **Organisms Isolated** | **Report** |  |
| *Staphylococcus aureus* | **Report with sensitivities** |
| Haemolytic streptococci groups A, C, G |
| Streptococci group B |
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|  | **Report if pure growth + comment****‘Significance uncertain at this site. Consider clinical factors when interpreting this result’** |  |
| Coliforms, pseudomonas and anaerobes  |  |
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| Skin flora and scanty mixed organisms | **No pathogens isolated** |  |
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| Non-haemolytic streptococci | **Do not report** |
| Enterococci |