**MICROBIOLOGY STANDARD OPERATING PROCEDURE**

**TITLE: CULTURE OF PUS, SKIN SWABS, AND NORMALLY STERILE FLUIDS**

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| Document no.: | Reviewed and Approved by: |  |
| Replaces document: | Date of original: 14th December 2012 |  |
| Applies to: Bacteriology  Created by: | Date of revision:  Date for review: | Page 1/7 |

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**1. Aim**

To describe the processing of fluids from normally sterile sites (excluding cerebrospinal fluid), pus, and skin/pus swabs.

**2. Principle**

The range of organisms causing superficial wound infections and superficial or deep tissue infections/abscesses is wide. Gram staining of clinical material may guide empiric antimicrobial treatment and aid selection of culture plates. Selective media may be required, especially for specimens from superficial or non-sterile sites. Enrichment cultures may be required for specimens from certain sites and to identify particular pathogens (e.g. *Burkholderia pseudomallei*). Culture results must always be interpreted in conjunction with clinical detail, especially for superficial swabs.

**3. Method**

***3.1. Specimen collection***

Wherever possible a specimen of pus/fluid collected aseptically into a sterile universal container is preferred over a swab. However, a swab collected into a suitable transport medium (e.g. Amie’s +/- charcoal) is acceptable if a pus/fluid specimen is not available. It is important to accurately record the anatomical site of a swab specimen, since cultures from swabs of a deep pus collection will require a different interpretation to those from a superficial site.

***3.2. Specimen transport and storage***

Specimens should ideally be stored and transported in sealed plastic bags. Laboratory processing should occur as soon as possible after specimen collection. Specimens should be refrigerated if delays in processing over two hours are unavoidable.

***3.3. Specimen processing***

***3.3.1. Reception***

Log the specimen in the appropriate specimen book / database and assign a specimen number.

**3.3.2. *Microscopic examination***

*Cell count (fluids from normally sterile site only)*

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*Gram stain (all specimens)*

Prepare a smear of the specimen and Gram stain. Consider the need for ZN/auramine staining (mandatory if TB is suspected).

If a swab is received, prepare the slide *after* performing culture.

For pus/fluid specimens, centrifugation should be carried out prior to Gram staining

*unless* the specimen is frank pus or clotted:

 Centrifuge the specimen in a sterile conical bottom container at 3,000 g for 10 minutes;

 Discard all but 0.5ml of the supernatant with a sterile pipette;

 Resuspend the pellet in the remaining 0.5ml fluid and use this for preparation of a smear and inoculation of culture media.

*Wet prep (selected specimens)*

A wet preparation may be required if amoebae, fungi, or parasites (e.g. paragonimus: for image, see faecal parasitology MOPSOP-002) are suspected. Place a drop of specimen onto a clean dry slide, cover with a coverslip, and examine using the x10 objective.

***3.3.3. Culture***

Inoculate and incubate culture media as indicated in Table 1.

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***Table 1. Culture media, conditions, and target organisms***

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| --- | --- | --- | --- | --- | --- | --- |
| **Clinical / Gram**  **strain** | **Standard media** | **Incubation** | | | **Cultures read** | **Target organism(s)** |
| **Temp (°C)** | **Atmosphere** | **Time** |
| **Swabs** | | | | | | |
| Alla | Blood agar | 35 – 37 | 5 – 10% CO2 | 48h | Daily | β-haemolytic streptococci  *Pasteurella* species (bite wounds)  *S. aureus*  *Vibrio* species |
| Chocolate agar | 35 – 37 | 5 – 10% CO2 | 48h | Daily | *Haemophilus* sp (cellulitis in children /  human bite wounds) |
| MacConkey agar | 35 – 37 | Air | 48h | Daily | Enterobacteriaceae  Pseudomonads |
| ASW  SBCT | 35 – 37 | Air | 5 days | Daily | *B. pseudomallei* |
| If yeasts seen,  burns, diabetic patient, intertrigo, paronychia | Sabouraud agar | 35 – 37 | Air | 5 days | Daily | Fungi |
| If Gram stain  indicates mixed infection (optional) | CNA-blood agar | 35 – 37 | Air | 48h | Daily | β-haemolytic streptococci  *S. aureus* |

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| --- | --- | --- | --- | --- | --- | --- |
| **Pus / Sterile fluids** | | | | | | |
| All | Blood agar | 35 – 37 | 5 – 10% CO2 | 48h | Daily | β-haemolytic streptococci  *Pasteurella* species  *S. aureus*  *Vibrio* species |
| Chocolate agar | 35 – 37 | 5 – 10% CO2 | 48h | Daily | *Haemophilus* sp |
| MacConkey agar | 35 – 37 | Air | 48h | Daily | Enterobacteriaceae  Pseudomonads |
| Sabouraud agar | 35 – 37 | Air | 5 days | Daily | Fungi |
| TSB | 35 – 37 | Air | 48h | Daily | Gram stain and subculture if turbid |
| If Gram stain  indicates mixed infection (optional) | CNA-blood agar | 35 – 37 | Air | 48h | Daily | β-haemolytic streptococci  *S. aureus* |
| ?Melioid / diabetic /  parotitisa | ASW  SBCT | 35 – 37 | Air | 5 days | Daily | *B. pseudomallei* |
| ?Nocardia | Blood agar  LJ slope | 35 – 37 | Air | 14 days | D3, D7, and  D14 | *Nocardia* sp |
| ?TB | LJ slope/liquid medium | 35 – 37 | Air | 9 weeks | Weekly | *M. tuberculosis* |

aRoutine culture for *B. Pseudomallei* may not be required outside of highly endemic areas

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**4. Interpretation**

Record semi-quantitative growth of each colony type.

***4.1. Minimum level of identification in the laboratory***

*Skin / pus swabs and pus specimens*

*Aeromonas* species level Anaerobes "anaerobes" level *Bacillus* species level if possible β-haemolytic streptococci Lancefield Group level *Burkholderia pseudomallei* species level

Coagulase-negative staphylococci “coagulase-negative” level

Corynebacteria “diphtheroids” level (species level if

diphtheria suspected)

*Eikenella corrodens* species level

Enterobacteriaceae “coliforms” level (species level in pus)

*Haemophilus* species level *Neisseria* species level *Pasteurella* species level

Pseudomonads “pseudomonads” level (species level in pus)

*S. aureus* species level

*S. milleri* (*S. anginosus* group) Lancefield group level

*S. pneumoniae* species level

Yeasts “yeasts” level (species level for

*Cryptococcus neoformans*)

*Vibrio* species level

*Fluids from normally sterile sites*

Anaerobes “anaerobes” level

β-haemolytic streptococci Lancefield group level Coagulase-negative staphylococci “coagulase-negative” level Mycobacterium sp. genus level (and refer for species ID) All other organisms species level

***4.2. Antimicrobial susceptibility testing***

All significant isolates should have antimicrobial susceptibilities determined, according to MOPSOP-004.

***4.3. Reporting***

Cell counts (if done).

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Gram stain results: WBC and organisms detected.

Wet prep results (if done): presence or absence of named organisms (e.g. paragonimus ova not seen).

Culture:

 Swabs: presence of significant isolates (e.g. *S. aureus*); no significant growth /

mixed growth of doubtful significance may be used; absence of growth.

 Pus: presence of significant isolates or absence of growth.

 Normally sterile fluids: organism(s) isolate or absence of growth.

**5. Quality assurance**

Media and identification tests should be quality controlled according to the relevant

MOPSOP.

**6. Limitations**

Prior antimicrobial use may result in negative cultures

**7. References**

Standard Operating Procedures from LOMWRU, SMRU and AHC. Health Protection agency, UK SOPs

([http://www.hpa.org.uk/ProductsServices/InfectiousDiseases/QualityAssurance/UKStan dardsForMicrobiologyInvestigations/TermsOfUseForSMIs/AccessToUKSMIs/](http://www.hpa.org.uk/ProductsServices/InfectiousDiseases/QualityAssurance/UKStandardsForMicrobiologyInvestigations/TermsOfUseForSMIs/AccessToUKSMIs/)):

BSOP 11: Investigation of skin, superficial and non-surgical wound swabs. BSOP 14: Investigation of abscesses and deep-seated wound infections. BSOP 26: Investigation of fluids from normally sterile sites.

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**Safety considerations**

|  |  |
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| **COSHH risk assessment - University of Oxford COSHH Assessment Form** | |
| **Description of procedure:**  Culture of skin or wound swabs / pus / body fluids | **Substances used:**  Variable, depending on organism cultured (may include Gram stain reagents; 3% hydrogen peroxide (catalase test); N,N,N',N'-tetramethyl-1,4-phenylenediamine (oxidase test); sodium deoxycholate (bile solubility test); bioMerieux API reagents). |
| **Quantities used:**  Small | **Frequency of use:**  Daily |
| **Hazards identified:**  Infection risk from specimens / culture plates Chemical exposure from bacterial identification test kits | **Could a less hazardous substance be used instead?**  No |
| **What measures have you taken to control risk?**  Training in good laboratory practices  Appropriate PPE (lab coat, gloves, eye protection)  Use of biosafety cabinet for reading of plates, follow-up of BSL-3 organisms (e.g. *B pseudomallei*) | |
| **Checks on control measures:**  Observation and supervision by senior staff | |
| **Is health surveillance required?**  No | **Training requirements:**  GLP |
| **Emergency procedures:**  Report all incidents to laboratory safety officer  Eye wash for splashes | **Waste disposal procedures:**  Sharps discarded into appropriate rigid containers for incineration.  Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration. Chemical waste disposed of according to manufacturer’s instructions. |