

Ministry of Health

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Acronyms:

BA	Blood agar
CA	Chocolate agar
MAC	Mackoncy
ID	Culture Identification
SOP	Standard operating procedure
MRSA	Methicilin Resistant Staph. Aureus
BHI media	Brain heart infusion
GC media	Gonococcus media

1. Scope

This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

2. Purpose

This procedure provides instructions for Abscess and deep seated wound investigation.

3. Definitions

- 3.1 Abscess: collection of pus in any part of the body
- 3.2 Deep seated: accumulation of pus in the tissues

4. Procedure:

- 4.1 Clinical background:
 - 4.1.1 Abscess or pus or aspirates (Except: anal, rectal, perianal, ischiorectal, perirectal, dental, orofacial, Tonsillar, peri-tonsillar and female genital tract sites). Abscesses may be due to *Staphylococcus aureus* or a mixture of different aerobic and anaerobic bacteria depending on the location of the abscess.
 - 4.1.2 Facial or dental Abscess:

Dental abscesses involve microorganisms colonizing the teeth that may become responsible for oral and dental infections, leading to dentoalveolar abscesses and associated diseases. Organisms most commonly isolated in acute are facultative or strict anaerobes. The most frequently isolated organisms are anaerobic Gram negative rods; however, other organisms have also been isolated. Examples are: α-haemolytic streptococci ,Anaerobic Gram negative bacilli ,Anaerobic streptococci ,"S. anginosus" group and Actinobacillus actinomycetemcomitans, Spirochaetes and Actinomyces species. The common pathogens of these infections are part of the normal oral flora including anaerobes of the oral cavity.

4.1.3 Ovarian, fallopian tube, tubo-ovarian, tubal Abscess:

These specimens are often mixed with aerobes and anaerobes and are usually surgically obtained. Sexual transmitted diseases organisms (STDs) such as *Neisseria. gonorrhoeae* may also be the cause.

4.1.4 Anal, rectal, perianal, ischiorectal or perirectal Abscess:

Perirectal abscesses are encountered in patients with predisposing factors. These include mmunodeficiency, malignancy, rectal surgery, ulcerative colitis. They are

often caused by; Anaerobes, Enterobacterales, Streptococci, *S. aureus*. These specimens commonly include normal stool or enteric flora; therefor the extent of the work-up of the enteric flora will be limited.

4.1.5 Pilonidal Abscess

Pilonidal abscesses are common in children, and result from infection of a pilonidal sinus. Anaerobes and Enterobacterales are usually isolated, but they may be caused by *S. aureus* and β-haemolytic streptococci.

4.1.6 Post-Operative Wound Infections

Post-operative wound infections arise when microorganisms contaminate surgical wounds during an operation or immediately afterwards. Colonised body sites are frequent sources of pathogens, although they may be transmitted via medical and nursing staff, via inanimate objects from other patients or elsewhere in the hospital environment. Organisms most commonly isolated include: *S. aureus* including MRSA, *Bacteroides* species, *Clostridium* species, Enterobacterales, Pseudomonads, β-haemolytic streptococci, Enterococci, *Peptostreptococcus* species. Coagulase negative staphylococci and coryneforms isolated from post-operative sites overlying implants or prostheses, may indicate infection.

4.2 Pre-analytic:

4.2.1 Sample:

- Sample type: pus, aspirates, or pus swab.
- Amount of sample required, including minimum requirements: 1 ml.
- Transportation media: leak proof sterile container for aspirates and pus, and charcoal swabs for pus swab.
- Sample stability and storage requirements: to be processed immediately within 2 hours. In case of delay, store the sample in 2-8 C.

4.2.2 Materials:

Reagents	Consumables/Supplies	Equipment
Chocolate media plate BA media plate SAB media plate GC media plate Gram stain reagents BHI enriched media	Disposable 0.001 ml Calibrated loop Incubating Rack slide cover slip	O2, CO2 incubator microscope 40X objective

4.2.3 Safety Precautions:

All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.

4.2.4 Quality Control:

- 1. Check the expiry dates of all media, reagents and stains before use.
- 2. All media, reagents, kits, and stains **MUST** be quality controlled before use.
- 3. Identification tests should be run with appropriate controls.
- 4. Record the quality control results in the appropriate QC sheet.

4.3 Analytical stage:

4.3.1 Direct Exam:

• (Gram's stain) Quantitate the presence of pus cells and organisms.

Cells grade	count
Scanty pus cells if	<5
+	if 5-10
++	if 10-25
+++	if >25

- Modified acid fast stain If Actinomyces or Nocardia is requested or suggested on Gram stain.
- Calcofluor white stain (if available) If fungus is requested.

4.3.2 Culture Set-up

Media	Incubation
Blood Agar (BA)	CO ₂ , 35°C x 48 hours
Blood agar	AnO ₂ , 35°C x 48 hours
MacConkey Agar (MAC)	O ₂ , 35°C x 48 hours
Chocolate Agar (CHOC)	CO ₂ , 35°C x 48 hours
GC media **	CO ₂ , 35°C x 72 hours
BHI enriched media	CO ₂ , 35°C x 24 hours, and then S/C in BA, MAC, CHOC, anaerobic plate
If Actinomyces is requested, set up a second set of	
anaerobic media	AnO ₂ , 35°C x reading to be taken every
	48 hrs, and incubated for 10 days
If fungus culture is requested or seen in gram stain,	
add	
1.Sabroud agar	1. O ₂ , 30°C x 14 days
2.Sabroud agar	2. O ₂ , 35°C x 5 days

^{*} lysed schedler : In case it is available for anaerobic organisms.

^{**} GC media: ** For vulva, bartholins gland, ovarian, fallopian tube, tubo-ovarian, prostatic abscess, and tubal samples.

4.3.3 Identification and Isolation

ABSCESS sites	Identification and Isolation
1. Pus or aspirates	• Any growth of potential pathogens such as <i>S. aureus</i> , β-haemolytic streptococci, <i>Streptococcus anginosus</i> group, <i>Pseudomonas aeruginosa</i> , anaerobe and yeasts are significant; work up
(Except: anal, rectal, perianal, ischiorectal, perirectal dental, orofacial Tonsillar, peri-tonsillar or female genital tract sites)	• Mixed growth of coagulase negative <i>Staph</i> , <i>Coryneform</i> bacteria (diphtheroids), <i>viridans Strep</i> or other skin organisms may be considered as "skin flora" except if culture was obtained intra-operatively (OR) from a joint or bone. If they present in pure growth (refer to a microbiologist or senior technologist for any extent of work-up).
	• For all aspirates, including liver abscess, identify all isolates, (for commensal organisms consult microbiologist).
	 Other organisms will be worked up only if there are ≤3 (up to 3) different bacterial types except skin flora. Otherwise >3 types simply list the morphotypes and consider it as mix growth.
2. facial or dental	 These specimens may be contaminated with oral flora due to the nature of the specimen.
Abscess	• Work-up all significant isolates including <i>Streptococcus</i> . <i>anginosus</i> group. Anaerobes & <i>S. Viridans</i> (<i>If only present in pure culture</i>). If <i>Actinomyces</i> requested and no growth in anaerobic plates after 48 hrs reincubate anaerobic plates for total 10 days.
	 Interpretation and extent of work-up of other organisms (e.g. Enterococcus, Aerobic gram-negative bacilli etc, please refer to a microbiologist or senior technologist.

3. Abscess from:

vulva, bartholins gland,ovarian, fallopian tube, tuboovarian and tubal or Pelvic abscess

- Examine plates carefully for *Neisseria gonorrhoeae* as per protocol described for cervical specimens.
- All potential pathogens (probable including anaerobes and possible pathogens) should be identified .See No.1 of this table for other details.
- Organisms resembling Lactobacilli or Gardnerella require identification only if they found as predominant or in pure culture. Otherwise they may be considered "vaginal/skin flora".
- **Mixed growth** of coagulase negative *Staph*, *Coryneform* bacteria (diphtheroids), β-*Strep*, *Lactobacilli*, enterococci, etc. may be considered as normal vaginal/skin flora.
- Simply list the skin/vaginal organism if it not mixed with other skin/vaginal organisms

4. Abscess: anal, rectal, perianal, ischiorectal or perirectal

- Work up any growth of *S. aureus*, beta-haemolytic *Streptococci*, *S. anginosus* group or *Pseudomonas aeruginosa*
- Identify and report other Gram-negative bacilli, enterococci, Anaerobes or yeast if present in pure culture or present in ≤ 3 (upto 3) morphotypes. If 3 enteric gram negative organisms other than salmonella and shigella to be discussed with microbiologist
- All other organisms can be considered as enteric or skin flora if they are >3 types (depending on what is appropriate). Screen any non-lactose fermenters (NLF) for Salmonella species and Shigella species

	 If there are questions about the extent of work-up, consult Microbiologist or senior technologist.
5. Tonsillar & peritonsillar abscess	 Identify any growth of Group A Strep, H. influenzae, S. aureus, Group C or G Strep, etc.), anaerobes, Archanobacterium Haemolyticum (hemolytic GPB) and C.diphtheriae (rare). Identify pure growth of any gram negative bacilli if presence
	 with moderate WBCs and no growth of anaerobes bacteria. If there are questions about the extent of work-up, consult Microbiologist or senior Technologist. Other organisms can generally be considered as "oral flora".

4.3.4 Susceptibility Testing:

• As appropriate for organisms that are identified.

4.4 Post – analytical stage:

- 4.4.1 Interpretation / Results / Alerts:
 - 4.4.1.1 Gram stain: Report with quantitation the presence of pus cells and organisms
 - 4.4.1.2 Culture report: report according to the following table:

ABSCESS sites	Reporting
Abscess / pus or aspirates:	 Negative Report: No growth" or "no significant growth or skin flora" "Normal respiratory flora"
Except anal, rectal, perianal, ischiorectal, perirectal dental, orofacial Tonsillar, peritonsillar or female genital tract sites	 Positive Report: Report all significant isolates with appropriate susceptibilities. If requested to identify some but not all GNB, etc. report either (whichever is most appropriate) "Growth of mixed bacterial flora" or "Growth of multiple Gram-negative bacilli" followed by "including:" name of organism(s) identified. Examples: Significant isolates - S. aureus, β-haemolytic streptococci, Streptococcus anginosus group, Pseudomonas aeruginosa, yeasts or other organisms ≤3 different bacterial types. Report all isolates with appropriate susceptibilities. >3 types non-significant isolates - Report as test comment "Mixed growth oflist morphotypes".
Facial, dental, peritonsillar or tonsillar Abscesses	 Negative report: "No growth" or "no significant growth or oral flora". Positive report: report significant isolates with appropriate susceptibility
ovarian, fallopian tube, tubo-ovarian, tubal abscess	 Negative report: "No growth" or "no significant growth or Skin flora or "Vaginal flora" Positive report: Report all significant isolates with appropriate susceptibilities. Phone all positive Neisseria gonorrhoeae cultures. See No.1 of this table for other details
Abscess: anal, rectal, perianal, ischiorectal or perirectal	Negative report: "No growth" or "no significant growth or Enteric flora" or "Skin flora" Positive report: Report all significant isolates with appropriate susceptibilities.

Abscess- vulva, bartholins gland	Negative Report: "No growth" or "vaginal/skin flora Positive Report: report the organisms identified with appropriate susceptibility.
	Phone all positive Neisseria gonorrhoeae cultures

5. Responsibilities

- 5.1 Responsible staff:
 - To ensure the adherence to critical result communication procedure
 - To facilitate the alternative channels once needed
- 5.2 Quality manager /officer
 - To follow up the implementation of the procedure
 - To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.
- 5.3 All lab staff:
 - To adhere to the procedure.
 - To document record and release results as recommended
 - To report test failures or incident

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publicati on	Page
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