HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Microbiology)		
Document:	Internal Policy and Procedures		
Title:	Anaerobic Culture		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 To establish system and set responsibilities for processing anaerobic culture.

2. DEFINITIONS:

2.1 Obligate anaerobes, are microorganisms that are killed by normal atmospheric concentrations of oxygen (21% O2).

2.2 Facultative anaerobe, an organism that is able to grow in the absence as well as in the presence of atmospheric oxygen.

3. POLICY:

3.1 Inoculate the sample into two blood agar plates one to be incubated aerobically and the other incubated anaerobically to differentiate between obligatory and facultative anaerobic bacteria.

3.2 Perform antibiotic susceptibility test using antibiotics suitable for anaerobic bacteria like Penicillin, Clindamycin, Vancomycin, Augmentin, Cefoxitin, Tazocin, Tienam , Metronidazole, the tested plates are incubated in anaerobic jar at 35+2 °C for 48 hours.

4. PROCEDURE:

4.1 **Principle:**

4.1.1 Oxygen from anaerobic jar is removed and replaced with inert gas.

4.1.2 In this Anaerobic system, hydrogen is generated which combines with free oxygen present in the jar.

4.1.3 The Anaerobic system comprises the following three items:

4.1.3.1 Envelope: it contains two types of tablets which release H2 and CO2 with addition of water; sodium borohydrate & sodium bicarbonate plus citric acid.

4.1.3.2 Catalyst replacement: oxygen present in an anaerobic jar is removed by the catalyst activity. The catalyst activates hydrogen (H2) gas to combine with free oxygen in the jar to form water which may be observed as condensate on the inner wall of the jar.
N.B.: The available sachet works in the absence of catalyst.

4.1.3.3 Indicator: it is used to check the activity of catalyst.

4.2 **Procedure:**

4.2.1 Inoculate the sample into two blood agar plates one to be incubated aerobically and the other incubated anaerobically to differentiate between obligatory and facultative anaerobic bacteria.

4.2.2 Note, it is better to use anaerobic media that contain reducing agents such as; schaedler agar, CDC anaerobic agar, fastidious anaerobic agar (with hemin & vitamin kl) if it is available.

4.2.3 Put the inoculated plate for anaerobic culture after streaking into the anaerobic jar.

4.2.4 Do not collect inoculated plates, but introduce them in the anaerobic jar and activate the anaerobic system as fast as possible.

4.2.5 Cut off the corner of gas pack envelope at dotted line and place it in jar with printed side facing outwards.

4.2.6 Place indicator at a specific place.
4.2.7 Cover the jar promptly and put it into the incubator at 37°C.
4.2.8 Open the jar after 48 – 72 hours of incubation for reading plates.
4.2.9 If actinomyces is suspected (Sulphur granules in Gram stain): incubate for not less than 2 weeks.
4.2.10 Read both blood agar plates, if there is growth in both aerobic and anaerobic plates, this means facultative anaerobic bacteria but if there is growth only in anaerobic plate this means obligatory Anaerobic bacteria.
4.2.11 Identify any obligatory anaerobic growth by Gram stain, catalase test & APIA (If available).
4.2.12 Perform antibiotic susceptibility test using antibiotics suitable for anaerobic bacteria like Penicillin, Clindamycin, Vancomycin, Augmentin, Cefoxitin, Tazocin ,Imipenem , Metronidazol, the tested plates are incubated in anaerobic jar at 35+2 °C for 48 hours.
4.2.13 Open the jar after 48 hours of incubation for reading plates & APIA.

4.3 **Quality control:**

4.3.1 Methylene blue indicator becomes colourless in an anaerobic condition, becomes blue when anaerobic is not achieved.

5. MATERIAL AND EQUIPMENT:

5.1 Microbiology Culture Media
5.2 Gram Stain Reagents
5.3 Anaerobic system (jar & anaerobic gas pack)
5.4 Vitek2 System ID (ANC) Cards

6. RESPONSIBILITIES:

6.1 Assigned Technician for Microbiology
6.2 Clinical Pathology Specialist/ Consultant

7. APPENDICES:

7.1 Selection and collection of specimens for anaerobes

8. REFERENCES:

8.1 Procedure Manual, Toronto Medical laboratories / Mount Sinai Hospital department of microbiology.
8.2 Bailey & Scott's Diagnostic Microbiology. Feingold& Baron; 12th. Ed.2007, C.V. Mosby Co. p. 301.
8.3 Clinical Microbiology Procedures Handbook, American Society of Microbiology, Washington DC, 2005.
8.4 Murray PA, et al. Manual of Clinical Microbiology, 1999. The ASM Press, 7th edition.

9. APPROVALS:

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Appendix 7.1

Selection and Collection of Specimens for Anaerobes

I. Absolutely unsuitable for anaerobic diagnosis (Insignificant specimens):

1. Sputum, throat, tongue, or tooth swabs
2. Pus from superficial wounds
3. Midstream or catheter urine
4. Stool (exception: c. difficile)
5. Skin swabs
6. Decubitus swabs
7. Generally: swabs with "flora" contamination

II. Conditionally acceptable specimens:

1. Vaginal swabs: only for diagnosis of non-specific vaginitis.
2. Urethral swabs: if anaerobic infection is suspected, after exclusion of other agents such as Chlamydia, gonococci.
3. Prostate secretions: if anaerobic infection is suspected, after exclusion of other agents such as Chlamydia, gonococci.
4. Bronchial secretions: only if collected with sheathed catheters to avoid contamination with throat flora.
5. Decubitus swabs: submit tissue or aspirate.
6. Dental pus: submit aspirate.

III. Important and well suitable specimens for anaerobes include:

1. Pleural aspirates
2. Transtracheal aspirates
3. Ascitic Fluid
4. Pericardial fluid
5. Extra orally collected pus
6. Peritoneal secretions
7. Pus from appendicitis
8. Suprapubically collected bladder urine
9. Cervical secretions (collected with speculum)
10. Pus from Douglas abscesses.
11. Any abscess materials
12. Biopsy from any sterile organs
13. Bone from osteomyelitis patients
14. Pus from deep wounds (also post-surgical)
15. Blood culture (especially post-surgical)
16. Cerebrospinal fluid (if anaerobic meningitis is suspected)