HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Microbiology)		
Document:	Internal Policy and Procedures		
Title:	Cultivation of Bacteria on Laboratory Media		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 To establish system and responsibilities for processing cultivation of bacteria on laboratory media.

2. DEFINITONS:

N/A

3. POLICY:

3.1 Media to be cultivated are chosen according to the type of specimen received.
3.2 In-use culture media should be kept at room temperature for at least 30 minutes before use.
3.3 The size of disposable loop used depends on sample type (1ul loop foe urine specimens, 10ul loop for others).

4. PROCEDURE:

4.1 **Inoculation of Culture Media:**
4.1.1 For microbiological investigations it is essential to know the skills of inoculating specimens onto culture media and sub culturing from one medium to another.
4.2 **Instructions for seeding media:**
4.2.1 This is selected according to the nature of the medium and inoculum.
4.2.2 Sterile plastic loop of different sizes are used.
4.3 **Seeding a culture plate:**
4.3.1 The inoculum from the clinical material or another plate is first spread out in the form of a primary inoculum (as at Appendix 7.1) which is also called as 'well-inoculum' or only 'well'.
4.3.2 The successive series of strokes B, C, D and E are made with the loop.
4.3.3 At each step the inoculum is derived from the most distal part of the immediately preceding strokes so as to gradually reduce the number of bacteria. This helps in obtaining isolated colonies.
4.3.4 When the inoculum is small or the medium is selective it can be more heavily inoculated (Appendix 7.2).
4.3.5 Several loop-full of the specimen are used to spread the primary inoculum.
4.4 **Seeding a liquid medium:**
4.4.1 Incline the tube containing the liquid medium to 45° and deposit the inoculum on its wall above the surface of the liquid at its lower end. Return the tube to a vertical position. Now the inoculum shall be below the surface of the liquid.
4.5 For culturing of tissue or biopsy, perform homogenization and grinding procedures involving tissue or biopsy specimen in safety cabinet then inoculate the media.
4.6 Mop up the workbench clean with any disinfectant at the start and close of work.
4.7 Wash hands with soap and water before and after handling infectious specimens.

5. MATERIAL AND EQUIPMENT:

5.1 Microbiology culture media
5.2 Platinum wires Or disposable sterile loops (1ul & 10ul size)
5.3 Benzene flame

6. RESPONSIBILITIES:

6.1 The assigned technician/ technologist for microbiology section.

7. APPENDICES:

7.1 Seeding a culture plate
7.2 Seeding with heavy inoculums

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 Isenberg HD(Ed) Clinical Microbiology Procedures handbook. American Society for Microbiology, Washington, DC, Vol 1, Section 1.4, 1992.

9. APPROVALS:

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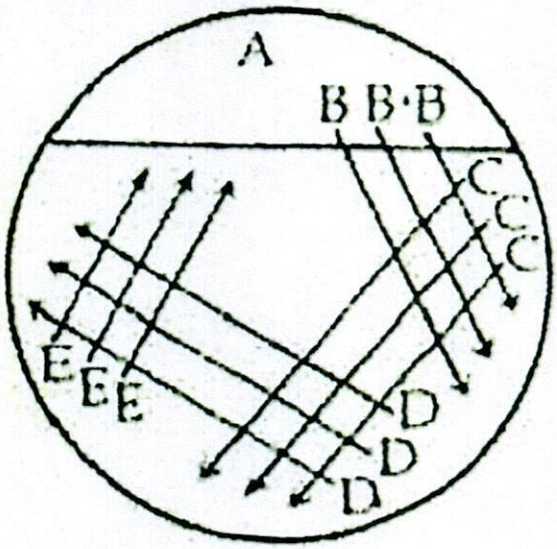
Appendix 7.1: Seeding a Culture Plate



Appendix 7.2: Seeding with Heavy Inoculums