HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Microbiology)		
Document:	Internal Policy and Procedures		
Title:	Ziehl-Neelsen Staining		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 To establish system and set responsibilities for processing Ziehl-neelsen staining smears.

2. DEFINITIONS:

2.2 **Ziehl–Neelsen staining**, is a bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria.

3. POLICY:

3.1 All sample preparation procedures must be performed inside Biological Safety Cabinet (BSC) - class II.
3.2 Telephone all positive results to ward / ordering physician and notify infection control.
3.3 Record in file of panic results.

4. PROCEDURE:

4.1 **Standard Precautions:**
- 4.1.1 Specimens collected from patients who have clinical signs of tuberculosis are sent to the laboratory in close containers that are opened in a **BSC class II**.
- 4.1.2 Because of the potential for aerosol generation, technician must **wear N95 mask**, specimen containers must be opened and direct smears prepared, air dried and alcohol-fixed in class II BSC.
- 4.1.3 Fixed smears may contain viable tubercle bacilli, but they are not easily aerosolized if dried on a slide.
- 4.1.4 Personnel may remove fixed slides from the BSC and stain them.
- 4.1.5 Stain reagents contain phenol, which kills tubercle bacilli during the staining process.

4.2 **Reagents:**
- 4.2.1 Carbol-fuchsin: Basic fuchsin solution (10 g basic fuchsin in 100 ml 95% ethyl alcohol) +(Phenol 50 gm + dist.water 1000 ml)
- 4.2.2 Acid Alcohol (concentrated HCl 3 ml +95% Ethyl alcohol 97 ml).
- 4.2.3 Methylene blue (Methylene blue 0.3 g/Distilled water 100 ml).

4.3 **Steps:**
- 4.3.1 Preparation of specimens:
  - 4.3.1.1 Thick tenacious samples like sputum:
    - 4.3.1.1.1 Must be liquefied and decontaminated by addition of equal volume of 4% Na OH incubate 15 min.
    - 4.3.1.1.2 Vortex then centrifuges the sample in closed centrifuge tube.
    - 4.3.1.1.3 Transfer the supernatant to the original container.
    - 4.3.1.1.4 Mix well the deposit, and then prepare the film.
    - 4.3.1.1.5 After vortex or centrifugation don't open the tube immediately but wait for 15 min to prevent dispersion of aerosols in the environment & opening of the tube must be done inside the safety cabinet.

4.3.1.2 All body fluids:

4.3.1.2.1 The samples transferred to centrifuge tube & centrifuged at 3000 rpm for 30 min, transfer the supernatant to the original container, mix the deposit well, and prepare the film.

4.3.1.3 For tissues:

4.3.1.3.1 Send the sample for histopathology department for preparing sections & fixation of smear for staining.

4.3.2 Smear Preparation:

4.3.2.1 Spread the sample over area 2x1cm and allow it to air dry.

4.3.3 Heat or alcohol fix.

4.3.4 Flood smear with carbol fuchsin.

4.3.5 Steam the slides gently for 5 minutes by flaming from below the rack not permitting the slides to dry out.

4.3.6 Rinse with tap water and tilt the slides to drain.

4.3.7 Decolorize with acid-alcohol until no more stain appears in washing.

4.3.8 Rinse with tap water and drain.

4.3.9 Counter stain with Methylene blue for 1-2 minutes.

4.3.10 Rinse with tap water, drain and air dry.

4.3.11 Examine with 100X oil immersion objective:

4.4 Interpretation and Reporting:

Quantity of Organism	Reporting Phrase / Criteria
0	No AFB seen
1-2/300 field	Doubtful, request another specimen
1-9/100 field	1+
1-9/10 fields	2+
1-9/ field	3+
More than 9/ field	4+

4.5 Telephone all positive results to ward / ordering physician and notify infection control.

4.6 Record in file of panic results.

5. MATERIAL AND EQUIPMENT:

5.1 Biological Safety Cabinet- class II

5.2 Ziehl Neelsen Stain Reagents +/- (4% Na OH)

5.3 Light microscope with oil immersion lens

5.4 Centrifuge

5.5 Disposables: sterile loops & glass slides

6. RESPONSIBILITIES:

6.1 Assigned Technician for Microbiology

6.2 Clinical Pathology Specialist/ Consultant

7. APPENDICES:

N/A

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.

9. APPROVALS:

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