



HEALTH HOLDING

HAFER ALBATIN HEALTH
CLUSTER
MATERNITY AND
CHILDREN HOSPITAL

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|--------------------------|--|-------------------------|----------------|
| Department: | Laboratory and Blood Bank (Microbiology) | | |
| Document: | Internal Policy and Procedures | | |
| Title: | Ziehl-Neelsen Staining | | |
| Applies To: | All Laboratory Staff | | |
| Preparation Date: | January 02, 2025 | Index No: | LB-IPP-085 |
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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

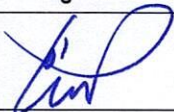
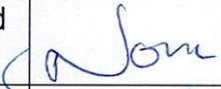



7. APPENDICES:

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:

| | Name | Title | Signature | Date |
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