PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20130 – Kinyoun Stain	Policy Number: 15-158-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 12/04/2024	Next Review Date: 12/04/2026
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 12/04/2024
Accreditation Canada Applicable Standard: NA	

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GUIDING PRINCIPLE:

The kinyoun stain is used in the microscopic detection of acid-fast microorganisms such as Mycobacterium. Acid-fast organisms have cell walls that are resistant to conventional staining by aniline dyes such as the Gram stain. Nonmycobacterial organisms with various degrees of acid-fastness include Rhodococcus species, Nocardia species, Legionella micdadei, and the cysts of Cryptosporidium, Isospora, Cyclospora and microsporidia.

PURPOSE/RATIONALE:

This standard operating procedure describes how to perform the kinyoun stain.

SCOPE/APPLICABILITY:

This standard operating procedure applies to Medical Laboratory Technologists (MLTs) performing the kinyoun stain.

SAMPLE INFORMATION:

Type	Sputum samples with STAT AFB stain requested

REAGENTS and/or MEDIA:

- Kinyoun Carbol-Fuchsin
- Carbol Fuchsin Decolorizer
- Carbol Fuchsin Counterstain (Methylene Blue)

SUPPLIES:

- Glass microscope slide
- QC slide
- Immersion oil
- Slide storage tray

EQUIPMENT

- Microscope

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

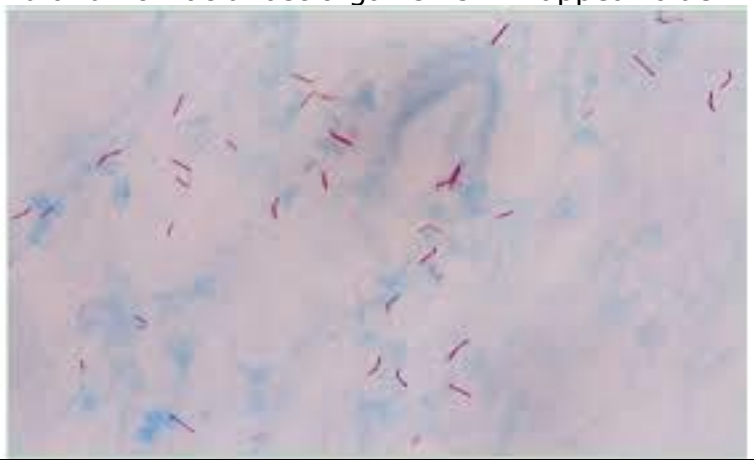
QUALITY CONTROL:

- Quality control is performed as tested
- A TQC order is automatically generated when test is ordered to record the QC results
- Refer to MIC60060-Microbiology Stain Quality Control

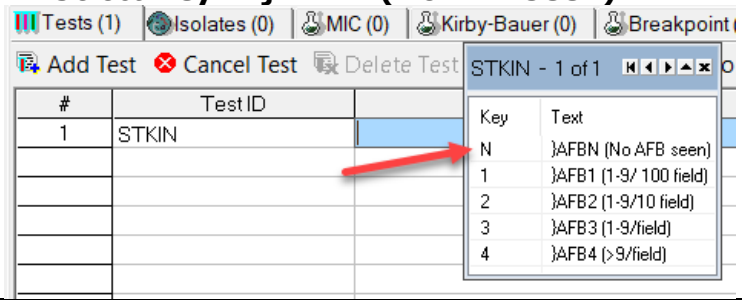
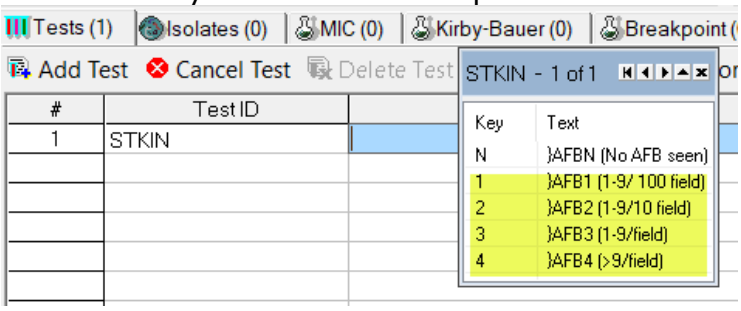
PROCEDURE INSTRUCTIONS:

Step	Action
Performing the kinyoun stain	
1	Prepare a smear of the specimen to be stained. Refer to MIC10000-Microbiology Specimen Handling for slide preparation instructions.
2	Place slide on the slide warmer in the BSC until dry.
3	Flood the entire slide with Kinyoun Carbol Fuchsin Stain for 2 minutes and rinse thoroughly with tap water.
4	Flood the slide with Carbol Fuchsin Decolorizer and decolorize until no more color drains from the slide (approx. 3 to 5 seconds). Rinse the slide thoroughly with tap water and shake off any excess moisture.
5	Flood the slide with Carbol Fuchsin Counterstain (Methylene Blue) and allow the slide to stain for 30 seconds. Rinse thoroughly with tap water and allow to air dry. Do not blot.
6	Examine the smear microscopically under a 100x oil immersion objective.

INTERPRETATION OF RESULTS:

Step	Action
1	• Acid-fast mycobacteria will appear as dark pink to red bacilli • Background and non-acid fast organisms will appear blue 
2	When a carbol fuchsin smear is read, a minimum of 300 fields should be examined before the smear is reported as negative

REPORTING INSTRUCTIONS:

IF	REPORT
No Acid-Fast Bacilli seen on Kinyoun-stained smear	• Result the test using the **"STKIN"** keypad • Select **Key N }AFBN (No AFB seen)** 
Acid Fast Bacilli seen on Kinyoun-stained smear	• Result the test using the **"STKIN"** keypad • Select Key 1 if 1-9 AFB seen per 100 fields • Select Key 2 if 1-9 AFB seen per 10 fields • Select Key 3 if 1-9 AFB seen per field • Select Key 4 if >9 AFB seen per field 

NOTE:

- Refer to Reportable Diseases-Public Health Act as of September 2009 for reporting to OCPHO (HPU1)
- Refer to LQM70620-Laboratory Critical Results List-Microbiology for results that need to be phoned to ordering location
- Refer to MIC36100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Prevention and Control
- Refer to MIC36200-Referral of Category A Specimens to APL for sending category A isolates to APL

LIMITATIONS:

1. Rapidly growing mycobacteria may vary in their ability to retain acid-fast dyes and may fail to stain
2. Be aware of adequate safety precautions and procedures required when handling specimens that are submitted for mycobacterial evaluation
3. Mycobacterial staining should always be used as an adjunct to culture methods since culture techniques are much more sensitive than all acid-fast staining procedures

CROSS-REFERENCES:

- MIC10000-Microbiology Specimen Handling
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36200-Referral of Category A Specimens to Alberta Precision Laboratories
- MIC60060-Microbiology Stain Quality Control
- LQM70620-Laboratory Critical Results List-Microbiology

REFERENCES:

1. Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
2. Dalynn Biologicals. *Kinyoun Carbol Fuchsin Stain* package insert, 2014

APPROVAL:

April 12, 2024
Date

Director, Laboratory and Diagnostic Imaging Services

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	07 Feb 17	Initial Release	L. Steven
2.0	31 Mar 22	Procedure reviewed and added to NTHSSA policy template	L. Steven
3.0	19 Feb 24	Procedure reviewed	L. Steve