

STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: Auramine Rhodamine Stain	Revision Date: 07-April-2017	Issue Date: 07-April-2015
Document Number: MIC81500	Status: Approved	
Distribution: Mycobacteria Manual	Page: 1 of 4	
Approved by: Gloria Badari, Director, Corporate Services and Chief Financial Officer	Signed by: (Original Signed Copy in Microbiology)	

PURPOSE:

To standardize the staining of direct concentrate smears using the fluorescent Auramine Rhodamine stain kit.

SPECIAL SAFETY PRECAUTIONS:

- Handle all patient samples and testing reagent using “Routine Practices”
- Please refer to the Northwest Territories Infection Prevention and Control Manual, march 2012
- Prior to testing all patient are to be identified as per I-0500 Use of Two Patient Identifiers.

REAGENTS and/or MEDIA:

Stain	Information
Stains for direct & culture smears:	
Modified Auramine Rhodamine (fluorescence for direct smears)	<ul style="list-style-type: none">• Suppler: Dalynn• Storage: Room Temperature• Kit contains: Auramine-Rhodamine, Decolorizer, Malachite Green

AURAMINE-RHODAMINE STAIN:

SUPPLIES:

- White slide rack
- 4x 4 gauze
- Dedicated TB timer
- Alcohol wipes
- Auramine-Rhodamine kit, use before expiration date.

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FILENAME: MIC81500AuramineRhodamineStainPRO.doc

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Step	Action
Follow the steps below to stain the direct smear with A/R Stain:	
1	<p>Direct smears are made after culturing the sample.</p> <ul style="list-style-type: none"> Refer to MIC80400 Mycobacteria Processing Procedure. <p>Ensure smears have been fixed for 10 minutes. See MIC80810 Mycobacteria Smear Fixing.</p> <p>There is no maximum fixation time. Smears that cannot be read the day of culturing are left fixed in the BSC for the next day.</p>
2	<p>If smears are to be read the day of staining, turn on the fluorescent microscope to "warm it up". See MIC81400 Leica Fluorescent Microscope.</p> <ul style="list-style-type: none"> Microscope is required to be turned on for 15 minutes before use to obtain maximum florescence.
3	<p>Using a gloved hand, carry one or two slides from the metal tray to the TB sink and place them on the TB rack.</p> <ul style="list-style-type: none"> Space slides out so that the stain and water rinse run-off will not touch another slide. This may cause cross-contamination resulting in false-positives. Do not carry the metal tray to the sink. Leave in the BSC.
4	Add one QC Stain slide to the rack.
5	Completely flood all smears with Auromine-Rhodamine. Leave for 5 minutes
6	Using gentle water pressure, rinse slides in running water.
7	Completely flood all smears at the same time with Decolourizer. Leave for 30 seconds.
8	Using gentle water pressure, rinse slides in running water.
9	Completely flood all smears at the same time with Malachite Green. Leave for 1 minute.
10	Using gentle water pressure, rinse slides in running water.
11	Place slides in a white slide holder lined with gauze to drip dry or place slides in the O2 incubator to speed up drying. Keeping smears in the dark helps preserve fluorescence.
Expected results:	
Acid fast bacilli	orange/yellow/green bacilli
Background	no fluorescence (black)

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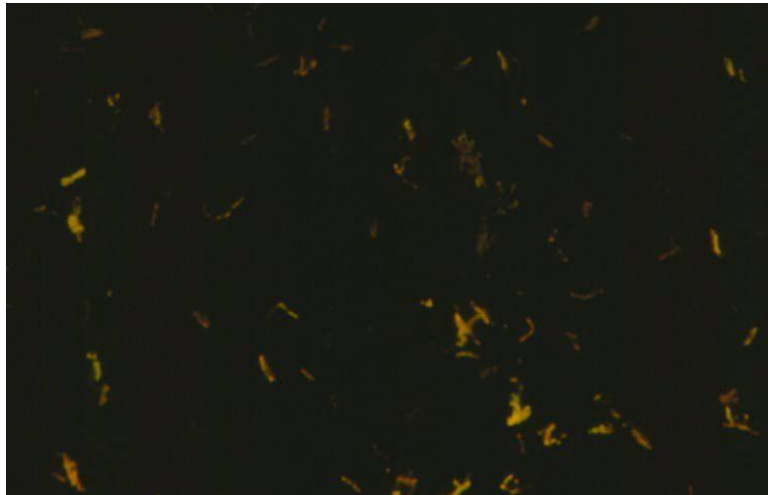


Image 1 (above) AFB seen in A/R direct smear.

Source: http://labmed.ucsf.edu/education/residency/sfgh_micro_img/index-sfgh-micro.html



Image 2 (above) Close-up of AFB seen in A/R direct smear.

Source: <https://www.studyblue.com/switch/cpt.html>

RELATED DOCUMENTS:

- MIC80400 Mycobacteria Processing
- MIC80810 Mycobacteria Smear Fixing.

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- MIC81300 AFB Stain QC & Slide Prep
- MIC81400 Leica Fluorescent Microscope

REFERENCES:

- Beckton Dickenson. (2014, August). TB Stain Kits and Reagents. *Product Insert*.
United States: Beckton Dickenson.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Jan2015	Initial Release	L. Driedger
	03Feb2015	Review	S. Webber