TITLE: AFB Stain QC & Slide Prep	Revision Date: 07-April-2017	Issue Date: 07-April-2015
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Distribution: Mycobacteria Manual	Page: 1 of 4	
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PURPOSE:

To standardize the preparation of AFB QC slides for Fluorescent and Kinyoun smears.

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.

MYCOBACTERIA STAIN QC SMEARS:

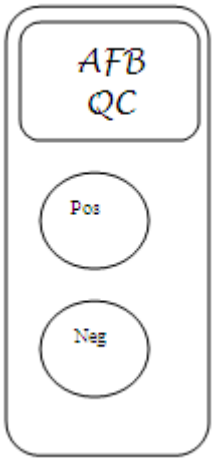
- Every batch of staining, either Fluorescence method or Kinyoun (cold ZN), require a control slide to be run in tandem with patient sample smears.
- The QC slide is the same for either staining method.
- QC slides are made in-house.
- The QC slide contains an AFB positive control (ie. *M. tuberculosis* ATCC# 25177) and negative control (*E.coli* ATCC# 25922).

PREPARATION OF AFB QC SLIDE:

Supplies:

- 0.9% NaCl (saline)
- Pencil
- Clean frosted glass slides
- Plastic Vitek tubes and caps
- Densicheck
- Vortex mixer
- Yellow 10 uL loops
- Bovine serum albumin
- Alcohol wipes

Step	Action
Prepare ½ a slide case or a full case if time permits. *Note: We're testing stain quality, not limits of detection, so an exact standardized microbial suspension is not required. Visually estimate that the density of E,coli and MTb are roughly the same and proceed with dispensing on the slide.*	
1	Place supplies inside TB BSC: • Slide box with clean frosted slides • Plastic test tubes, one filled with sterile 0.9% NaCl (saline). • Plastic caps • Pencil if not already inside BSC • Alcohol wipes
2	These steps may be performed outside the BSC: • Obtain *E.coli* # 25922 in the red QC organism box beside the Urine bench • Ensure Densicheck is QC'd. • Label a plastic tube. Make an approx 1-2 mF standard with *E.coli* in saline. • Bring the suspension into the TB lab when prepared. Add several drops of albumin into the tube. Albumin helps the bacteria adhere to the slides. • Place a yellow loop inside tube.
3	Obtain Mycobacteria control isolate from LJ slant in the 37° Celsius incubator. Bring into the TB BSC.

	• Match the density of the Mycobacteria suspension to the E.coli. • Add several drops of albumin into the tube. • Place a yellow loop inside tube.
4	Wipe slides with an alcohol wipe to remove glass particles or dust. Label slides with "*AFB QC*" using a pencil and place on metal tray rack.
5	Work one slide at time with one QC suspension at a time: • Place one yellow loopful of QC isolate to one half of the slide. • The positive control should be placed on top closest to the frosted end. Smear all slides with the one isolate and then switch QC suspensions • Place one yellow loopful of QC isolate to the other half of the QC slide, working one slide at a time. • The negative control should be placed at the bottom on the slide, farthest from the frosted end. 
6	Let smears completely air dry. Leave the TB BSC sash open.
7	Fix with 5% phenol alcohol. Leave the TB BSC sash open for 10 minutes Allow phenol to dry overnight in the BSC. TB BSC sash may be closed at this point.
8	Stack the QC slides in a slide box and place near the TB stains by the sink.

Expected results:

Stain	Expected results
Direct (A/R) and Kinyoun QC stain results:	
Modified Auramine Rhodamine	Positive: orange/yellow/green bacilli Negative: no fluorescence
Kinyoun	Positive: fuchsia/ red bacilli Negative: very pale blue/green background

If:	Then:
QC slide fails to produce expected results	Must re-stain all smears in the run

RELATED DOCUMENTS:

- MIC81500 Auramine Rhodamine Stain
- MIC81600 Kinyoun Stain
- MIC80810 Mycobacteria Smear Fixing

REFERENCES:

- Clinical and Laboratory Standards Institute (CLSI). *Laboratory Detection and Identification of Mycobacteria; Approved Guideline*. CLSI document M48-A (ISBN 1-56238-669-7). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2008.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31-Jan-2015	Initial Release	L. Driedger
	03Feb2015	Review	S. Webber