Reduction of Time For Heat Fixation of Mycobacteria Smears

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Backround

Previously SHL heat fixed smears for AFB stain at 65-75° C for two hours. Can we shorten this time?
Based on Bailey & Scott's Diagnostic Microbiology (12th Ed.) SHL proposed the new heat fixing protocol of heat fixing slides for 15 minutes at 80° C.

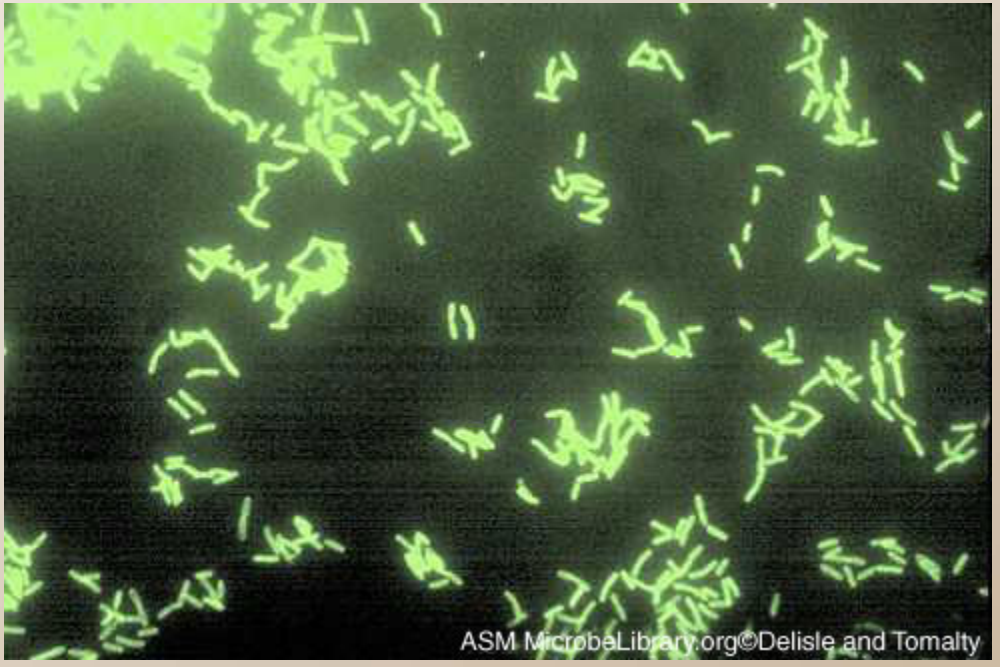
Questions

1. Will the quality of the Auramine-Rhodamine be affected by the higher temperature?

2. Will the shorter fixation time and higher temperature be able to sufficiently reduce the residual viability of the organism to the level of the original slide fixation time and temperature?

Goals

- ✓ Faster turn-around-time (TAT) for smear results
- ✓ Faster turn-around-time (TAT) for NAAT results



Protocol Step 1 – Slide Prep/Specimen Spike

Slide Prep

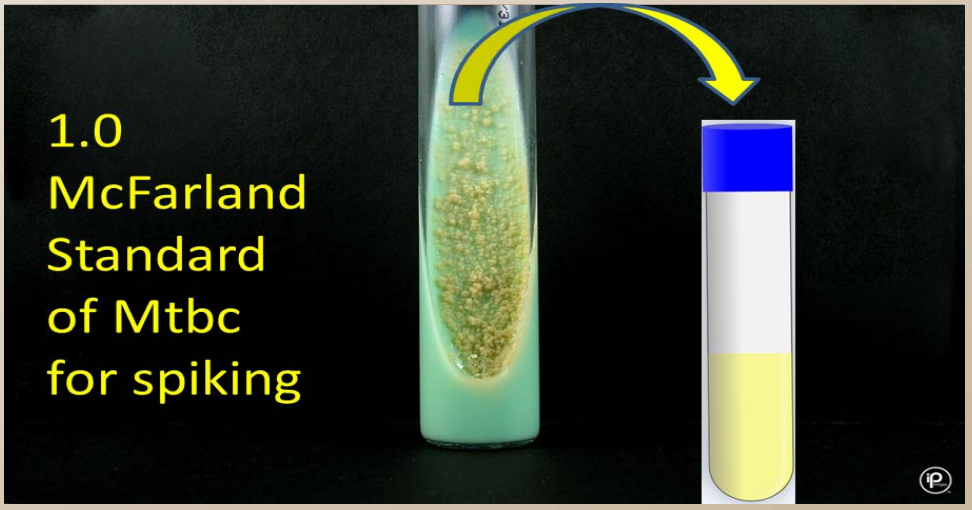
1.At least three days prior to starting the validation all slides to be used were cleaned by soaking them in straight bleach for 24 hours.

2.Slides were then placed in sterile DI water for 24 hours to rinse.

3.Slides were then placed in a 37° C incubator to dry.

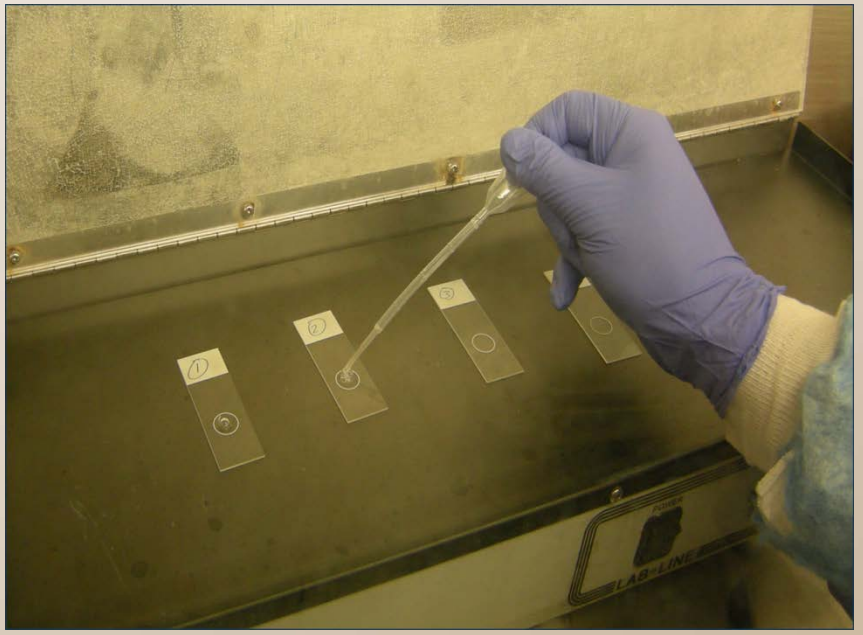
Specimen Spike

When ever possible patient sediment known to contain Mtbc were used. The remaining numbers of specimens were sediments spiked with a patient isolate.



Protocol Step 2 – Making Smears

- Validation was conducted by two different technologists on two different days to account for consistency and variability.
- 30 specimens tested; 20 by Ryan and 10 by Elizabeth
- Slide warmer was allowed to reach proper temperature before making smears.



#1 70°	

#1 80°	

Protocol Step 3 – Heat Fixing/Inoculation to MGIT

- All specimens had smears made in duplicate.
- One set was heat fixed at 70°C for 2 hours and the other set was heat fixed at 80°C for 15 minutes.
- 7H10 plates were inoculated with sediment from each sample to demonstrate viability of the organism.
- MGITS were inoculated by using a 10 ul loop to scrape off a portion of the smear material.
- MGITS were incubated for 10 weeks
- All slides underwent Auramine-Rhodamine staining to determine if the increased temperature affected the results.

Results

- Of 30 specimens tested, one specimen fixed at 70°C grew AFB.
- Zero specimens fixed at 80° C grew AFB.

Validation of Method to Heat Fix Smears for AFB at 80^{O} C for 15 minutes

				Fluorescent stain**		70^{O} C MGIT Broth^		80^{O} C MGIT Broth^		
Date	Tech	Sample #	7H10 plate*	70^{O} C	80^{O} C	Date	Result	Date	Result	Comments
3/18/2012	RJ	1	c/w TB	1+	2+	5/31/2012	Pos	5/31/2012	Neg	70 degree Controls
3/18/2012	RJ	2	c/w TB	Neg	3+	5/31/2012	Neg	5/31/2012	Neg	Pos = Pos Neg = Neg
3/18/2012	RJ	3	c/w TB	3+	2+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	4	c/w TB	4+	4+	5/31/2012	Neg	5/31/2012	Neg	80 degree Controls
3/18/2012	RJ	5	c/w TB	4+	2+	5/31/2012	Neg	5/31/2012	Neg	Pos = Pos Neg = Neg
3/18/2012	RJ	6	c/w TB	3+	3+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	7	c/w TB	4+	4+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	8	c/w TB	4+	4+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	9	c/w TB	2+	3+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	10	c/w TB	1+	4+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	11	c/w TB	1+	4+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	12	c/w TB	2+	3+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	13	c/w TB	2+	3+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	14	c/w TB	4+	4+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	15	c/w TB	3+	2+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	16	c/w TB	Neg	3+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	17	c/w TB	4+	4+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	18	c/w TB	Neg	2+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	19	c/w TB	Neg	3+	5/31/2012	Neg	5/31/2012	Neg	
3/18/2012	RJ	20	c/w TB	1+	3+	5/31/2012	Neg	5/31/2012	Neg	

*document that growth on 7H10 is consistent with TB (c/w TB) or if you do a Kinyoun document those results and write Kinyoun in the comment field

** document the results using the quantitaion normally used for patients (+/-; 1+ through 4+); record your QC results in the comments field

^document the date the MGIT flags as positive and the result of the Kinyoun OR document the last date of incubation and no growth (NG) if applicable

Conclusion

- Our validation demonstrated that heat fixing AFB smears at 80°C results in less viability of Mtbc on the slide. We also demonstrated that the quality of the Auramine-Rhodamine stain was not adversely affected by the increased temperature. In addition, the overall time from start of processing to reading fluorescent smears was decreased by about 1.5 hours, allowing staff to perform and complete other critical work in a timelier manner.

Some people have asked…

- SHL uses a Labline Slide Warmer (Barnstead International) Model 26025

- Potential Replacement
    1. Fisher Scientific Slide Warmer – 120 volts Cat. No. 11-474-521 ambient to 100°C

Acknowledgements

1. Forbes, B.A., Sahm, D.F., Weissfeld, A.S.(2007). Bailey & Scott's Diagnostic Microbiology (12th Ed.). St. Louis, MO.
2. Dr. Michael Pentella
3. Mary DeMartino
4. Elizabeth Albaugh

Questions?