

Verification of the Scientific Devices Modified Auramine O Stain Set in a low incidence TB setting.

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Abstract

Objective: As a result of the supply shortages caused by the SARS-CoV-2 pandemic, the State Hygienic Laboratory (SHL) at the University of Iowa completed a verification of the Scientific Device Modified Auramine O Stain Set.

Study Design: Evaluation of the Auramine O stain was completed by comparing to Remel Auramine-Rhodamine stain and acid fast bacillus culture.

Results: A total of 318 specimens, positive (n=24), negative (n=288) were included in the study. Eight samples failed and were excluded from the final analysis as a result of wash over due to user error. The sensitivity of the Modified Auramine O stain as compared to the Auramine-Rhodamine had a sensitivity of 83.3%, a specificity of 99.3%, and an overall accuracy of 95.6%. When the Auramine O and Auramine-Rhodamine stains were both compared to the acid fast bacillus culture results, each had a sensitivity of 37.2% (37.2%), a specificity of 97.7% (97%), and accuracy of 89.3% (88.7%) respectively.

Conclusion: The Modified Auramine O Stain Set offers the advantage of a rapid (2 minutes) with two steps versus the (19 minute) three step Auramine-Rhodamine stain procedure. The Scientific Device's Modified Auramine O Stain Set was also more readily available than the Remel Auramine-Rhodamine during supply shortages related to the COVID-19 pandemic.

OBJECTIVES

- Highlight multiple reagent options for fluorochrome staining
- Demonstrate the effectiveness of Scientific Devices Modified Auramine O Stain as compared to Remel Auramine-Rhodamine Stain and acid fast bacillus culture
- Describe the performance of fluorochrome stain in a low incidence TB setting

Acknowledgments/Sources

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Study Design

As outlined in the technical insert: "The SDL Modified Auramine O Stain Set uses a modified stain and quenching/decolorizing agent to make a rapid (2 minute) two step fluorescent staining procedure for the visualization of Mycobacteria organisms in clinical specimens". The Modified Auramine O stain set combines the decolorizer/quencher in the second step where the Remel Auramine-Rhodamine has a separate decolorizer and separate quencher (counterstain) step.

The preparation of patient smears using the Modified Auramine O stain used the following procedure:

1. Heat fix smear.
2. Apply Auramine O Stain on smear for 1 minute.
3. Rinse with deionized or tap water and drain slide.
4. Apply Decolorizer/Quencher reagent for 1 minute.
5. Rinse with deionized or tap water and drain slide.
6. Allow slide to air dry.
7. Slides were read with the Leica DM6 B Microscope using the FITC Long Pass Fluorescent Filter LED light source. Technical insert Rev 03 recommends to "read slide under mercury or halogen light source fluorescent microscope".

Verification Guidelines

- Test smears in parallel using Remel Auramine-Rhodamine staining method and Scientific Devices Modified Auramine O staining method for a minimum of three weeks.
- Positive/Negative Result agreement = 100%
- Smear grade (1+, 2+, 3+, 4+) difference between methods ≤ 1
- Minimum number of positive smears n = 20
- Minimum number of technologists n = 3

Figure 1: Scientific Device to Remel Result Agreement by Technologist

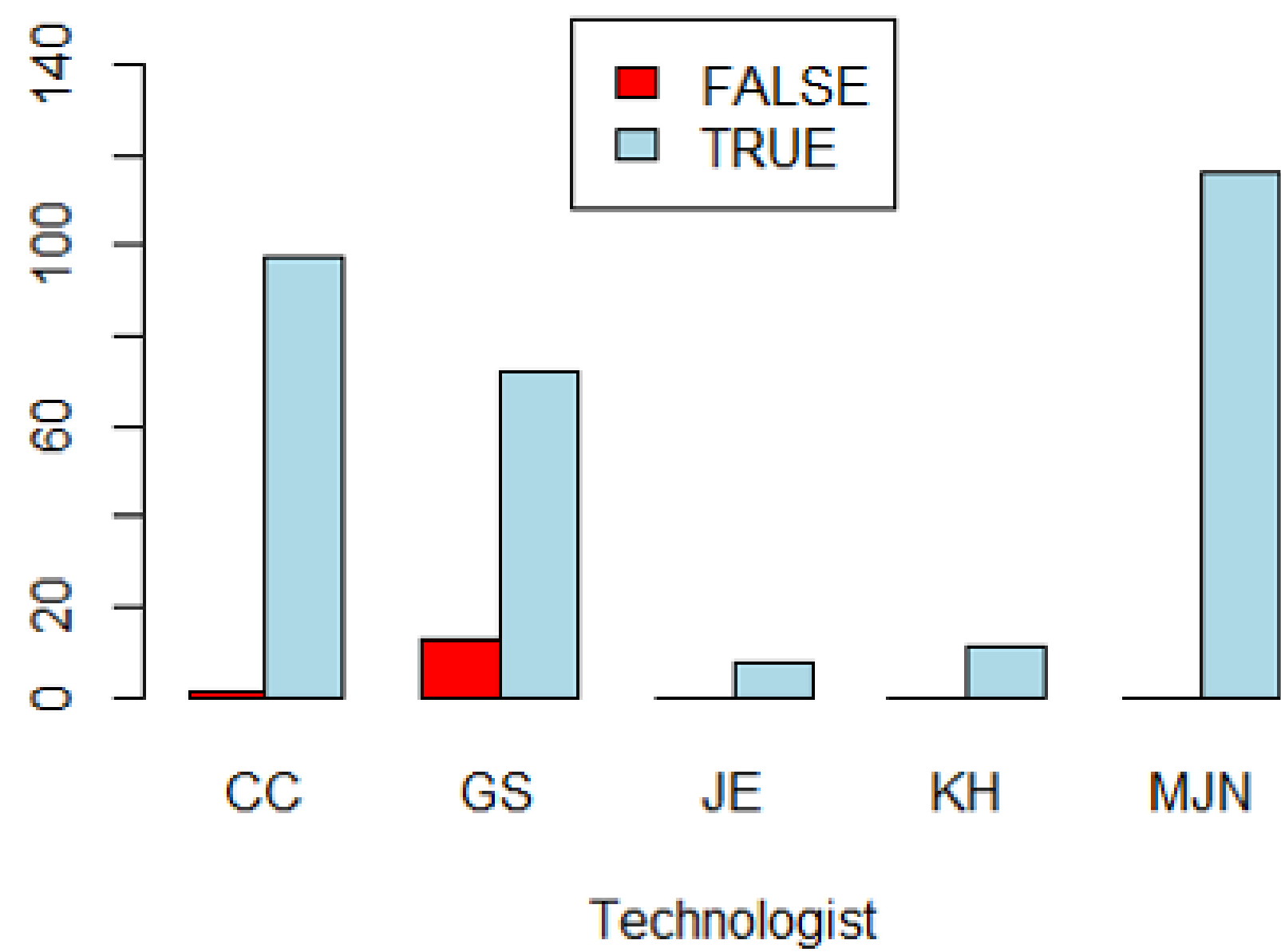
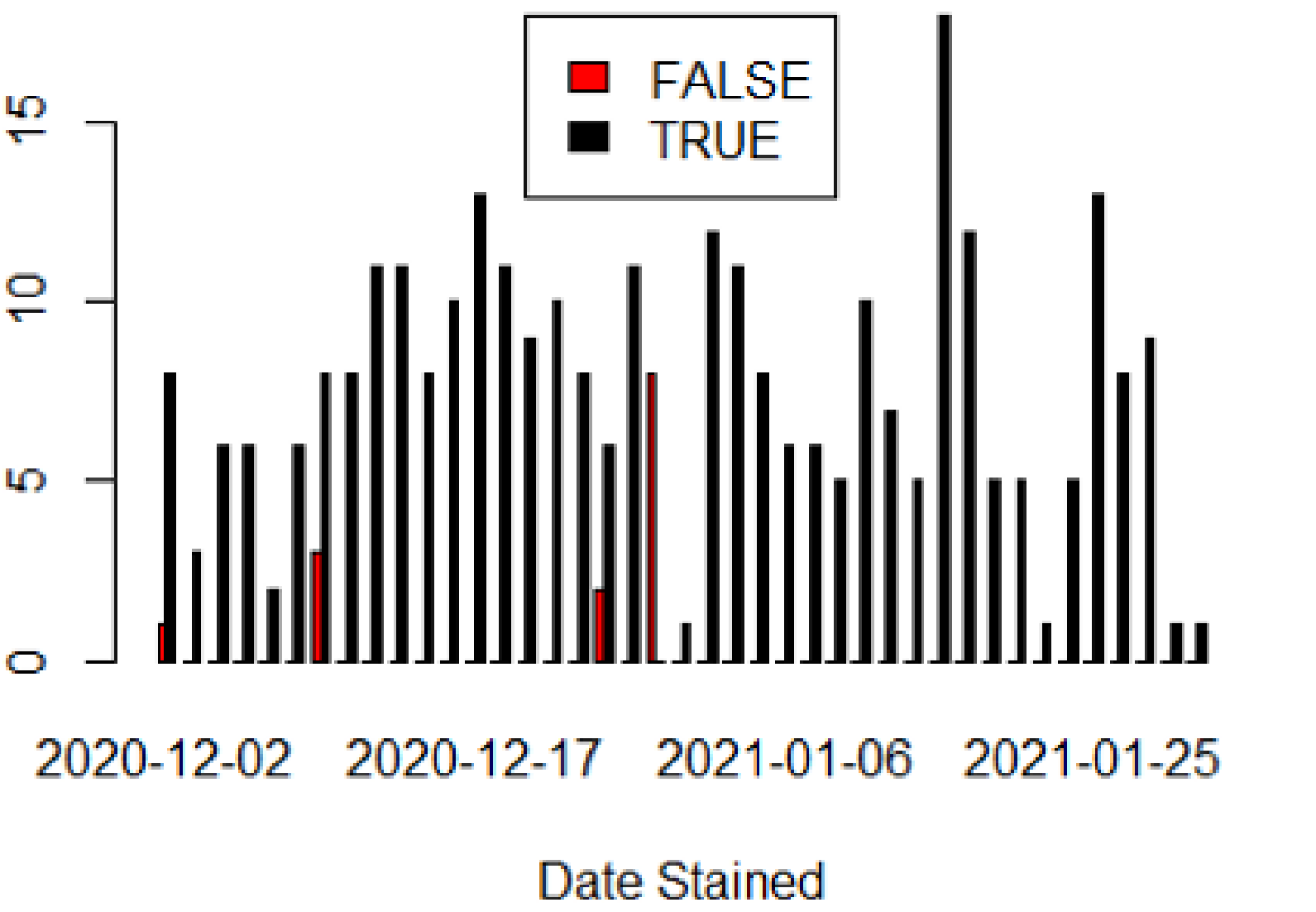


Figure 2: Scientific Device/Remel Agreement by Date Range



RESULTS

Results: A total of 318 specimens, positive (n=24), negative (n=288) were included in the study. Eight samples failed and were excluded from the final analysis as a result of wash over due to user error. The sensitivity of the Modified Auramine O stain as compared to the Auramine-Rhodamine had a sensitivity of 83.3%, a specificity of 99.3%, and an overall accuracy of 95.6%. When the Auramine O and Auramine-Rhodamine stains were both compared to the acid fast bacillus culture results, each had a sensitivity of 37.2% (37.2%), a specificity of 97.7% (97%), and accuracy of 89.3% (88.7%) respectively (Table 1).

- **Pass** - Minimum number of technologists n = 3 (Figure 1)
- **Pass** - Test smears in parallel using Remel Auramine-Rhodamine staining method and Scientific Devices Modified Auramine O staining method for a minimum of three weeks. (Figure 2)
- **Pass** - Positive/Negative Result agreement = 100%
- **Fail** - Smear grade (1+, 2+, 3+, 4+) difference between methods $\leq 1 = 6/7 = 86%$ (Figure 2)
- **Pass** - Minimum number of positive smears n = 20

Table 1: Remel and Scientific Devices Compared to AFB Culture

	Remel	Scientific Devices
Sensitivity	37.2%	37.2%
Specificity	97.0%	97.7%
Accuracy	88.7%	89.3%

CONCLUSION

In 2019, Iowa had a Tuberculosis case rate of 1.6 per 100,000 population according to the Centers for Disease Control and Prevention. While the verification failed to meet the smear grade guideline, SHL was able to demonstrate performance of the Scientific Devices stain set by comparing both stain sets to afb culture where it was shown that the two stains perform almost identically. The Modified Auramine O Stain Set offers the advantage of a rapid (2 minutes) with two steps versus the (19 minute) three step Auramine-Rhodamine stain procedure. The Scientific Device's Modified Auramine O Stain Set was also more readily available than the Remel Auramine-Rhodamine during supply shortages related to the COVID-19 pandemic.