Evaluating the Significance of Equivocal Mycobacterial Smear Results

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BACKGROUND

In 2014, in an attempt to increase smear sensitivity, the Indiana State Department of Health (ISDH) TB Lab began resulting certain Acid Fast Bacillus (AFB) smears as equivocal. Previously, when only one, two, or three AFB were observed on the entire slide, the smear was resulted as negative; however, these smears were now resulted as equivocal. This reporting change was prompted by a significant staffing turnover, which triggered a review of our reporting processes.

For all patients with an unknown TB status, real time PCR for *Mycobacterium tuberculosis* complex (MTBC) was performed on all sputa with equivocal results in order to rule out the presence of MTBC.

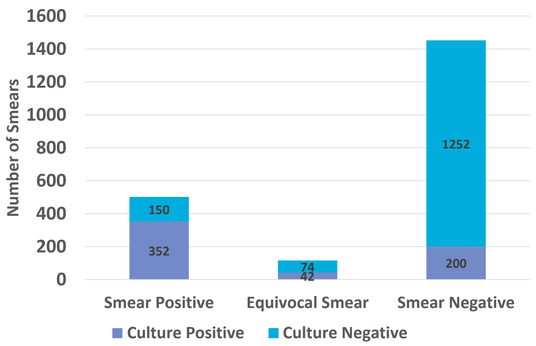
OBJECTIVE

To determine the significance of mycobacterial smear results when very few acid fast bacilli are observed in the concentrated smear

Table 1: Total Specimens Received (Sept 2015-Aug 2016)

Smear Result	Number	% of Total
Positive	516	24.6%
Equivocal	119	5.7%
Negative	1462	69.7%

Figure 1: Culture Results, Stratified by Smear

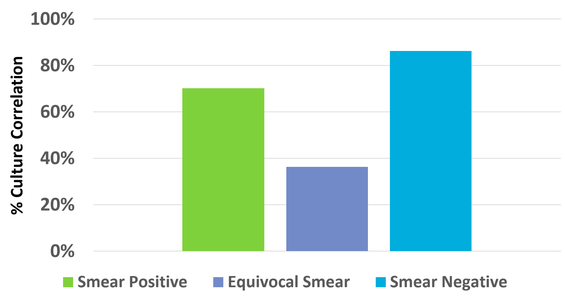


STUDY DESIGN

All sputum specimens were digested and decontaminated using standard protocol. Concentrated smears were prepared, fixed using 5% phenol in 70% ethanol, and stained using Auramine O-phenol stain. Using a fluorescent microscope, smears were examined for AFB at 200x magnification for a minimum of 30 fields. When AFB were observed, they were counted at 500x magnification, and the results were reported qualitatively. All results were blindly verified by a second analyst. When reading discrepancies occurred, a third analyst served as a tie-breaker. A smear was reported as equivocal if only one, two, or three AFB were observed on the entire slide, and as positive if greater than three AFB were observed.

Smear and culture data were collected for all specimens tested during a twelve month period and the smear-to-culture correlation was calculated separately for smear positives, smear negatives, and equivocal smears (Figure 2). Equivocal results were counted as smear positives for the purpose of calculating the culture correlation rate.

Figure 2: Smear-to-Culture Correlation Rates



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RESULTS

From September, 2015 through August, 2016, a total of 2097 sputum specimens were processed for smear and culture (Table 1). Of these, 24.6% (n=516) were smear positive, 69.7% (n=1462) were smear negative, and 5.7% were equivocal (n=119). 70.1% of the smear positive sputa resulted in a culture that was positive for one or more mycobacteria. 13.8% of the smear negative sputa resulted in a culture that was positive, and 36.2% of the equivocal sputa resulted in a positive culture (Figures 1 and 2).

For smear positive and equivocal smears with conflicting culture results, patients were categorized into three groups:

1. Sputa collected from a known TB patient
2. One or more NTMs were isolated from another sputa
3. All cultures for the patient were culture negative

The culture discordant rates were calculated for both equivocal and positive smears for comparison. Of the 77 equivocal smears that did not result in a positive culture, 71.6% (n=53) were collected from a previously diagnosed MTBC patient or were accompanied by one or more positive non-tuberculous mycobacteria (NTM) cultures (Figure 3).

Figure 3: Equivocal Smear, Culture Negative Specimens

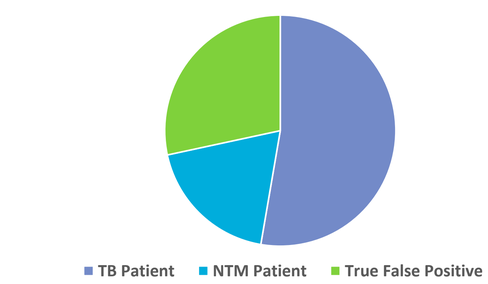
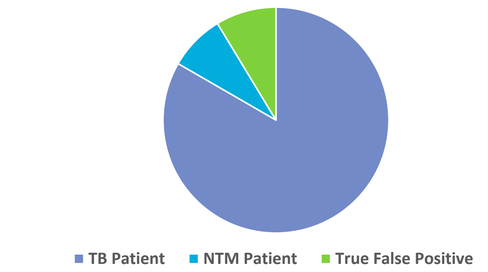


Figure 4: Smear Positive, Culture Negative Specimens



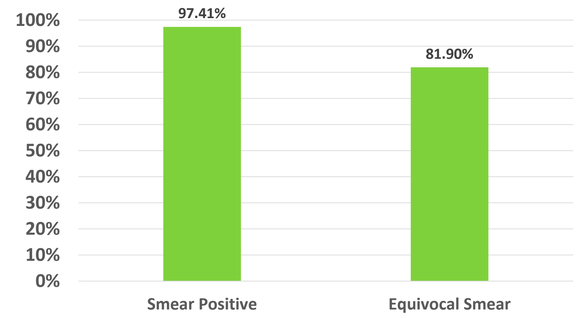
DISCUSSION

The majority of culture discordant equivocal and positive smears were collected from previously diagnosed MTBC patients or were accompanied by one or more positive NTM cultures, 71.6% and 91.3%, respectively (Figures 3 and 4). It seems likely that the majority of these smear results represent either non-viable MTBC bacteria or low levels of NTM present in the specimen.

Although none of the equivocal results in this limited data set became culture positive for MTBC, the results suggest that additional follow up and testing should be considered.

After adjusting the data to exclude discordant positive and equivocal smears from known TB patients and NTM patients, the specificity was calculated at 97.4% and 81.9% respectively (Figure 5).

Figure 5: Smear Specificity



CONCLUSION

In this data set, equivocal smears resulted in positive cultures approximately three times as often as negative smears. Therefore, even smears with very few visible AFB should be considered significant, as they are more likely to indicate an infectious patient status than a negative smear. For equivocal smear results collected from patients with an unknown TB status, reflex PCR testing should be performed to rule out MTBC, increasing the accuracy and speed of a TB diagnosis.