Dear Editor,

Microscopic examination of respiratory specimen for Acid Fast Bacilli (AFB) plays an important role in the initial diagnosis of tuberculosis as well as for monitoring of antitubercular treatment. Direct microscopy of sputum has a low sensitivity.[1] Previous studies have shown that concentration and liquefaction of sputum significantly improves the sensitivity of direct microscopy.[2] In the present study, we have compared the sensitivity of direct AFB smears to that with smear made after concentration of the specimen utilizing two different methods, N-acetyl-L-cystine (NALC)-NaOH concentration method and NaOCl liquefaction and sedimentation method, so as to evaluate an easy, less cumbersome, and cost-effective method to improve the sensitivity of direct microscopy for AFB, especially for developing countries and smaller peripheral centers.

Four hundred and fifty early morning sputum samples from suspected cases of pulmonary tuberculosis received at Designated Microscopy Centre (DMC) of Subharti Medical College, Meerut, Western Uttar Pradesh, were screened for AFB. Slides were prepared directly from the sputum samples (direct AFB smears).[3] The remaining sputum was thoroughly mixed and divided into two parts in 10 ml conical screw-cap tubes. The first part of sputum was processed by standard NALC with 2% NaOH digestion and decontamination method.[4] To NALC 2% NaOH solution equal volume of sputum was added and the mixture was vortexed for 20–30 seconds. For effective decontamination the tubes were kept at room temperature for 15 minutes. Then the tubes were filled with phosphate buffer (pH 6.8) and concentrated by centrifugation at 3,000 rpm for 15 minutes. The supernatant was poured off, the resulting sediment was resuspended in 1–2 ml of phosphate-buffered saline and the suspension was used to prepare the concentrated smears.

The second part of the sputum was processed by the NaOCl liquefaction and sedimentation method.[5] In an equal volume of sputum, 5% NaOCl was added. The tubes were incubated at room temperature for 15 minutes and shaken by hand at regular intervals. The tube was left on the bench at room temperature overnight (12–15 hours). The supernatant of each tube was carefully poured off, the sediment was mixed well with the remaining fluid, and 1–2 drops were transferred with a sterile pipette to a slide.

The slides made from all the three methods were coded, air dried, heat fixed, and stained by Ziehl–Neelsen technique.[1] The slides were reported as per the recommendation of the American Thoracic Society.[6]

Statistical analysis was performed by using Z test for proportion, to test the significant difference between the methodologies of detecting the positivity of AFB by NaOCl, NALC, and direct methods at 5% level of significance.

No significant difference between NALC and NaOCl was observed at 5% level of significance ($P > 0.05$), whereas a significant difference was observed between direct and NALC/NaOCl methods ($P < 0.05$). Further, by applying one-way ANOVA $F$ test for testing the difference between gold standard and the three methods, viz. direct, NALC, and NaOCl, a significant difference was observed between gold standard and direct methods. However, by Tukey’s method, it was found that gold standard was better.

The prevalence of *Mycobacterium tuberculosis* in clinically suspicious cases was 128/450 (28.4%). The NALC-NaOH concentration method and NaOCl liquefaction and overnight sedimentation method showed comparable results of detecting 28.4% and 28% cases, respectively. Direct microscopy, however, was the least sensitive detecting only 98 (21.7%) cases (Table 1). Interestingly, NALC-NaOH and NaOCl methods were also able to detect more positive samples 55 (42.9%) and 53 (41.4%), respectively, even with lower concentration of bacteria (i.e., scoring 1+ and scanty bacilli) as compared to direct microscopy, detecting only 26 (20.3%) cases. The gold standard (culture on L.J. media) showed 130 (28.9%) positive samples.

Though NALC-NaOH concentration method has been identified as the most sensitive method, due to limitation in funds and equipments this method is not being performed in smaller laboratories and hospitals in many parts of the country, leaving direct smear microscopy as the only

<table>
<thead>
<tr>
<th>Methods used</th>
<th>Positive</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Culture on L.J. Media</td>
<td>130</td>
<td>28.9</td>
</tr>
<tr>
<td>NALC-2% NaOH concentration</td>
<td>128</td>
<td>28.4</td>
</tr>
<tr>
<td>NaOCl liquefaction and sedimentation</td>
<td>126</td>
<td>28.0</td>
</tr>
<tr>
<td>Direct smear</td>
<td>98</td>
<td>21.7</td>
</tr>
</tbody>
</table>

*Number of samples ($N = 450$)
available method used for diagnosis of tuberculosis. Though direct smear microscopy has the advantage of being simple to perform, rapid, less cumbersome, and above all it is inexpensive it has a major disadvantage of having a discouragingly low sensitivity. Therefore, if only direct microscopic method was used for diagnosis, around 22.6% cases with lower AFB counts would have been missed in our study. The significant increase in the smear sensitivity from 98 in direct microscopy to 126 by NaOCl treatment in our study may be attributable to clearer microscopic field due to reduction of the debris. Further, NaOCl method has the advantage of being available at low cost as household bleach, which also limits the risk of laboratory infection as a potent disinfectant. However, it was observed that all the three methods were equally sensitive in detecting positive cases with higher count (i.e., scoring 2+ and more).

To conclude, the overnight delay in obtaining the results may be a drawback of the NaOCl sedimentation method, but the method has an advantage of being less cumbersome and cost effective to improve the sensitivity of direct microscopy without requiring any special equipments, thus can be easily used under the existing conditions of smaller laboratories and primary health centers particularly in a developing country like ours.

References

4. Kent PT, Kurbica GP. Public health Mycobacteriology, a guide for the level III laboratory. 1985; Centers for Disease Control Development of Health and Human Services, Atlanta, Ga.

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