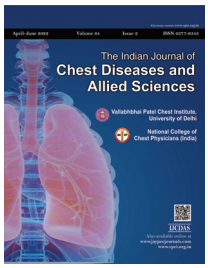
BRIEF COMMUNICATION

Evaluating a Modified Ziehl-Neelsen Technique with Triton X-100 for Detecting Acid-fast Bacilli in Sputum



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Anisha Garg[1], Biswaroop Chatterjee[2], Aarti Kotwal[3], Rishabh Bansal[4], Indranil Saha[5]

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Abbreviations used in this Article

AFB = Acid-fast bacilli; Z-N = Ziehl-Neelsen; PTB = Pulmonary tuberculosis; RNTCP = Revised national tuberculosis control program; CSF = Cerebrospinal fluid

Microscopy for acid-fast bacilli (AFB) is the first step in the laboratory diagnosis of pulmonary tuberculosis (PTB). Although fluorescent microscopy is more sensitive, light microscopy is widely used in low-resource settings because the equipment is cheaper. Therefore, in an attempt to improve the efficiency of light microscopy for AFB, we evaluated a published modification of Ziehl-Neelsen (Z-N) staining by comparing it with conventional Z-N staining for sputum smears. Both Z-N staining techniques were 81% sensitive and 99% specific when compared independently with fluorescent staining. Both techniques had positive predictive value (PPV) and negative predictive value (NPV) of 93% and 96%, respectively with a Kappa coefficient of 0.74, indicating a good agreement between the two. It was concluded that the modified Z-N technique with additional Triton-X 100 did not perform better than conventional Z-N staining when applied to homogenized sputum smears.

Mycobacterium tuberculosis is the leading cause of death from an infectious disease, ranking above the human immunodeficiency virus.[1] According to the World Health Organization, there were an estimated 10 million new TB patients in the year 2019 with 1.4 million deaths; India alone accounted for approximately one-fourth of each.[1] This is tragic because most patients of TB can be cured with timely diagnosis and correct treatment.

Pulmonary TB (PTB) is the commonest clinical form of TB. Microscopy remains the cornerstone of its diagnosis, and about 41% of all the patients are diagnosed by microscopic examination of sputum for AFB using either a light or fluorescent microscope.[2] The biggest shortcoming of light microscopy is its relatively low sensitivity with a detection threshold as high as 10^4 AFB per gram of sputum.[3] This has been partly addressed by staining smears with phenolic auramine O and examining them under a fluorescent microscope.[4] This significantly increases the diagnostic yield; unfortunately, fluorescent microscopes are several times more expensive than light microscopes, and therefore, not available in many laboratories in the developing countries.[5]

In this context, a recent publication describing an improved Z-N staining technique is significant as it has the potential to improve the sensitivity of microscopy even with a light microscope. The enhanced sensitivity of the technique depends on the ability of Triton X-100 to permeabilize both the lipid-rich cell wall of mycobacteria and the cell membranes of host phagocytes to improve the staining of AFB lying both outside and inside phagocytes.[6]

Since sputum comprises the majority of the specimens in a diagnostic laboratory, this study was planned to compare the modified Z-N technique with the conventional Z-N technique on smears of homogenized sputum specimens. The study population consisted of patients presenting with productive cough of more than 2 weeks duration to Himalayan hospital, Uttarakhand during the period of data collection; these patients yielded a total of 84 non-replicate sputum specimens, and thus, a complete enumeration method was followed.

Modified Z-N staining with additional Triton X-100 was performed as described in the article by Chen et al.[6] Z-N staining by standard technique and Auramine O staining were performed as specified by the Revised National Tuberculosis Control Program (RNTCP) of India.[7,8] Each technique was controlled every day using smears of *M. tuberculosis* strain H37Ra. The slides were examined and results were recorded according to the grading scheme of the RNTCP. Smears that would have been labelled "doubtful" in the RNTCP scheme, were considered scantily positive and labelled "scanty" for the purpose of the present study after getting the identity of the AFB confirmed by two independent and experienced observers. The specimens were neither cultured nor assessed with CBNAAT.

[1–3]Department of Microbiology, IQ City Medical College, Durgapur, West Bengal, India; Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India

[4]Maulana Azad Medical College, New Delhi, Delhi, India

[5]Department of Community Medicine, IQ City Medical College, Durgapur, West Bengal, India

Corresponding Author: Biswaroop Chatterjee, Department of Microbiology, IQ City Medical College, Durgapur, West Bengal, India, e-mail: doctorbiswaroopchatterjee@gmail.com

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Auramine O-stained smears were clearly positive (1+, 2+ or 3+) in 13 specimens, clearly negative in 68 specimens and scanty in 3 of the 84 specimens. The performance of both the modified Z-N staining with Triton X-100 and the conventional Z-N staining was found to be similar when compared to the fluorescent staining in the 84 specimens.

Both conventional Z-N and modified Z-N staining performed equally well with a sensitivity of 81% and a specificity of 99% when compared to fluorescent staining. PPV and NPV were also identical for both the techniques at 93% and 96%, respectively. Since both the techniques, i.e., conventional and modified Z-N staining, had been applied to the same set of specimens, the two tests were compared with each other by Kappa coefficient for agreement. Kappa coefficient was found to be 0.74, which indicates good agreement. Our study failed to show any difference between the conventional and the modified Z-N staining techniques.

The findings of the present study are very different from that of Chen et al.,[6] who found that the modified Z-N staining technique had a sensitivity of 100% compared to only 16.8% for the conventional Z-N staining technique in a sample of 48 cerebrospinal fluid (CSF) specimens from 29 patients with "gold standard" culture-positive tuberculous meningitis. Feng et al. reported the sensitivity of the modified Z-N technique was 82.9% in contrast to only 3.3% for conventional Z-N staining in a sample of 37 patients with tuberculous meningitis diagnosed by positive cultures and 203 patients with probable or possible tuberculous meningitis diagnosed by the published clinical criteria.[9]

There are two possible reasons for the lack of an additional advantage of the modified Z-N technique, in comparison to the experience of the inventors of the technique: (i) the main advantage of the modified Z-N technique lies in the ability of Triton X-100 to permeabilize the cell membranes of phagocytes and the mycolic-acid containing waxy cell walls of mycobacteria to allow better penetration of carbolfuchsin. However, this advantage did not apply to our specimens, all of which had been homogenized with Petroff's technique which disrupts phagocytes and partially damages the cell walls of AFB. We homogenized our sputum specimens to ensure the uniformity of all three smears to eliminate the confounding variable of uneven smear quality during the analysis of results. This limitation does not apply to CSF specimens which are liquid and homogeneous, ensuring the uniformity of all smears made from them, and (ii) the presence of mucus and other substances, which are present in sputum but absent in CSF, possibly interfered with the action of Triton X-100 in some or the other way.

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