

Performance of Modified Universal Sample Processing Method in a Field Study in Uganda

We developed the universal sample processing (USP) methodology and demonstrated its excellent performance in several validation studies using blinded samples (1–4). An article titled “Poor performance of Universal Sample Processing Method for Diagnosis of Pulmonary Tuberculosis by Smear Microscopy and Culture in Uganda” was published recently in the *Journal of Clinical Microbiology* (5). In this study, smear sensitivities and specificities were not significantly different in a modified USP protocol that was used and the standard *N*-acetyl-L-cysteine-NaOH (NALC-NaOH) method. The authors also noted that mycobacterial cultures were more frequently negative in the former method. This in itself is not entirely surprising since it is well known that even established diagnostic techniques, not to mention new and novel methodologies, vary widely in performance from very poor to excellent depending on the study settings.

It is most important that new diagnostic techniques are evaluated in well-designed studies, and we thank Cattamanchi and coworkers for carrying out an independent assessment of USP methodology in Uganda (1). However, we wish to highlight some concerns that we have about the misrepresentation of “USP methodology.” While referring to it as the “USP method,” the authors have actually used a *modified* version of the USP protocol. Based on a careful examination of their findings, we believe that the “poor” performance of the USP method is likely to be a consequence of using a modified USP protocol.

Critical aspects of the method were altered. First, bacteria were sedimented at a lower centrifugation speed (3,000 × *g* instead of the recommended 5,000 to 6,000 × *g*). This is likely to be a crucial modification since the USP protocol advocates a higher centrifugation speed. Second, the authors do not mention how much of the processed sample was applied; while the NALC-NaOH method advocates the use of only two loopfuls, the USP method prescribes the application of 10% of the processed sample to the slide (2–5). Third, the use of fluorescence microscopy (which is not advocated by USP methodology) likely increased the sensitivity of the NALC-NaOH method without enhancing the detection of acid-fast bacilli on USP slides that are remarkably free of background interference. Fourth, culture sensitivity could be compromised by inefficient bacterial sedimentation, incomplete removal of guanidinium hydrochloride (GuHCl), or the use of 4 to 6 M GuHCl as opposed to the 4 M GuHCl recommended for optimal culture sensitivity. Finally, our previous validation studies were carried out under laboratory conditions, where the finer technicalities of the USP method were strictly adhered to. These stringent procedures might not have been possible in this first field application of the “modified USP” method.

The authors mention that the results of the USP smear method were not significantly different from those of the NALC-NaOH method but contradict this statement by defining its performance as “poor.” They also noted that compared to the NALC-NaOH method, the USP method yielded a greater proportion of scanty positive results in support of its stated efficacy in detecting paucibacillary specimens. On this basis and in light of the modifications in the protocol, the title

“poor performance” is not justified and the authors should refer to it as “modified USP method.”

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Authors' Reply

We thank Dr. Chakravorty and colleagues for their letter giving us the opportunity to discuss accurate comparison and reporting of smear microscopy methods for the diagnosis of tuberculosis.

The primary goal of our study was to determine whether sputum processing by the universal sample processing (USP) method instead of the standard *N*-acetyl-L-cysteine-NaOH (NALC-NaOH) method increases the accuracy of smear microscopy for diagnosis of pulmonary tuberculosis. We found no difference in sensitivity or specificity between the two methods when performed at a national reference laboratory in a low-resource, high-tuberculosis-burden country.

We acknowledge that the USP method was modified in our study, as described in Materials and Methods in our article. These modifications standardized key parameters of the two sputum-processing techniques, including centrifugation speed and amount of specimen smeared on a slide (two loopfuls for each method) and may account for the poor performance of

the USP method in our study relative to that in previous validation studies. However, unlike these previous studies, our standardized approach avoided biasing results in favor of the USP method, which calls for a higher centrifugation speed and a greater amount of specimen per slide than the NALC-NaOH method. Indeed, our findings suggest that when tested under equivalent conditions, the novel chemical processing associated with the USP method does not increase the diagnostic accuracy of smear microscopy.

Dr. Chakravorty and colleagues also suggest that fluorescence microscopy is not “advocated” with the USP method and that it would not enhance “detection of acid-fast bacilli on USP slides that are remarkably free of background interference.” To address this concern, we went back and analyzed results that were available from 241 patients who had both USP- and NALC-NaOH-processed smears read by light microscopy (Ziehl-Neelsen stain). The USP sensitivity was actually lower (45% versus 58%, a difference of 13%; 95% confidence interval [CI], 2 to 22%; $P = 0.01$) and the specificity similar (82% versus 87%, a difference of 5%; 95% CI, -4% to 13%; $P = 0.22$) compared to that of NALC-NaOH when using light microscopy.

Lastly, we agree with Dr. Chakravorty and colleagues that the stringent conditions in their laboratory-based evaluations were not likely replicated in the non-research setting employed in our study. However, it is worth noting that the Uganda National Tuberculosis Reference Laboratory has greater experience and expertise than is present in most peripheral laboratories in low-income countries. Our intent was to test the USP method in a field setting. The efficacy of the USP method under conditions typically present in field laboratories should

be demonstrated if it is to have significant impact on the global tuberculosis epidemic.

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