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Read the new microscopy handbook: even the Ziehl-Neelsen technique has changed

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The recent publication in this *Journal* by Das et al. highlighted the Ziehl-Neelsen (ZN) staining method currently in use around the world.[1] We would like to point out that the authors have referenced and used an older recommended concentration of carbol fuchsin, 0.3%, as the comparative method, although the World Health Organization (WHO), the International Union Against Tuberculosis and Lung Disease (The Union) and partners revised the recommended concentration of carbol fuchsin to 1% after much deliberation,[2] taking into account comparisons with different concentrations of stains.[3] The authors' use of the previously recommended lower concentration of carbol fuchsin may have caused bias, as the currently recommended 1% concentration has been shown to be more reliable, in particular improving sensitivity.

As microscopy is still the primary diagnostic tool for tuberculosis (TB), with an estimated 83 million smears performed for diagnosis each year, most using the ZN staining method, it is important to reiterate the recommended method and the fuchsin concentration.[4] To provide some background, the original stain concentration was mentioned as 1% carbol fuchsin.[5–7] The visualisation of acid-fast bacilli (AFB) by the ZN staining method is primarily dependent upon the quality of the basic fuchsin, and questions about the concentration and quality of fuchsin in country settings led to the above-mentioned studies optimising the fuchsin concentration for ZN. A detailed explanation of the recommended use of 1% hot carbol fuchsin was also provided in the 2007 counterpoint.[2] The recent Global Laboratory Initiative (GLI) publication also recommends use of 1% carbol fuchsin concentration.[8]

One possible reason for using the older WHO/Union manuals as reference is that it may not be apparent in the literature that the GLI manual is based on participation and consensus between the WHO and The Union and therefore replaces the older manuals. The apparent confusion over the recommended carbol fuchsin concentration in this study, and its subsequent publication in the IJTLD, highlights the need for technical partners and countries to focus more on the quality of this primary diagnostic method in parallel with efforts to implement the fluorescence microscopy method and newer molecular diagnostics.

Conflicts of interest: none declared.

Furthermore, the Ebola outbreaks have highlighted the global health security agenda, which includes reliable sputum smear microscopy as both an indicator and a core test for strengthening national laboratory systems.[9]

Reliable sputum smear microscopy remains a key priority for the diagnosis of TB. We encourage continued research on methods with more practical application to improve the diagnosis of TB, and we strongly encourage national tuberculosis programmes to review current AFB microscopy manuals in use and align them with the GLI microscopy handbook and technical resources for best practice in TB control.

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