Possible Sources of Error
In Examination of
Stained Bacteriology Slides

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From time to time, microscopic results are not confirmed by culture techniques, and in some cases, the results of staining procedures are incompatible with the clinical picture. These variances occur primarily with acid-fast stains and the gram stain on a wide variety of specimens.

From past experience, a review of methodology was made and it was found that these discrepancies could be accounted for by the contamination of the immersion oil, the oil immersion lens or from blotting or bibulous paper used to dry slides. Thick or heavy slides, as generally prepared for tuberculosis examination, more often were problems, as excess material tended to flake off into the oil or onto the blotting paper.

These situations can readily be reproduced in the laboratory. If one takes a thick, positive TB slide—particularly from a culture or sputum concentrate—the organisms can be transferred to the oil reservoir by the rod or dropper if it touches the slide, and sufficient oil and stained organisms remain on the oil immersion lens.

If one examines a heavy positive TB slide, removes the slide and substitutes a clean slide, on refocusing one can readily find acid-fast organisms on the clean slide. If accidental transfer occurs to the oil, the organisms generally can be noted to be floating in the oil and generally are situated above the background counter stain, which often requires a slight readjustment of focus.

With gram or TB stains, blotting paper can be readily contaminated and this contamination can be transferred either to clean slides or other stained slides. Take stained blotting paper previously used to dry stained slides, moisten a clean slide, dry by blotting with the contaminated paper and examine microscopically. More often than not, stained microorganisms can be found on the clean slide.

A review of standard texts and references generally used in the laboratory reveals little or nothing. Cruikshank\textsuperscript{1} is the only author who notes the possible contamination of the oil reservoir, the oil immersion lens and the hazards of blotting paper and then only in relation to TB stains. Bailey and Scott\textsuperscript{2} and Stokes\textsuperscript{3} suggest use of clean blotting paper, but with no reasons stated.

As one must be meticulous in the preparation of slides for microscopy and in staining, one must also avoid negating the precise technique by contamination of the immersion oil or the transfer of microorganisms from previously used blotting paper.

It is recommended that one avoid these potential sources of false-positive findings by never smearing immersion oil on slides, and by wiping the oil immersion lens after each examination or at least after every positive TB slide. Air dry slides, preferably, or be sure that the blotting or bibulous paper is used only once. Books of blotting paper should not be used.

References

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