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Lab automation

Staining of acid fast bacteria using the new RAL-stainer automate

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Background:

The RAL-stainer is a compact and fully automated commercial unit for the staining of microscopy slides. The automate can be used for several staining protocols such as Gram-staining or staining for acid fast bacteria (AFB). In this study we compared the results of staining for AFB with the Cold ZN kit on the RAL-stainer with the current in house modified Ziehl-Neelsen (MZN) protocol for both direct smears of clinical samples and smears from culture media for Mycobacteria.

Material/methods:

In total, smears of 42 samples (mostly respiratory) were included; 18 smears of clinical samples and 24 smears of positive mycobacterial culture media (Mgit tubes, Löwenstein slants and BacT alert bottles). All smears were prepared in duplo. One slide was stained manually after heat fixation for 15 minutes with Ziehl-Neelsen carbol-fuchsin solution (Merck 1.09215.0500), and counter-stained and decolorized with acidified methylene blue solution. The remaining slide was colored on the RAL-stainer according to the instructions of the manufacturer (1 minute in a fixative solution, 10 minutes in fuchsine solution, 3 minutes in a decolorizing solution followed by 1 minute counter-staining with methylene blue; Kit RAL Stainer Cold ZN, ZR360240-0000, MLS nv). All samples with a positive AFB smear were analyzed by PCR for identification purpose.

Results:

The result of all sixteen smears of clinical samples were concordant (15 negative, 3 positive). Nineteen of the smears (79%) of the positive cultures were positive with the MZN staining of which 1 was negative on RAL-stainer. It was a smear made of a colony of *Mycobacterium chelonae* on a Löwenstein-Jenssen slant. All negative stainings were concordant.

Conclusions:

In conclusion, the RAL-stainer combined with the Cold ZN kit seems to be a promising and easy method for the staining of AFB. Since the limited amount of samples in this study, these results should be confirmed by other studies. The RAL-stainer reduces the biohazard by its design (closed containers and a closed ventilated system during staining).