Comparison of the RAL Stainer System and the BD TB Fluorescent Stain Kit T for staining smears from pulmonary specimens: quality and time study

Sunday, May 11 • P 0963

C. Caukovitis, A. Adam
Ege University Medical Faculty, Department of Clinical Microbiology, Bornova Izmir, Turkey

Objectives:
The aim of this study was to compare an automated mycobacteria staining system – the RAL stainer system – with a manual staining method in terms of performance and quality of staining and to demonstrate that the RAL system can be used without generating cross-contamination. In addition, 2 protocols for the RAL stainer were evaluated: the conventional protocol according to the package insert (39 min) and a shorter variant (20 min).

Methods:
In this study, 100 pulmonary samples (80 sputum and 20 pulmonary specimens other than sputum: bronchoalveolar lavage, bronchoscopic aspirate, postbronchoscopic sputum and deep tracheal aspirate) from routine testing were investigated. 10 negative and 10 positive samples were selected based on the culture results and were processed in the same run to evaluate cross-contamination. Clinical specimens were processed by the conventional N-acetyl-L-cysteine–NaOH method for decontamination. Then, three smears were prepared on slides for staining: one for manual staining (BD TB Fluorescent Stain Kit T), one for RAL stainer with conventional protocol and one for RAL stainer with shorter protocol. One positive control slide (+H37Rv) and one negative control slide were used for each run. Smears were examined blind with a fluorescent microscope at a 450 x magnification according to Kent/Kubica criteria. The presence of artifacts, color brightness and easiness of reading were scored for each slide.

Results:
All positive controls were found positive, all negative controls were found negative for each run. The levels of agreement between results obtained with the manual staining protocol and the RAL stainer using conventional and short protocols were 84% and 82% for smear grade, 92% and 85% for presence of artifacts, 86% and 89% for brightness of color, and 91% and 89% for easiness of reading respectively. Cohen’s Kappa coefficients between manual and RAL stainer using conventional or short protocols were 0.79 and 0.76 respectively which is considered as excellent. When we tested negative and positive samples according to the culture results in the same run, no case of cross-contamination was observed.

Conclusions:
The study demonstrated the safety of the RAL system and showed equivalent performance using the conventional or short protocol compared to manual staining for smear grade evaluation. The RAL system offers improved brightness of color over the manual protocol and there is no significant difference in terms of easiness of reading between two methods. Using the short protocol, slides can be ready to read for mycobacteria screening within 20 minutes compared to more than 30 min currently using manual protocol. The RAL system can be used in specialized labs requiring a medium to high throughput system for mycobacteria staining in their daily practice.

Performance of VIDAS® Anti-HCV as a second-line test for the detection of anti-HCV antibodies

Monday, May 12 • P 1400

Furlini G., Foschi C., Nardini P., Galli S., Mezzoni MG., Landini MP.
Microbiology Unit, Sant’Orsola-Malpighi Hospital, Bologna, Italy.

Objectives:
Detection of specific antibodies is crucial for routine diagnosis of Hepatitis C virus (HCV) infection. Nowadays, many commercial tests based on chemiluminescence (CMIA) technology are available as highly-sensitive screening methods. In case of weak reactivity on first-line assays, further investigations are required to discriminate true-positively from biological false-positively. For this purpose, immunoblot tests are used due to their high specificity. Nonetheless, final interpretation issues and unsatisfactory cost-effectiveness strongly limit their usefulness in daily practice. In this study, we evaluated the performance of VIDAS® Anti-HCV (Innogenetic) as a second-line test for the detection of anti-HCV antibodies in order to limit the use of immunoblot in our laboratory setting. As preliminary data, we report on the concordance of VIDAS® Anti-HCV with RecomLine HCV IgG (Mikrogen) in a group of selected sera.

Methods:
In our laboratory, testing for anti-HCV antibodies was first performed on ARCHITECT Anti-HCV (Abbott), a fully-automated, high-throughput chemiluminescent assay. Sera were scored as positive or negative on the basis of the signal/cut-off result (cut-off=1). In case of weak positivity and in certain clinical or epidemiological settings, further tests based on a different technology were performed to clarify doubtful results. Until June 2012, a second-line test based on enzyme-linked immunosassay (INNOTEST® HCV/Ab N) was used. From July 2012, INNOTEST® HCV Ab IV was replaced by VIDAS® Anti-HCV, a third-generation test which combines a two-step enzyme immunoassay with a final fluorescent detection. Discordant results were arbitrated by RecomLine HCV IgG, a line immunoblot assay for qualitative detection of antibodies against individual antigens of HCV. To evaluate the performance of VIDAS® Anti-HCV, we compared the total amount of immunoblot assays performed during the year before and after the introduction of the new second-line test, in respect to the number of screening tests on ARCHITECT Anti-HCV. The concordance of VIDAS® Anti-HCV with RecomLine HCV IgG was calculated testing a panel of 127 sera with a s/co result ranging from 0.7 to 7.0 on ARCHITECT Anti-HCV.

Results:
During the year before VIDAS® Anti-HCV introduction (July 2011-June 2012) a total of 634 immunoblot assays were performed. On the other hand, after its introduction (July 2012-June 2013) only 404 immunoblot tests were used as confirmatory method. Considering the total amount of anti-HCV screening tests, we noted a significant decrease of cases requiring immunoblot from 7.5% (634/83802) to 4.8‰ (404/83769). Among selected sera, VIDAS® Anti-HCV showed a concordance with RecomLine HCV IgG of 73% (93/127). Border-line results of RecomLine HCV IgG were considered as positive.

Conclusions:
In our experience, VIDAS® Anti-HCV showed a good diagnostic performance as second-line test for anti-HCV antibodies detection, limiting the use of immunoblot tests.