Evaluation of a “combination” ELISA kit and genotyping performance of Restriction Fragment Length Polymorphism among Hepatitis C Virus infected patients’ sera.

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ABSTRACT

Many developing countries are reluctant to intensively screen blood bank samples and employ genotype specific treatment strategy for Hepatitis C virus (HCV). This is mainly due to high costs, time and high technical skill requirements associated with Nucleic Acid Amplification Technology (NAT)-based tests. The study aimed to evaluate *Monolisa® HCV Antigen-Antibody Ultra* (Bio-Rad Laboratories Limited, Marnes La Coquette, France), a new combination ELISA assay designed to detect in parallel antigens for and antibodies to HCV, and further determine the genotyping performance of Restriction Fragment Length Polymorphism (RFLP) assay on HCV genotypes 1 to 4 samples. The study involved retrospective and prospective analysis of samples stored at the Max von Pettenkofer Institute (MvPI) and samples obtained from patients attending HCV treatment at the two main Ludwig Maximillian University hospitals in Germany. Sensitivity, Specificity and Predictive values of the new ELISA kit was evaluated and compared with the AXSYM HCV version 3.0 (Abbot Diagnostics, Germany), an antibody based ELISA kit. Seventy four samples were tested on the two ELISA assays while fifty PCR positive samples were genotyped by Restriction Fragment Length Polymorphism assay. The study further measured the viral loads of twelve samples using random primers and compared the results with the measurements obtained by 5'UTR specific primers. The two ELISA assays realized comparable results both recorded a similar sensitivity of 91% with positive predictive values of 100% and 98% for the two assays respectively. Specificity of *Monolisa® HCV Ag-Ab Ultra* was recorded as 100% with a negative predictive value of 87% against a specificity of 93% with a negative predictive value of 86% recorded for AxSYM. Two samples with high viral loads of 780.000 and 8.900.000 IU/mL were not detected by the *Monolisa® HCV Ag-Ab Ultra* assay. Genotyping of these two samples revealed genotype 1b, a HCV-subtype. Restriction Fragment Length Polymorphism assay genotyped and showed clear results on forty two (84%) samples, unclear results on six (12%) samples and conflicting results on two (4%) of the fifty samples genotyped. Although RFLP realized difficulty with genotype 2 samples, all other genotypes were easily genotyped. Finally the study showed similarity in the viral load measurements between random and specific primers. The study concludes that although *Monolisa® HCV Antigen-Antibody Ultra* assay depicts high sensitivity and specificity in detecting antibodies to HCV, it does not add further benefit to detect HCV infections by enhanced sensitivity due to the potential contingency to trace viral capsid antigens, a fact that needs further evaluation. On the other hand, RFLP is an effective genotyping tool among HCV genotypes 1 to 4. The study also reveals the importance of random primers and asserts the primers as a point of focus in the future projection in Hepatitis C genotyping. It recommends further evaluations of these assay platforms using Kenyan samples, as their introduction would be instrumental in HCV diagnosis and management in Kenya. This work provides a baseline for further studies on evaluation of antigen sensitivity of *Monolisa® HCV Antigen-Antibody Ultra*, restriction performance of various enzymes used in RFLP genotyping and performance of random primers in HCV diagnosis.