Comparative Evaluation of Three Commercial Automated Immunoassays: Architect (Abbott), Vidas® (BioMérieux) and LIAISON® XL (Diasorin), for the Detection of Antibody to Hepatitis C Virus

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Abstract: Serological diagnosis of HCV infection is primarily based on assays that detect specific IgG anti-HCV antibodies. However many of these tests share similar antigenic targets as the solid phase of the assay and therefore shared “non-specific” reactions are common. Thus, screening in a setting of low HCV prevalence can generate false-positive results. This has necessitated confirmatory algorithms to confirm the initial reactivity and have included an alternate EIA with a different assay format to the screening assay, recombinant immunobLOTS or HCV RNA detection. However, in May 2013, the Centre for Disease Control (CDC) published updated anti-HCV diagnostic guidance which focused on detection of active infection and the nucleic acid test is the cornerstone of this paradigm.

Annually over 70,000 samples are received at the National Virus Reference Laboratory for anti-HCV investigation. This study evaluated three commercial automated immunoassays: Architect (Abbott), Vidas® (Biomerieux) and LIAISON® (DiaSorin) for the detection of antibody to Hepatitis C virus using a panel of 95 well characterised serum samples. The assays showed comparable performance for the detection of anti-HCV. We identified that the samples with S/CO ratios of between 1.0-2.0 are often, based upon confirmatory testing, false positive reactions. However correct selection of anti-HCV assays is required to avoid duplication of “false positive” results, minimise costs and accurately identify HCV infected individuals.

Keywords: Anti-hepatitis C antibody, automated immunoassays.

1. INTRODUCTION

Hepatitis C virus (HCV) infection remains a major public health burden with up to 3% of the world's population having evidence of HCV infection with more than 170 million chronically infected [1]. New identifications of HCV infection will increase in the future with the associated health costs due to the morbidity, such as end-stage liver disease, cirrhosis, and hepatocellular carcinoma and mortality associated with HCV infection [2].

The diagnosis of HCV infection is generally based on serological assays that detect specific anti-HCV antibodies, HCV antigen and HCV RNA. Previously, the CDC recommended a testing algorithm based upon testing of blood specimens for anti-HCV by screening immunoassays followed by supplemental confirmatory tests if the screening assay is reactive but below a specific signal-to-cutoff ratio threshold [3]. Recently, the CDC updated their guidance for HCV testing by indicating that individuals with reactive results after HCV antibody testing should be evaluated for the presence of HCV RNA in their blood [4].

Anti-HCV assays have improved significantly since the first generation tests which utilised a single HCV recombinant antigen to the current assays which incorporate core (structural), NS3 protease/helicase (non-structural), NS4 (non-structural) and NS5 replicase (non-structural) proteins and demonstrates significant improvements in sensitivity, particularly, with regard to increased reactivity with the NS3 antigen and earlier detection of seroconversion [5]. However, despite these improvements, as the assays are still based upon the detection of only IgG and an indirect assay format, the time between HCV infection and the detection of anti-HCV is generally more than 40 days [6,7]. In addition, non-specific reaction can frequently occur which creates difficulty in the diagnosis of HCV infection.

The current study compares three commercial automated anti-HCV immunoassays: Architect Anti-HCV test (Abbott), Vidas® Anti-HCV (Biomerieux) and LIAISON® XL HCV Antibody assay (DiaSorin).

2. STUDY DESIGN

2.1. Patient Samples

Ninety five serum samples previously received at the National Virus Reference Laboratory for anti-HCV investigation were tested in parallel in the three assays.
2.2. CE Marked Automated Assays

2.2.1. ARCHITECT Anti-HCV Test (Abbott) is a Two-Step Chemiluminescent Microparticle Immunoassay

Firstly, sample, recombinant HCV antigen (HCV core, NS3 and NS4 proteins) coated paramagnetic microparticles and assay diluent are combined. Anti-HCV in the sample binds to the HCV coated microparticles. After washing, anti-human IgG acridinium labelled conjugate is added in the second step. After another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A relationship exists between the amount of anti-HCV in the sample and the RLUs measured. This assay was used to initially investigate the samples. Diagnostic sensitivity and specificity of this assay with 95% confidence intervals are 99.1% (96.77-99.89%) and 99.6% (99.45-99.71%). The intra- and inter-assay coefficient of variations were <6% and <10% respectively.

2.2.2. VIDAS Anti-HCV (bioMérieux) Test Combines a Two-Step Enzyme Immunoassay Indirect Sandwich Method with a Final Fluorescent Detection

Antigens (HCV core, NS3 and NS4 proteins) are coated on the interior of the solid phase receptacle (SPR) which also serves as a pipetting devise. In the first step, the sample is diluted and then cycled in and out of the SPR several times. The anti-HCV antibodies present in the sample binds to the HCV antigens. After washing, mouse monoclonal anti-human IgG (Fab) conjugated to recombinant alkaline phosphatase is cycled in and out of the SPR. After further washing, during the detection step, the substrate (4-Methylumbelliferyl phosphatase) is catalyzed by the conjugate enzyme into a fluorescent product (4-Methyl-umbilifarone) which is measured at 45nm. The intensity of the fluorescence is proportional to the concentration of antibody present in the sample. Diagnostic sensitivity and specificity of this assay with 95% confidence intervals are 99.1% (96.77-99.89%) and 99.6% (99.45-99.71%). The intra- and inter-assay coefficient of variations were <6% and <10% respectively.

2.2.3. The LIAISON® XL HCV Antibody Assay (Diasorin) Uses Chemiluminescence Immunoassay Technology for the Qualitative Determination of Specific Antibodies to Hepatitis C Virus

This method is an indirect assay designed with two recombinant antigens (core and NS4) specific for HCV coating magnetic particles (solid phase), while a third HCV antigen (biotinylated NS3) is provided lyophilized, as a separate reagent. In the first incubation, the biotinylated antigen is captured by strepavidin-coated magnetic particles, and HCV antibodies if present bind to the solid phase via the recombinant HCV antigens. In the second incubation step, a mouse monoclonal antibody to human IgG, linked to an isoluminol derivative reacts with the IgG. After further washes, a starter reagent is added and a flash chemiluminescence reaction is induced. The light signal which is proportional to the amount of isoluminol-antibody conjugate, is measured as relative light units (RLU) and is proportional to the amount of HCV antibody present in the sample. Diagnostic sensitivity and specificity of this assay with 95% confidence intervals are 100% (96.46-100%) and 99.5% (99.51-99.83%). The intra- and inter-assay coefficient of variations were <10% and <13% respectively.

2.2.4. INNO-LIA HCV Assay

The INNO-LIA HCV Score is a third generation line immunoassay which incorporates HCV antigens derived from the core region, the E2 hypervariable region, the NS3 helicase region, the NS4A, NS4B and NS5A regions. The antigens are coated as 6 discrete lines on a nylon strip and a further 4 control lines are coated on each strip. The test sample is incubated in a trough with the test strip. If present in the sample, HCV antibodies will bind to the HCV antigen lines on the strip. An affinity-purified alkaline phosphatase-labelled goat anti-human IgG (H+L) conjugate is added and reacts with specific HCV antigen/antibody complexes if present. Incubation with the enzyme substrate produces a colour, the intensity of which is proportional to the amount of HCV-specific antibody captured from the sample on any given line. Colour development is stopped with the addition of sulphuric acid. The intensity of the reaction on the control lines on each strip is used to assign the reactivity ratings for each antigen on that strip. Diagnostic sensitivity and specificity of this assay with 95% confidence intervals are 100% (98.9-100%) and 94.5% (91.8-96.3%).

2.3. Statistical Analysis

Statistical analyses were performed using Spearman rank correlation coefficient tests. ROC curve analysis was performed using Medcalc statistical software programme.

3. RESULTS

Ninety five samples were investigated: For 37 anti-HCV negative and 33 anti-HCV positive specimens, all
three assays were in agreement. According to WHO recommendations, all anti-HCV positive sera were retested. Ten samples generated an equivocal result on the Architect (S/CO between 0.9-1), however all were nonreactive on the XL HCV Antibody assay. Two samples were reactive using the Vidas, one was weakly positive (S/CO 1.6) and gave an indeterminate result when tested on the INNO-LIA HCV confirmatory assay. A follow up sample from this patient tested negative indicating that the original result was due to non-specificity and not HCV infection. The second sample gave a S/CO of 3.94 on the Vidas, however this specimen was negative on the XL assay. Fifteen samples gave weak positive results using the Architect (S/CO 1.001 to 4.999) and are shown in the Table 1. Thirteen of the 15 samples gave similar results using the Vidas and XL platforms. Further analysis revealed that 7 samples had S/CO values of 1-1.8, and 5 of these samples were negative on the Vidas and XL assays. Of the remaining 8 samples which had S/CO values of 2-4.5, six samples were reactive on the Vidas and XL assays. The remaining 2 samples were negative on the Vidas (0.89, 0.25) and weakly positive using the XL (1.1, 1.7). A follow up sample was requested to confirm and to clarify the patients HCV status, however none was received. Eight samples that were positive on all three automated systems were collected from patients who had previously confirmed HCV infection (Samples 3,4,8,9,12-15).

As shown in Figure 1, high value correlation coefficients between all three automated assays were observed; Architect vs XL (r=0.964) (Figure 1A), XL vs Vidas (r=0.948) (Figure 1B) and Vidas vs Architect (r=0.966) (Figure 1C). ROC curve analysis using results from the Architect as classification variable, gave an AUC value (95% CI) for the XL (0.948, 0.877-0.984) and the Vidas (0.927, 0.850-0.972). Sensitivity values (95% CI) were 89.6% (77.3-96.5) for the XL and 85.4% (72.2-93.9) for the Vidas. Both the XL and Vidas assays showed specificity of 100% for anti-HCV screening when compared to the Architect assay. However, a gap in the coverage of S/CO values can be seen in Figure 1A and 1B which appears to be due to the Vidas assay.

4. DISCUSSION

HCV infection may have an asymptomatic presentation and therefore, particularly in a population with low prevalence of HCV infection, accurate anti-HCV assays are essential. However, as many assays utilise similar HCV antigens as a solid phase, shared “non-specific” reactions frequently occur. Therefore,

<table>
<thead>
<tr>
<th>Sample</th>
<th>Architect</th>
<th>Vidas</th>
<th>Diasorin XL</th>
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<tr>
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Reference ranges for the Architect as negative, equivocal, weak positive and positive are (S/CO ratio <0.799, 0.800-1.000, 1.001-4.999 and >5.000) as defined by the National Virus Reference Laboratory, Dublin, Ireland. For the Vidas assay, values of >1 are positive and <1 are negative. In the Diasorin XL assay, >1 is reactive and <1 is nonreactive.
careful selection of anti-HCV assays is required to reduce these shared “non-specific” reactions. Recently a number of commercial automated anti-HCV assays have recently been launched. To our knowledge, this is the first study to assess the performance of these assays in relation to each other.

In the current study, 100% concordance was observed in all three assays for those samples which were clearly anti-HCV positive or negative. However, discrepancies were identified in specimens generating results which were close to the assay cut-off for each assay. The findings in our study show that the samples that produce results within a S/CO of 1-2 are often “false positive” and are negative for anti-HCV when they are analysed with supplementary tests such as the INNO-LIA HCV confirmatory assay or following the investigation of subsequent samples. Therefore, a S/CO threshold could be introduced, above which samples could be regarded as containing anti-HCV antibody. However, although our sample size is small and further studies are underway to include a larger panel of samples, it can be observed that 75% (6/8) of samples which gave relatively weak positive results using the Architect assay (S/CO of 2-4.5), were from patients with previously confirmed HCV infection or patients who had resolved HCV infection. Therefore, very careful consideration should be applied to determining this cut-off value. Based upon these results, it would appear that the Architect assay generated equivocal results which were negative in the other two assays, suggesting that the other two assays may be more suitable for screening for anti-HCV. However, there could be selection bias as the samples were initially tested using the Architect assay. The Vidas format is however not applicable for large scale screening whereas the Liaison XL is more suitable. In addition, a testing algorithm could be established using the different automated assays to identify anti-HCV infection and minimise the occurrence of shared “non-specific” reactions.

The specificity of serologic HCV diagnostics is problematic to ascertain since an appropriate gold
standard is lacking. Although several immunoblots for the confirmation of positive HCV EIA results are available, these tests have lost their clinical importance since the development of highly sensitive methods for HCV RNA detection as can be seen in the recent CDC guidelines [4]. Immunoblots however are mandatory to make the exact identification of serologically false-positive-tested individuals possible.

This study underlines that, since differences in the sensitivities and specificities of assays may be observed, the concomitant use of at least a supplemental confirmatory assay is necessary to establish an accurate HCV profile. These performance differences within commercial assays have to be known for a proper interpretation of the serological results and suggest using more than one assay to resolve difficult or borderline anti-HCV reactive samples.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interest exists.

DISCLAIMER

The use of trade names and commercial sources is for identification only and does not imply endorsement by the National Virus Reference Laboratory.

REFERENCES


