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Performance characteristics of the ARCHITECT® Anti-HCV assay

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Abstract

Introduction: The ARCHITECT Anti-HCV assay is a fully automated high throughput chemiluminescent microparticle immunoassay (CMIA) for the detection of antibodies to structural and nonstructural proteins of the hepatitis C virus (HCV). To further enhance the performance of this test, the assay was modified to improve the specificity for blood donor specimens.
Methods: The specificity of the enhanced ARCHITECT Anti-HCV assay was evaluated by screening blood donor samples randomly collected from various German blood banks, as well as hospitalized patient samples derived from Germany and the US. Additionally, antibody sensitivity was determined on commercially available anti-HCV seroconversion panels and on a commercially available worldwide anti-HCV genotype performance panel.
Results: Apparent specificity of the modified ARCHITECT Anti-HCV assay in a blood donor population consisting of 3811 specimens was 99.92%, compared to 99.76% for the current on-market assay. Additionally, antibody sensitivity was determined on commercially available anti-HCV seroconversion panels. Seroconversion sensitivity equivalent to or better than the current on-market product was observed by testing 33 seroconversion panels.
Conclusion: This study demonstrates that the modified version of the ARCHITECT Anti-HCV assay shows improved specificity for blood donor specimens compared to the current assay on market without compromising sensitivity. With the availability of the improved ARCHITECT Anti-HCV assay and the recent launch of the ARCHITECT HIV Ag/Ab Combo assay, the ARCHITECT system now offers a full hepatitis/retrovirus menu with excellent performance on a high throughput, random access, automated analyzer, ideally suited for blood screening and diagnostic applications.

Keywords: Hepatitis C; Anti-HCV; Immunoassay; Performance evaluation; Specificity

1. Introduction

The hepatitis C virus (HCV) is an enveloped positive-stranded RNA virus of the *Flaviviridae* family and has been demonstrated to be the etiologic agent of 90% of chronic non-A, non-B hepatitis (Bendinelli and Pistello, 2000; Alter, 1999; Choo et al., 1989).

HCV is a major global healthcare problem. The WHO estimates that up to 3% of the world's population has been infected with the virus, equating to more than 170 million carriers of HCV worldwide. Within this broad estimate, there is considerable variability in the prevalence of infection (Wasley and Alter, 2000). HCV infection is often asymptomatic; however, of those individuals exposed to HCV, the vast majority of HCV-infected individuals (>85%) become persistently infected, and up to 30% of these develop progressive liver disease, including persistent chronic infection and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) (Lauer et al., 2001; Richter, 2003; Poynard et al., 1997; Tong et al., 1995). Transmission is mainly associated with infected blood products or intravenous drug abuse, although other less common routes such as vertical or sexual transmission are reported (Hallam et al., 1993). HCV infection is infrequently diagnosed during the acute phase of infection. Clinical manifestations can occur, usually within 7–8 weeks after exposure to HCV, but the majority of persons have either no symptoms or only mild symptoms. In cases in which symptoms of acute hepatitis have been documented, they usually consisted of jaundice, malaise, and nausea. The

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infection becomes chronic in most cases, and chronic infection is typically characterized by a prolonged period in which there are no symptoms (Poynard et al., 1997). Virological diagnosis and monitoring of hepatitis C virus (HCV) infection are based on two categories of laboratory test, namely serologic assays detecting specific antibody to HCV (anti-HCV) (indirect tests) and assays that can detect, quantify, or characterize the components of HCV viral particles, such as HCV RNA and core antigen (direct tests). Direct and indirect virological tests play a key role in the diagnosis of infection, therapeutic decision-making, and assessment of the virological response to therapy (Pawlotsky, 2002).

The presence of anti-HCV antibodies indicates that an individual may have been infected with HCV and/or may be capable of transmitting HCV infection while active infection is marked by the presence of HCV RNA detected by reverse transcriptase PCR (Alter et al., 2003; Polyak and Gretch, 1997). Serological testing of HCV antibody in individuals has improved in both sensitivity and specificity in recent years. The implementation of blood donation screening for anti-HCV by enzyme immunoassays (EIAs) has led to a marked decline in the risk of transfusion-transmitted hepatitis (Japanese Red Cross, 1991; Donahue et al., 1992). However, the risk of post-transfusion HCV in the USA and Western Europe is still estimated at one per 100,000 and per 400,000, respectively, and is attributed to the seronegative 'window' period of HCV infection (Majid and Gretch, 2002). It is thus essential to identify individuals infected with HCV, to treat them and to prevent dissemination of the virus.

2. Objective

The ARCHITECT Anti-HCV assay is a fully automated high throughput two-step assay, using chemiluminescent microparticle immunoassay (CMIA) technology, for the qualitative detection of anti-HCV in human serum and plasma launched in 1999. Since introduction of this assay to the market, the performance characteristics of this assay were observed and false-reactive ARCHITECT Anti-HCV samples were collected to further optimize the assay performance regarding specificity. Extended laboratory work resulted in a modification of the assay diluent of the ARCHITECT Anti-HCV reagent kit. This study shows the result of the performance evaluation of the modified versus the current ARCHITECT Anti-HCV assay with regards to precision, specificity and sensitivity.

3. Materials and methods

ARCHITECT Anti-HCV reagent kit (6C37), ARCHITECT Anti-HCV calibrator kit (6C37–01) and ARCHITECT Anti-HCV control kit (6C37–10) from Abbott GmbH & Co. KG, Wiesbaden, Germany. The modified ARCHITECT Anti-HCV assay consists of a reagent kit, whereby the assay diluent (6C37J) was replaced with a modified assay diluent. The ARCHITECT Anti-HCV assay is a chemiluminescent microparticle immunoassay (CMIA) that is based on the enzyme immunoassay (EIA) principle. In contrast to the EIA, where the antigen-antibody complexes will be detected by enzyme-labeled conjugate, the CMIA uses acridinium labeled conjugate as detection system. The reactions of the ARCHITECT Anti-HCV assay occur in the following sequence: in the first step of the procedure, sample, assay diluent and HCV antigen coated paramagnetic microparticles are combined. The HCV antigens used in this assay are recombinants, derived from NS3, NS4 and the core region of the HCV genome. Anti-HCV present in the sample binds to the HCV coated microparticles. After washing, anti-human acridinium-labeled conjugate is added in the second step. Following 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). ARCHITECT Anti-HCV has been designed to detect antibodies to structural and nonstructural proteins of the HCV genome (NS3, NS4, core).

The ARCHITECT Anti-HCV assay cutoff value is calculated by the following formula: calibrator 1 mean RLU value $\times$ 0.074 = cutoff RLU. ARCHITECT delivers specimen results as a ratio of the specimen signal (in RLU) to the cutoff value (S/CO). S/CO ratios of greater than or equal to 1.00 are considered reactive for anti-HCV; ratios less than 1.00 are nonreactive for anti-HCV.

The confirmatory assay used to discriminate between a true positive or negative result, was the Chiron RIBA HCV 3.0 SIA from Ortho Clinical Diagnostics, Emeryville, CA, USA.

Specificity was evaluated on 3811 unselected fresh serum and plasma samples from four different German blood banks. Additionally, specificity testing was performed on 1984 randomly selected specimens from hospitalized/diagnostic patients collected from Germany and the USA.

Specimens from individuals with medical conditions unrelated to HCV infection were obtained from ProMedDx, Norton, Massachusetts, USA.

For assessment of seroconversion sensitivity 33 commercial HCV seroconversion panels with 300 bleeds were used (Boston Biomedica Incorporation (BBI), West Bridgewater, MA, USA: PHV901, PHV904, PHV905, PHV907, PHV908, PHV909, PHV910, PHV911, PHV912, PHV913, PHV914, PHV915, PHV916 and Zeptometrix, Buffalo, NY, USA: BCP6211, BCP6212, BCP6213, BCP6214, BCP6215, BCP6216, BCP6222, BCP6224, BCP6225, BCP6226, BCP6227, BCP6228, BCP6229, BCP9044, BCP9045, BCP9046, BCP9047, BCP9054, BCP9055, BCP9058).

In addition, sensitivity for different HCV genotypes and low titer anti-HCV samples was assessed using the WWHV301 and the PHV102 panel (Boston Biomedica Incorporation (BBI), West Bridgewater, Massachusetts, USA).