

## DETECTION OF HEPATITIS C VIRUS CORE ANTIGEN IN BLOOD DONORS USING A NEW ENZYME IMMUNOASSAY

**Alhusain J. Alzahrani, PhD, Obeid E. Obeid, PhD.**

Department of Microbiology, College of Medicine, King Faisal University, Dammam, Saudi Arabia

**هدف الدراسة :** الكشف عن وجود الأنتجينات الداخلية لفيروس التهاب الكبد الوبائي ج في عينات متبرعي الدم في مستشفى الملك فهد الجامعي بالخبر بواسطة كاشف جديد من نوع الاليزا ومقارنة ذلك بالكواشف المستعملة حالياً (ميا - أبوت) .

**طريقة الدراسة :** تم تحليل 898 عينة من المتبرعين بالدم بواسطة الكاشف الجديد لتحديد وجود الأنتجينات الداخلية لفيروس التهاب الكبد الوبائي ج . تم التأكد من النتائج عن طريق الكاشف التأكيدي ومن ثم مقارنة هذه النتائج بنتائج الكواشف المستعملة حالياً (ميا - أبوت) .

**نتائج واستنتاجات الدراسة :** الكشف المبدئي حدد وجود الأنتجينات الداخلية لفيروس الكبد الوبائي ج في 18 عينة من عينات المتبرعين بالدم . الكشف التأكيدي حدد وجود الأنتجينات في 3 عينات من عينات المتبرعين بالدم بنسبة 0.33% . كل هذه العينات لا تحتوي على أجسام مضادة لفيروس التهاب الكبد الوبائي ج وذلك باستعمال ميا - أبوت . يمكن استعمال الكاشف للكشف عن وجود الأنتجينات الداخلية لفيروس التهاب الكبد الوبائي لفحص عينات المتبرعين بالدم وذلك لتقليل احتمال نقل العدوى بواسطة الدم .

**الكلمات المرجعية :** فيروس التهاب الكبد الوبائي ج ، الأنتجينات الداخلية ، متبرعي الدم ، الاليزا .

**Objectives:** The purpose of the study was to evaluate the presence of hepatitis C virus [HCV] core antigen (HCV core Ag) in blood donors at King Fahd Hospital of the University, Al-Khobar, using the new HCV core Ag assay, and to correlate this finding with anti-HCV antibodies detected in the same samples using the standard Abbott MEIA (microparticle enzyme immunoassay).

**Materials and Methods:** A total of 898 samples from blood donors were analyzed using the new assay prototype designed to detect and quantify total HCV core Ag in serum (Ortho-Clinical Diagnostics). Positive results were confirmed by the neutralization assay. The results of the HCV core Ag assay were compared with the results of the standard Abbott MIEA which measures anti-HCV antibody.

**Results and Conclusions:** Out of the 898 samples tested, 18 samples were found to be positive by the HCV core Ag assay (2%). Out of these, 3 samples were confirmed positive by the neutralization protocol (0.33%). All the HCV core Ag positive samples were negative for anti-HCV antibodies (using MEIA by Abbott). These 3 donors may have been in the window period of HCV infection, or may be low responders for the HCV antigens, and are thus unable to mount detectable antibody level. The HCV core Ag assay is a potentially useful assay for screening blood donors, which will minimize the risk of using HCV positive blood from a patient in the window period of HCV infection.

**Key Words:** Hepatitis C virus, Core Antigen, Blood Donors, ELISA.

**Correspondence to:**

**Dr. Alhusain J. Alzahrani,** Department of Microbiology, College of Medicine, King Faisal University, P.O. Box 2114, Dammam 31451, Saudi Arabia

## INTRODUCTION

Hepatitis C virus (HCV) was identified and cloned in 1989. The genome was found to consist of a positive sense single stranded RNA molecule that codes for 10 individual proteins: C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B8. Immunoassays were subsequently developed to detect antibodies to this virus and HCV was found to be the agent responsible for the majority of cases of post-transfusion hepatitis.

During hepatitis C viral genome replication, frequent mutations occur. Phylogenetic analysis of the NS5 gene, in a series of HCV isolates from Europe, North and South America and the Far East, showed that HCV can actually be structured into six groups based on genome sequence (Types 1-6).

The most striking features of HCV are its propensity to persist in a large proportion of infected individuals and the broad spectrum of liver diseases that result from infection. It is estimated that 85% of HCV infected patients progress to chronic hepatitis.

The routine laboratory diagnosis of HCV infection is based on the detection of HCV-specific antibodies (Ab) by enzyme linked immunosorbent assay (ELISA) or microparticle enzyme immunoassay (MEIA). The most commonly applied supplemental assay is the recombinant immunoblot assay (RIBA).

Using 3<sup>rd</sup> generation ELISA, anti-HCV positivity was 5.87% in Saudi patients visiting out-patients clinics in a private hospital in Jeddah. Seroprevalance of anti-HCV among blood donors varies from 0.5% to 4.2%.<sup>1-6</sup> Shobokshi<sup>6</sup> reported a notable decrease among Saudi male blood donors nationwide. At Riyadh central blood bank, a decline from 1.3% in 1997 to 0.8% in 1999 was observed.

Inherent problems with enzyme immunoassays for anti-HCV antibody detection include inability to detect antibodies against conformational epitopes and the genetic diversity of HCV. In addition, the anti-HCV antibody are either absent or very low in the incubation period and during the prodromal phase (window period). This may lead to false

negative results.

Seronegativity was found to be common during an acute illness, as antibodies can be intermittently positive and negative during a follow up.<sup>7</sup> Moreover, incomplete serological responses were reported in young children.<sup>8</sup> Indeed, antibody tests cannot distinguish between persons with anti-HCV antibodies who have recovered and patients exhibiting an active infection, and they are inadequate for the monitoring of therapy.

The direct detection of HCV has depended on methods of nucleic acid amplification or hybridization of HCV RNA. The Amplicor HCV test (Roche Diagnostics Corporation, Branchburg, N.J.) and branched chain DNA signal amplification assay (bDNA) (Bayer Corporation, Tarrytown, N.J.) have been used to detect HCV RNA in HCV antibody-positive individuals, and to monitor the efficacy of therapy.<sup>9</sup> Of late, both assay systems have become semi-automated.<sup>9</sup> However, several problems still persist when these methods are used for the screening of patients: the bDNA assay requires a long incubation period, while reverse transcription (RT)-PCR requires considerable skills with limited reproducibility.<sup>10</sup> In addition, both assays are costly.

Detection of hepatitis C virus [HCV] core antigen (HCV core Ag) may well be an alternative to the molecular techniques, and is particularly important during the window period of HCV infection occurring after HCV infection and before the appearance of antibodies. An assay prototype designed to detect and quantify total HCV core Ag in serum and plasma in the presence or absence of anti-HCV antibodies has recently been developed by Ortho-Clinical Diagnostics.<sup>11-16</sup> This assay is currently used in the routine screening of blood donors in various European countries such as Italy and Spain.<sup>12</sup> Currently, there is no assay that is used routinely for the detection of HCV core antigen in the blood samples of donors in Saudi Arabia. To the best of our knowledge, there is no published data about the detection of HCV core Ag in blood donors in Saudi Arabia.

The aim of the study was to evaluate the presence of HCV core Ag in blood donors at King Fahd Hospital of the University, Al-Khobar, and to compare this finding with anti-HCV antibodies detected in the same samples.

## MATERIALS AND METHODS

### Detection of anti-HCV antibodies

Anti-HCV antibodies were investigated by a third-generation HCV enzyme-linked immunosorbent assay (AxSYM HCV version 3.0, Abbott Diagnostics, Chicago, Ill). and HCV 3.0 ELISA test system (Ortho-Clinical Diagnostics, Raritan, N.J.) and by a third-generation recombinant immunoblot assay (RIBA HCV 3.0; Ortho-Clinical Diagnostics).<sup>9</sup>

### Quantification of total HCV core Ag

A simple enzyme immunoassay for the detection and quantification of total HCV core Ag has recently been developed (Ortho-Clinical Diagnostics). This test was used as instructed by the manufacturer. Briefly, 100  $\mu$ l of one serum aliquot from each patient was pretreated with an immune complex dissociating buffer for 30 minutes at 56°C. Subsequently, samples and diluted standards were incubated for 1 hour and shaken in a microwell coated with anti-core MAb at 25°C. After washing, an anti-core-specific conjugate was added and incubated for 30 minutes at 25°C. After a second wash, the antibody-core Ag conjugate was detected by the addition of *o*-phenylenediamine and hydrogen peroxide. Sample optical density was measured at 492 nm, and core Ag concentrations were calculated against a curve obtained from standards. The results were expressed in picograms per milliliter, with a limit of detection established by the manufacturer at 1.5 pg/ml.

### Neutralization assay

Positive samples for total HCV core Ag obtained in the specificity study were subjected to a neutralization procedure. Briefly, pretreated samples were incubated in quadruplicate for 1 hour at 25°C while being

shaken. Before the addition of the conjugate, two wells were treated with 20  $\mu$ l of neutralizing antibody reagent and the remaining two were treated with a similar volume of control antibody reagent. Following the addition of the conjugate, the assay was completed by the standard procedure. A sample was considered truly positive when a drop in concentration of 50% was observed in the neutralized versus the control treated sample.

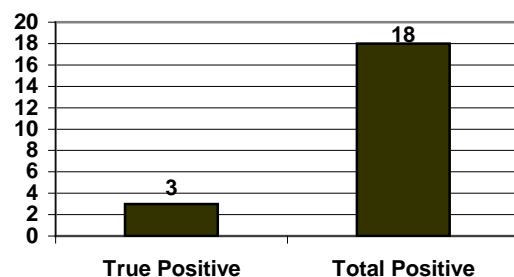
### Statistical analysis

Statistical analysis was done by linear regression and Student's *t* test. All statistical significance was assessed at the  $p \leq 0.05$  level (for results under the cut-off value, log<sub>10</sub> of half the cut-off value was used).

## RESULTS

### Assessment of HCV core Ag in Blood Donors

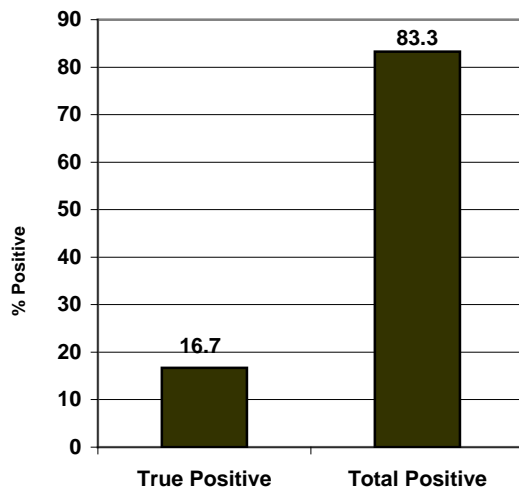
Eight hundred and ninety eight samples from blood donors were analyzed using HCV core Ag assay. As shown in figure 1, out of the 898 samples tested, 18 were found positive (total positive) by the HCV core Ag assay (2% of the 898 samples). All the HCV core Ag positive samples were negative for anti-HCV antibodies.



**Figure 1:** Detection of HCV core Ag in blood donors. Total positive represents the total number of positive samples before neutralization assay. True positive represents the total number of positive samples after neutralization assay.

### Neutralization of positive samples

Positive samples for total HCV core Ag obtained (18 samples) were subjected to a neutralization procedure. As shown in figure 2, out of the 18 positive samples, 3 samples were confirmed positive by the neutralization protocol (0.33% of total). Hence, the percentage of true positives was 16.7% (3 out of 18) and the percentage of false positives was 83.3% (15 out of 18).



**Figure 2:** HCV core Ag in blood donors: Percentage of true positive (after neutralization) and false positives (before neutralization) samples.

## DISCUSSION

Hepatitis C infection is a major health concern. The main method of transmission is through blood transfusion. The availability of highly sensitive and highly specific tests for anti HCV antibodies has led to a dramatic reduction in post-transfusion hepatitis C infection.

However, antibody detection is known to have its limitations as mentioned above. In addition, the lack of antibodies in the incubation period and low level of antibodies early in the infection limits the usefulness of antibody detection techniques. Molecular techniques are useful when antibodies are low or absent. However, they are not easy to apply to a large number of samples (see above).

All the samples that were positive in the HCV core Ag test were negative for anti-HCV antibodies. The lack of anti-HCV antibodies in the three true positive donors may well be due

to the fact that the three donors were in the window period of HCV infection. The window period is characterized by the absence of detectable antibodies, especially in the first few days following HCV infection. During this period, there is evident viremia, which may be detected directly by molecular techniques (i.e. PCR) or by HCV core Ag assay.

Another possibility for the lack of antibodies in the three samples positive for HCV core Ag is that the three donors may be low responders for the HCV, and are thus unable to mount detectable antibody level. For each antigen in nature, humans are either high responders (produce high levels of antibodies to the particular antigen) or low responders (produce low levels of antibodies to the particular antigen). Low and high responder status of an individual is genetically controlled, probably through the HLA gene cluster.

The neutralization assay showed that 15 out of 18 total positive samples were false positives (83.3%). This is rather high. This may well be due to cross reacting antigen in the sera of the 15 samples. It may also be due to improper washing.

The HCV core Ag assay is easy to perform, relatively cheap and does not require a special set up in the laboratory. This is in contrast to the molecular techniques for the direct detection of HCV. Moreover, the results of the HCV core Ag assay correlate very well with the PCR-based assays.<sup>17</sup>

The serious consequences of a blood transfusion that is contaminated with HCV makes the search for a better assay a priority. The advantages of the HCV core Ag assay over the molecular techniques are the cost and the simplicity of its use.

## CONCLUSION

The HCV core Ag assay is a potentially useful assay for screening blood donors to minimize the risk of using HCV positive blood from a patient in the window period of HCV infection. The HCV core Ag assay may well be an alternative to the molecular techniques. However, more extensive evaluation involving various centers in Saudi Arabia is needed

before this assay is included in the routine screening of all blood donors.

#### REFERENCES

1. Fathalla SE, Al-Jama AA . Prevalence of hepatitis C viral antibodies in blood donors, pregnant women and hemodialysis patients in the Eastern Province of Saudi Arabia: a preliminary study. *Saudi Med J* 1993; 14:265.
2. Bernvil SS, Andrews VJ, Kariem AA. Hepatitis C antibody prevalence in Saudi Arabian blood donor population. *Ann Saudi Med* 1991;11:563-7.
3. Bernvil SS, Andrews VJ, Sasich F. Second-generation anti-HCV screening in a Saudi Arabian donor population. *Vox Sang* 1994; 66: 33-6.
4. Abdelaal M, Rowbottom D, Zawawi T. Epidemiology of hepatitis C virus: a study of male blood donors in Saudi Arabia. *Transfusion* 1994; 34:135-7.
5. Mehdi SR, Pophali A, Al-Abdul Rahim KA. Prevalence of hepatitis B and C and blood donors. *Saudi Med J* 2000; 21(10):942-4.
6. Shobokshi OA. Keynote address. Overview of viral hepatitis A through to TT virus. The Sixth Saudi Gastroenterology Association meeting, Jeddah, Saudi Arabia. 30 November - 2 December 1999.
7. Courouce AM, Le Marrec N, Bouchardeau F, Razer A, Maniez M, et al. Efficacy of HCV core antigen detection during the pre-seroconversion period. *Transfusion* 2000;40(10):1198-202.
8. Nagai T, Nagai T, Okazaki T. Relationship between cryoglobulins and hepatitis C virus (HCV) core antigen or antibody titers. *Clin Chem Lab Med* 2004;42(1):105-6.
9. Veillon P, Payan C, Picchio G, Maniez-Montreuil M, Guntz P, Lunel F. Comparative evaluation of the total hepatitis C virus core antigen, branched-DNA, and amplicor monitor assays in determining viremia for patients with chronic hepatitis C during interferon plus ribavirin combination therapy. *J Clin Microbiol* 2003;41(7):3212-20.
10. Icardi G, Bruzzone B, Gota F, Torre F, Giannini E, Massone L, et al. A new assay for hepatitis C virus (HCV) core antigen detection: an alternative to nucleic acid technologies in positive or indeterminate anti-HCV subjects? *Ann Ig* 2003;15(6):863-70.
11. Bouvier-Alias M, Patel K, Dahari H, Beaucourt S, Larderie P, Blatt L, et al. Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. *Hepatology* 2002;36(1):211-8.
12. Komatsu F, Takasaki K. Determination of serum hepatitis C virus (HCV) core protein using a novel approach for quantitative evaluation of HCV viraemia in anti-HCV-positive patients. *Liver* 1999; 19(5):375-80.
13. Maynard M, Pradat P, Berthillon P, Picchio G, Voirin N, Martinot M, et al. Clinical relevance of total HCV core antigen testing for hepatitis C monitoring and for predicting patients' response to therapy. *J Viral Hepat* 2003; 10(4):318-23.
14. Nakamuta M, Shimohashi N, Tada S, Kinukawa N, Enjoji M, Uchimura K, et al. Serum levels of HCV RNA and core protein before and after incubation at 37 degrees C for 24 h. *Hepatol Res* 2001; 19(3):254-62.
15. Netski DM, Wang XH, Mehta SH, Nelson K, Celentano D, Thongsawat S, Maneeakarn N, et al. Hepatitis C virus (HCV) core antigen assay to detect ongoing HCV infection in the injection drug users. *J Clin Microbiol* 2004;42(4):1631-6.
16. Dwyer R. The ADVIA Centaur(R) infectious disease assays: a technical review. *J Clin Virol* 2004; 30 Suppl 1:S1-4.
17. Raker CA, Tabor E, Okayama A, Yu MY, Kohara M, Mueller NE, et al. HCV core antigen as an alternative to NAT to detect HCV viremia. *Transfusion* 2004; 44(2):307-8