

## The usefulness of Umelosa hepatitis C vírus qualitative kit as supplemental test for confirmation of hepatitis C virus infection

### Utilidade do teste Umelosa vírus da hepatite C qualitativo como teste suplementar para confirmação da infecção pelo vírus da hepatite C

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#### ABSTRACT

Forty voluntary blood donors from two different blood banks in Havana, Cuba, who were repeatedly reactive on the routine screening of antibodies to hepatitis C virus, by Umelisa HCV test, were analyzed for the presence of HCV RNA using a nested PCR assay of the HCV 5' untranslated region, Umelosa HCV qualitative. Sera from 45 patients of a specialized gastroenterology consultation, positive to Umelisa HCV, were also assayed with the Umelosa HCV qualitative, to establish their condition related to the presence of HCV RNA previously to the indication of a treatment or after three, six or twelve months of antiviral therapy. Serum HCV-RNA was detected in 21/40 (52.5%) donors who had repeatedly positive ELISA results, confirming the HCV infection for them. In specialized consultation HCV-RNA was detected by PCR analysis in 30/45 (66%) analyzed sera.

*Key-words:* Hepatitis C virus. Polymerase chain reaction. Anti-HCV testing. False positive blood donors. Confirmatory test.

#### RESUMO

Quarenta doadores de sangue voluntários de dois bancos de sangue em Havana, Cuba, repetidamente reativos ao exame de rotina para anticorpos contra o vírus da hepatite C, pelo teste Umelisa HCV, foram analisados para a presença de RNA HCV através de um ensaio de PCR da região 5' não-traduzida do HCV denominado Umelosa HCV qualitativo. Amostras de soro obtidas de 45 pacientes atendidos em consulta gastroenterológica especializada, positivos para Umelisa HCV, também foram avaliados através do Umelosa HCV qualitativo, para estabelecer sua condição em relação à presença de RNA HCV previamente à indicação de um tratamento ou após três, seis ou doze meses de terapia anti-viral. O RNA HCV sérico foi detectado em 21/40 (52,5%) doadores que haviam apresentado resultados ELISA positivos repetidos, confirmando a infecção por HCV. Nos pacientes atendidos em consulta especializada, o RNA HCV foi detectado por análise de PCR em 30/45 (66%) amostras de soro analisadas.

*Palavras-chaves:* Vírus da hepatite C. Reação da polimerase em cadeia. Exame anti-HCV. Doadores de sangue falso positivos. Teste de confirmação.

It is estimated that there are more than 170 million people infected with hepatitis C virus (HCV) worldwide<sup>19</sup>. The prevalence of HCV infection varies from country to country and the natural history of infection is not well understood<sup>16</sup>. HCV is responsible for the majority of cases of transfusion-related hepatitis.

When the first generation of enzyme immunoassays (EIAs) for detection of antibodies to hepatitis C virus (anti-HCV) was approved in May 1990, blood banking agencies recommended testing of all blood units<sup>15</sup>. In Cuba first screenings for anti-HCV began in 1993 and since 1995 all donations are assayed with the Umelisa HCV test (Immunoassay Center, Havana, Cuba) for the presence of antibodies to HCV.

It is known that serological diagnosis of HCV infection suffers from a high number of false positives to the real presence of antibodies and cannot answer the question regarding the virus

replication. A very high prevalence of anti-HCV antibodies has previously been noted among commercial plasma donors negative for HCV-RNA, as well as the low predictive value of EIAs for anti-HCV in a low-prevalence blood donor population and the need for additional confirmatory testing of anti-HCV-reactive sera to refute a large proportion of false-positive results<sup>3,13,16</sup>.

The introduction of the third-generation anti-HCV tests resulted in the increased number of anti-HCV reiterated positive donors, mainly due to false-positive results<sup>18</sup>. The exact significance of this response in healthy blood donors remains unknown. Some studies have demonstrated the occurrence of positive results for supplemental tests and abnormal results of liver function tests only in those subjects with high antibody titers, which suggests that reactive samples of low optical density, near the cut-off value,

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require further confirmation<sup>17</sup>. Low antibody titers have been more prevalent during the winter months, suggesting that seasonal intercurrent infections may increase the percentage of false positives<sup>4</sup>.

Some authors have suggested the combination of two different screening assays for anti-HCV confirmation<sup>2</sup>. The ALT determination in conjunction with anti-HCV testing is another way proposed to improve the quality of screening for potentially infectious donors<sup>6</sup>. It has been reported that among ELISA-reactive donors, those with elevated ALT had a significantly higher probability of being positive to HCV RNA compared to those with normal ALT<sup>14</sup>.

Recombinant immunoblot assay (RIBA) used for years as supplemental confirmatory test for anti-HCV ELISAs, to assess the prevalence of anti-HCV, is not suitable for HCV infection confirmation<sup>5</sup>. Nucleic acid detection assays became substitutes of RIBA tests for the diagnosis and confirmation of HCV infection, allowing the direct measurement of viral replication. There is no technique based on the detection of antibodies, including RIBA test, that could be considered confirmatory for the diagnosis of HCV infection. Samples negative by polymerase chain reaction (PCR) could result RIBA negative, positive or indeterminate.

To determine infectivity of the anti-HCV positive cases, the introduction of ribonucleic acid (RNA) testing is essential. PCR, considered the gold standard screening test for HCV RNA, is vital, irrespective of symptoms and ALT levels. It helps to resolve weakly positive or negative ELISA results when the clinical context is compatible with hepatitis C<sup>11</sup>.

In the present work we show the utility of a nested polymerase chain reaction assay Umelosa HCV qualitativo<sup>9,10</sup> (Immunoassay Center, Havana, Cuba) as confirmatory test in blood banks, to distinguish healthy seropositive donors or those with false-positive ELISA results, from individuals who are really infected and might benefit from further investigation and treatment. The usefulness of this assay, to follow patients on therapy in specialized gastroenterology consultation, is also described.

## MATERIAL AND METHODS

Aiming at evaluating the real rate of infection in positive anti-HCV voluntary blood donors, coming from two different blood banks in Havana, 40 test samples repeatedly positive by UMELISA HCV (twenty-six plasmas and fourteen sera) were subsequently assayed by a nested PCR assay Umelosa HCV qualitative.

Forty-five anti-HCV positive sera, from 45 patients of a specialized gastroenterology consultation, were also assayed with the Umelosa HCV qualitative test, to establish their condition regarding the presence of HCV RNA, prior to the indication of treatment or after three, six or twelve months of antiviral therapy.

**Umelisa HCV test.** The Umelisa HCV is an indirect enzyme immunoassay which uses as solid phase ultramicroplate strips coated with one recombinant protein of the virus NS3 region and synthetic peptides corresponding to the CORE, NS4 and NS5 regions. The enzyme in the anti-human IgG/Alkaline Phosphatase (AP) conjugate (Immunoassay Center, Havana, Cuba) hydrolyzes the fluorogenic substrate 4-Methylumbelliferyl Phosphate (Koch Light Ltd. Haverhill, Suffolk, England) producing a fluorescent signal with an intensity proportional to the HCV antibodies concentration in the sample. In all steps of the assay volumes of 10µL per well were used.

**Umelosa HCV qualitative test.** Is an assay based on nucleic acid amplification technology (NAT), for the detection of HCV RNA in human serum or plasma. The test is performed in three steps: extraction of viral RNA employing a variant of the phenol-chloroform method of Chomckzynsky<sup>7</sup>; one single tube reverse transcription of target RNA and PCR coupled, with a second round of amplification; and hybridization of the amplified DNA in ultramicroplate strips coated with a complementary probe. The conjugate Streptavidine/AP (Immunoassay Center, Cuba) is bound by biotin-labeled, during PCR, product, and the AP fluorogenic substrate 4-Methylumbelliferyl Phosphate (Koch Light Ltd. Haverhill, Suffolk, England) is hydrolyzed by the enzyme, producing a fluorescent signal which is measured on a SUMA PR-521 plate reader (Immunoassay Center, Havana, Cuba).

## RESULTS

Hepatitis C virus - ribonucleic acid (HCV RNA) was detected (PCR+) in 21/40 (52.5%) donors who had repeatedly positive ELISA results (Ac+). In specialized consultation hepatitis C virus - ribonucleic acid was detected in 30/45 analyzed sera (Table 1).

*Table 1- Results by Umelisa and Umelosa HCV tests.*

Origin of samples	Sera/Plasmas	Ac +	PCR +	Total
Gastroenterology Consultation	45/-	45	30	45
Confirmation			15	15
months of therapy (3)			7	10
months of therapy (6)			6	10
months of therapy (12)			2	10
Blood Bank	14/26	40	7/14	40

## DISCUSSION

Of the 40 anti-HCV positive blood donors screened, 21 were confirmed for the presence of HCV RNA. Despite the small size of the sample, the analysis made for blood banks allowed us to estimate the real positive rate of HCV infection in blood donors in Cuba. The overall agreement found between the two assays (Umelisa HCV, for the screening of antibodies against the virus and Umelosa HCV qualitative, for detection of HCV RNA) in Cuban blood banks (52.5%), coincided with the reports for similar studies, carried out in other countries<sup>18,12</sup>.

The assay Umelosa HCV qualitative allowed distinguishing healthy seropositive donors or those with false-positive ELISA results, from those who were really infected and needed medical assessment. This test should be very useful in Cuban blood banks in the surveillance of anti-HCV positive blood donors with normal ALT levels, identifying donors who might benefit from further investigation and treatment, and avoiding unnecessary liver biopsies or other analyses.

In specialized consultation HCV RNA was detected, by PCR analysis, in 30/45 analyzed sera. All the samples assayed to confirm the presence of the virus, resulted positive to the Umelosa test. More than 50% of Umelosa HCV positive and Umelosa negative samples were cases of resolved infection: patients who had completed twelve months of treatment with Interferon or Interferon combined

with Amantadine, and did not have a detectable viral load at the moment of the test. The other PCR negative samples belonged to patients with three or six months of therapy. See Table 1.

Umelosa positive results provided doctors criterion to begin therapy, in the case of non-treated studied patients (15), or to continue or stop it on those whose HCV RNA test was still positive, depending on the duration of the therapy.

The samples positive to Umelisa HCV and negative by PCR can indicate an already resolved infection or active viral replication, but with a viral load inferior to the detection limit of the technique. That is why, not only analytical but also clinical aspects should be considered for the analysis of the results of any analytical test.

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