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Effectiveness of three types of rapid tests for the detection of hepatitis C virus antibodies among blood donors in Alexandria, Egypt

Ghamdan Al-Tahish[a,b,*], Mohamed A. El-Barrawy[b], Mona H. Hashish[b], Zeinab Heddaya[c]

[a] Department of Microbiology, Faculty of Medicine and Health Sciences, Hodeidah University, Hodeidah, Yemen
[b] Department of Microbiology, High Institute of Public Health, Alexandria University, Alexandria, Egypt
[c] Director General for Fever Hospitals Department, Ministry of Health and Population, Egypt

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A B S T R A C T

Hepatitis C is one of the most important diseases transmitted through screened improperly blood donation. The detection of HCV antibodies is performed by enzyme immunoassays (EIA) or supplementary assays (immunoblots). However, these methods are not well-suited to developing countries due to their high cost and technicality.

The effectiveness of three different rapid tests for the detection of anti-HCV antibodies was evaluated compared to third-generation ELISA among blood donors attending the blood bank of Medical Research Institute in Alexandria, Egypt. The results were compared subsequently to the results of HCV RNA obtained by qualitative reverse transcriptase polymerase chain reaction (RT-PCR).

The three types of rapid tests showed a specificity of 100% and sensitivities of 96–98% compared to ELISA. Compared to RT-PCR, ELISA and all three types of rapid tests showed an almost equal specificity (77–78.5%). ELISA showed 100% sensitivity while all three types of rapid tests showed equal sensitivities of 97% compared to RT-PCR.

The rapid tests showed good performance for detecting anti-HCV antibodies in the sera of blood donors compared to ELISA. Therefore, the present study recommends the use of the tested rapid tests to screen for anti-HCV among blood donors in resource-limited countries as an alternative for conventional ELISA.

1. Introduction

Hepatitis C virus (HCV) infection is a major worldwide public health problem due to its high prevalence and to the high risk of chronicity (WHO, 1999). Over 170 million people worldwide are estimated to be infected with HCV (Lauer and Walker, 2001). Chronic hepatitis, cirrhosis and hepatocellular carcinoma may develop in more than 70% of patients (Yaari et al., 2006). The prevalence of HCV is higher in developing countries compared to the developed world (Wild and Hall, 2000). HCV infection varies widely across regions, ranging from as low as 1% in Nigeria to as high as 20–30% in some rural communities of Egypt (Frank et al., 2000; Muktar et al., 2006).

HCV infection is diagnosed conventionally based on the detection of virus antibodies using screening tests such as enzyme immunoassays (EIA) or immunoblots (Erensoy, 2001; Richter, 2002). The used third-generation HCV enzyme-linked immunosorbent assay (ELISA) includes recombinant or synthetic antigens from the putative core and non-structural regions NS3, NS4 and NS5 (WHO, 2001a). Following detection of HCV antibodies by EIA, current clinical practice relies on RNA amplification techniques, such as polymerase chain reaction (PCR), for the detection and quantification of HCV RNA (Richter, 2002). ELISAs are the HCV screening tests used most widely due to their ability to screen large numbers of specimens on a daily basis, as is the case in blood transfusion services in industrialized countries. However, many blood transfusion services in resource-limited countries process only limited numbers of specimens; hence, individual tests would be more appropriate (WHO, 2001a).

High cost and time-consuming procedures make these conventional tests inappropriate for developing countries. Furthermore, there is a lack of implementation of routine screening for HCV-infected blood units in developing countries (Mbanya et al., 2001). It has been determined that 17% of all blood donations in the world are not tested reliably for transfusion transmissible infections (STIs). This number increases to 43% if only the non-industrialized countries are considered (WHO, 2001b). Rapid tests have several advantages compared to conventional ones for anti-HCV. First, only a very small amount of blood or serum is required. Second, rapid

* Corresponding author at: Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria, Egypt. Tel.: +20 1224255625.
E-mail address: ghamdani75@yahoo.com (G. Al-Tahish).

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tests are read visually with internal control within a very short time compared to third-generation ELISA and can be performed on an individual sample basis (Montebugnoli et al., 1999; Jourbert et al., 2003; Daniel et al., 2005). Third, rapid tests do not require technical expertise that is beneficial in centers where such expertise is lacking or 24-hour laboratory services are not available. Finally, the costs of many currently available rapid tests are no more expensive than the EIA tests (Yuen et al., 2001; Lee and Allain, 2004; ICBS Blood Screening Test Evaluation Center at PEI, 2006; Desbois et al., 2008). In addition, even if there is no prophylactic HCV treatment after a needle-stick injury, it can be important to know rapidly the HCV status of a source patient. If negative, health care workers can be reassured (Desbois et al., 2008).

The demanding identification of diagnostic test kits that are sensitive, specific, rapid and affordable was the motive to the current work on blood donors in Alexandria. The present study evaluated the effectiveness of one-step test device, the flow-through test and the indirect solid-phase EIA HCV rapid tests compared to third-generation ELISA. The results of HCV antibodies obtained by ELISA and the three rapid tests were compared with the results of HCV RNA obtained by qualitative RT-PCR.

2. Subjects and methods

This study was carried out through the period from July 2009 to February 2010. It included 100 blood donors (50 anti-HCV positive and 50 anti-HCV negative) who attended the blood bank of MRI in Alexandria. The anti-HCV results of attendees were taken from blood bank records, and the samples were retested by ELISA technique to obtain the absorbance values. The study protocol was approved by the Institutional Ethics Board and informed consent was obtained from all participants.

2.1. Collection of blood samples

Three ml blood were collected from each patient, sera or plasma were separated by centrifugation and divided into two aliquots. One aliquot was stored at −20 °C (for anti-HCV testing) and the other at −70 °C until tested (for HCV RNA testing).

2.2. Detection of HCV antibodies by ELISA

HCV antibodies were detected by Dialab HCV Ab ELISA kit (Dialab, Wr. Neudorf, Austria) that was purchased from Dialab distributor.

2.3. Detection of HCV antibodies by rapid tests

The ImmunoComb II HCV test (Inverness Medical Innovations, Waltham, MA, USA) is an indirect solid-phase EIA. The solid phase is a card with 12 projections (teeth). Each tooth is sensitized at three spots: upper spot (human IgG internal control), middle spot (HCV core antigen) and lower spot (HCV non-structural antigens NS3, NS4 and NS5). The developing plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise, by moving the card from row to row, with incubation at each step.

HCV one-step test device ACON HCV (ACON Laboratories, San Diego, CA, USA) is a qualitative, membrane-based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is coated with recombinant HCV antigen on the test line region of the device. During testing, the serum or plasma specimen reacts with the protein A coated particles. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a red line in the control region will appear confirming that the test has been performed properly.

The fourth- generation HCV TRI-DOT (J. Mitra, New Delhi, India) has been developed and designed with increased sensitivity for core and NS3 antibodies using a unique combination of modified HCV antigen. They are for the putative core, protease/helicase NS3, NS4 and replicase NS5 regions of the virus in the form of two test dots "T1" & "T2" to provide a highly sensitive and specific diagnostic test.

2.4. Detection of HCV RNA by nested RT PCR technique

The protocol for HCV RNA detection via RT-PCR amplification proceeds in a stepwise manner from: RNA isolation to RT-PCR amplification for detection on an agarose gel. RNA was isolated using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), the sample (serum or plasma) is first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding of the RNA to the QIAamp membrane, and the sample is loaded onto the QIAamp Mini spin column. The RNA binds to the membrane, and contaminants are washed efficiently away in two steps using two different wash buffers. High-quality RNA is eluted in a special RNase-free buffer. The purified RNA is free of protein, nucleases, and other contaminants and inhibitors.

The QIAgen OneStep RT-PCR kit (Qiagen, Hilden, Germany) contains optimized components that allow both RT and PCR amplification for extracted RNA to take place sequentially in the same tube. The primers amplify the 5′UTR fragment of the HCV genome where the first round of PCR was performed with two external primers and the second round of PCR was performed with two internal primers. The amplified 5′UTR fragment was a 256-bp product that extended from nucleotide position −276 to −21(Gao et al., 2002) (Table 1).

PCR products were loaded on 2% agarose in Tris buffer EDTA (TBE) containing 0.5 μg of ethidium bromide per ml. After electrophoresis, the gels were photographed under ultraviolet light.

2.5. Statistical analysis

Data were entered, verified and analyzed using SPSS version 15.0 (IBM, Chicago, USA). The sensitivity, specificity, predictive values, Kappa statistics and confidence limits of anti-HCV tests were tested for calculation the performance for these tests.

Table 1
HCV primers, sequences and their positions in two round PCR (according to Gao et al., 2002).

Nested RT PCR	Name	Sequence	Positions
First round PCR	External sense primer	ACT GTC TTC ACG CAG AAA GCG TCT AGC CAT	−285 to −256
	External antisense primer	CGA GAC CTC CCG GGG CAC TCG CAA GCA CCC	−14 to− 43
Second round PCR	Internal antisense primer	TCC CGG GGC ACT CGC AAG CAC CCT ATC AGG	−21 to −50
	Internal sense primer	ACG CAG AAA GCG TCT AGC CAT GGC GTT AGT	−276 to −247