Evaluation of First Response ® HCV card test as a reliable rapid test for HCV detection

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Abstract: Hepatitis C Virus (HCV) infection is a global health issue causing approximatively 500000 deaths each year. Untreated, chronic HCV infection can lead to progressive hepatic fibrosis, cirrhosis, end-stage liver disease, and hepatocellular carcinoma. There is a strong need for an effective vaccine that protects against the different genotypes of HCV but the prevention still represents an enormous challenge.

HCV was first identified in 1989 using molecular methods at the Chiron Corporation, but to date, the virus has never been visualized or grown in cell culture. The general nowadays method of detecting infection with HCV is to observe the presence of antibodies to the virus by an EIA enzyme immunoassays method followed by confirmation with Western Blot. A Rapid Anti-HCV Test; based on immuno-chromatography; is a simple, visual qualitative test that detects antibodies in human serum or plasma within 15 minutes.

The objective of this work is to evaluate the performance and compare the results obtained by two HCV detection platforms with high throughput Advia Centaur XP (Siemens Healthcare Diagnostics, USA) and First Response ® HCV Card Test HCV (Premier Medical Corporation Limited).

These data could help in HCV control and understanding and hopefully, in vaccine design and development, moreover to identify a reliable test to evaluate and prevent HCV infection.

Keywords: HCV, infection, prevention, test, vaccine.

Introduction

Hepatitis C virus (HCV) is a single-stranded RNA virus with a genome of about 10 000 nucleotides containing a single large, continuous open reading frame. This blood borne virus is the commonest cause of chronic hepatitis, liver cirrhosis and liver cancer [1].

The infection occurs through unsafe injection practices; inadequate sterilization of medical equipment; and the transfusion of unscreened blood. Hepatitis C instead, is not spread through breast milk, food or water or by casual contact with an infected person. HCV represents a global healthcare problem and the World Health Organization (WHO) estimates that at least 170 million people (3 % of the world's population) are infected with HCV worldwide and most of the patients are concentrated in developing countries in Africa and Central and East Asia. Furthermore, approximately 500 000 people die each year from hepatitis C-related liver diseases [2].

The incubation period for hepatitis C is 2 weeks to 6 months. Following initial infection, approximately 80% of people do not exhibit any symptoms. Those who are acutely symptomatic may exhibit fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, grey-colored feces, joint pain and jaundice. Few people are diagnosed during the acute phase, the majority often go on to develop chronic HCV infection which remains undiagnosed until liver has serious damage [3- 5].

Spontaneous eradication of HCV occurs in 15%-50% of acute infections, and clearance is associated with specific immune responses. Although neutralizing antibodies to HCV (anti-HCV) have been identified, they are isolate-specific and poorly correlate with viral clearance. Moreover, HCV isolates are classified into 6 major genotypes and more than 80 subtypes, recently, a seventh genotype has been characterized which delay the virus

detection and make the diagnosis more complicated. Antiviral medicines can cure approximately 90% of persons with hepatitis C infection, but access to diagnosis and treatment is low. Therapy is expensive and long lasting with many adverse effects [6, 7].

In the absence of HCV antibodies, HCV specific Cell-mediated immunity (CMI) may represent the only biomarker of HCV infection. Understanding the immune mechanisms of patients who successfully have cleared the infection will remain essential to the controlling of HCV infection and the design and development of an effective vaccine [8, 9].

Screening and diagnosis

Conventional methods fail to isolate the virus in cell culture or visualize it by electron microscope. Cloning the viral genome has made it possible to develop serologic assays that use recombinant antigens. Multiple antigens using recombinant protein and/or synthetic peptides increased the specificity, sensitivity and reduced the cross-reactivity of the HCV antibody tests [11].

Screening for anti-HCV antibodies is first performed through serological test to identify infected people. Then a nucleic acid test for HCV RNA through PCR or a western blot assay for the virus proteins are needed to confirm chronic HCV infection. Only 15-45% of infected people spontaneously clear the infection by a strong immune response without treatment but will still test positive for anti-HCV antibodies [2].

Chronic hepatitis C infection induced - liver damage is assessed by liver biopsy and manifests in fibrosis and cirrhosis. Moreover, laboratory test identify the genotype of the hepatitis C strain from 6 genotypes and which respond differently to treatment. It is possible for a person to be infected with more than one genotype. The degree of liver damage and virus genotype are used to guide treatment decisions and management of the disease [12, 13].

The availability of diagnostic tests with high level of sensitivity, specificity, robustness and reliability, combined with automation and traceability of the results may improve the turnaround time of tests execution, reducing the overall costs of additional tests and investigations, long hospitalization and personnel costs.

These tests may be performed routinely in the future, and detect rapidly and early any possible infection allowing a rapid action to improve the diagnosis and prognosis of the patient [10].

Objective

The aim of this study is to evaluate the performance of two HCV screening tests, one through the automated platform ADVIA Centaur XP (Siemens Healthcare Diagnostics, USA) and the other is the First Response ® HCV Card Test HCV (Premier Medical Corporation Limited). Here, we compare the obtained results in order to validate the HCV Card test as reliable for HCV infection screening.

Material and Methods

HCV chemiluminescent enzyme immunoassay for the qualitative detection of IgG antibodies to HCV is directly proportional to the amount of anti-HCV present in the sample. The results are expressed as an Index. According to the manufacturer instructions, samples with an index value <0.8 are negative or non-reactive to IgG antibodies for HCV. Samples with an Index value ≥ 0.8 and <1 are equivocal. While those with an Index value ≥ 1 are positive or reactive. Equivocal samples were reported but excluded from this analysis.

The ADVIA Centaur assay is a two-wash antigen/antibody sandwich immunoassay in which antigens are bridged by antibody present in the patient sample and antigen (p24) in the sample is bridged by antibody present in the reagents. The technique is fully automated. The analyzer incorporates a dedicated software package for instrument control, data collection, results analysis, calibration and quality control. The ADVIA Centaur system has a throughput of up to 240 tests per hour and has a capacity to accommodate 180 samples and up to 30 reagent packs (see Figure 1)
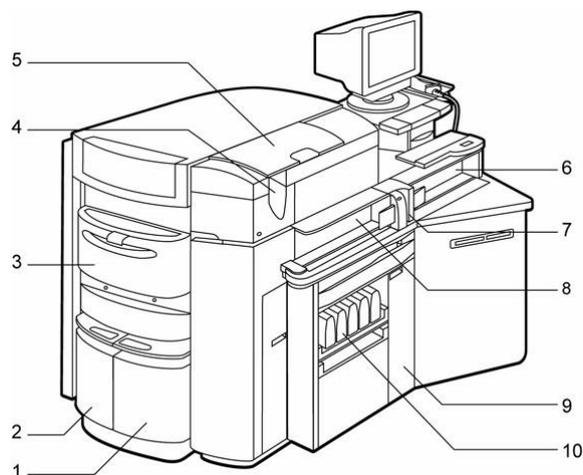
1 Water bottle, **2** Waste bottle, **3** Primary reagent compartment, **4** Sample tip loader, **5** Cuvette loader, **6** Sample exit queue, **7** Stat entry, 8 Sample entry queue, 9 Cleaning solution cabinet, 10 System fluids

Figure 1. The ADVIA Centaur analyzer [14]

Collecting Specimens

The assay can be performed on serum specimens collected using recommended procedures for collection of diagnostic blood specimens. Samples are processed by centrifugation, typically followed by physical separation of the serum from the clot. Specimens with obvious microbial contamination should not be used.

Storing Specimens

The specimens should be tested as soon as possible after collecting. Processed specimens may be stored at 2° to 8℃ if not tested within 24 hours of collection. Specimens that have been stored at room temperature for longer than 24 hours should not be used. Separated specimens are stable for 24 hours at room temperature, and up to 14 days at 2–8℃. For longer storage, specimens may be frozen for up to 8 months at -20℃ or colder. Samples may be subjected up to 5 freeze/thaw cycles.

Transporting Specimens

Samples should be shipped at 2–8℃ or frozen.

HCV-Card Rapid Test

The HCV-Card Rapid Test 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 card. During testing, the serum or plasma specimen reacts with the recombinant HCV antigen coated colloidal gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with another 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 (see Figure 2). To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.
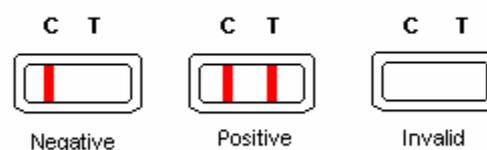
Figure 2. HCV Card Rapid Test [15]

The evaluation was conducted on established blood donors previously been screened using the ADVIA Centaur XP system (Siemens ADVIA Centaur XP HCV assays). Sample collection and analysis using Hepatitis C Detection Card Test (Premier Medical Corporation Limited), were performed in parallel from September to January 2015 at the Department of R&D at the Egyptian Company for Blood Transfusion Services (EgyBlood/Vacsera).

Results and Discussion:

A total of 725 blood samples of young donors were collected and screened using Siemens ADVIA Centaur XP HCV assays with the following parameters:
• Mean age of all donors (years) = 31.56 (7.04)
• Median age all donors (years) = 32 (IQR:26-37)
• No. of Male donors = 501 (69.1%)
• No. of Females donors = 224 (30.6%)
• Mean age females donors = 31.01 (6.46)
• Mean age males donors = 31.8 (7.28)

Table 1. Results of IgG antibodies to HCV using Siemens ADVIA Centaur XP HCV assays

Siemens ADVIA Centaur XP HCV assays	Number
No. Nonreactive (negative) samples (<0.80)	460
No. Reactive (positive) samples (>1.00)	256
No. Equivocal Samples (>0.80 - <1.00)*	9
Total No.	725

* excluded from this analysis

As we can see in Table 1, only 9 samples out of 725 were considered equivocal and excluded from the analysis. This shows the high specificity and reliability of Siemens ADVIA Centaur XP HCV assay.

After excluding the 9 equivocal samples, we performed an assay on the 716 left samples with the Hepatitis C Detection Card Test as shown in Table 2.

Table 2. Number of previously screened donors evaluated using the Hepatitis C Detection Card Test

Hepatitis C Detection Card Test	Number
No. of True Positive	223
No. of False Positive	0
No. of True Negative	460
No. of False Negative	33
Total No.	716

Comparing the results obtained with both screening tests, we got the same number of negative samples, since 460 samples were non-reactive with Siemens ADVIA Centaur XP HCV assay and evaluated as true negative with the Hepatitis C Detection Card Test. The positive samples obtained with Siemens ADVIA Centaur XP HCV assay instead, were not all considered positive using the Hepatitis C Detection Card Test. Only 223 samples

out the 256 were considered true positive, with the other 33 evaluated as false negative. No samples were considered as false positive (see Table 2).

The Hepatitis C Detection Card Test is capable of discriminating false negative and false positive which indicates that this assay is more reliable and specific and may be considered a perfect candidate as rapid screening test for HCV infection.

Table 3. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of the Hepatitis C Detection Card Test.

Reference		First Response® HCV Card Test		Total
Method	Results	Positive	Negative	
Siemens ADVIA Centaur XP	Positive	223 (TP)	0 (FP)	223
	Negative	33 (FN)	460 (TN)	493
Total results		256	460	716

From Table 3 we can clearly that the Hepatitis C Detection Card Test is very specific, sensitive and reliable.
Moreover, we have calculated the sensitivity and specificity together with other important parameters and all indicate the high performance of this assay.
Sensitivity = 87.1%

Specificity = 100%
Positive predictive value: 100.00%
Negative predictive value: 93.3%

Table 4 shows the evaluation of the equivocal samples by the Hepatitis C Detection Card Test.

Table 4. List of Samples with equivocal values

	Sample Bar code	Siemens ADVIA Centaur XP HCV Result	Hepatitis C Detection Card Test Result
1	1509090041034	0.8	Positive
2	1510290031014	0.81	Positive
3	1512060031062	0.87	Negative
4	49716000277	0.89	Negative
5	49716000289	0.92	Negative
6	1509110041016	0.93	Negative
7	1510170021005	0.93	Negative
8	1509060031019	0.94	Negative
9	1509010041059	0.96	Positive

The amino acid sequence and the purity of the HCV antigen used for assay development are significant factors influencing both the specificity and the sensitivity of anti-HCV immunoassays.

The high IgG concentration in human blood (>5 mg/ml)-e.g. in paraproteinemia or auto-antibody production may lead to false signals. With Ig G denaturation caused by repeated freezing and thawing or by heat-inactivation of serum samples, some of the IgG molecules gain a strong tendency to be bound to the micro-well surface by direct adsorption or by indirect capture via the surface molecules, giving false-positive results. This problem might be more serious when the samples are from patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), portal cirrhosis, and some infectious diseases due to the very complicated, higher concentration of immunoglobulin components in their blood [1].

The Hepatitis C Detection Card Test is a qualitative test but it may also considered semi-quantitative since the thickness or weakness of its bands give a clear idea on the high or low concentration of the antibody. There should always be a purplish red control band in the control region, regardless of the test result to improve the specificity and sensitivity of the test.

The Hepatitis C Detection Card Test request clear, fresh, free flowing Serum or Plasma which may represent sometimes a limitation. If a sample has been frozen, it should be allowed to thaw in a vertical position and checked for fluidity.

Conclusion

In front of the high incidence of HCV infection, especially in underdeveloped countries, universal and appropriate precautions should be adopted for all patients regardless the suspect of bearing an infection.

The clinical laboratory remains an active structure for the virus detection and, with the availability
of diagnostic tests with high level of sensitivity and specificity together with high throughput automation, the time and procedure test would improve giving reliable results and reducing the need for additional investigations with the corresponding costs.

The First Response ® HCV Card Test shows high sensitivity and reliability compared to ADVIA Centaur XP platform, which makes it a simple and rapid perfect candidate for HCV infection detection.

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