Research Article

Validation of a rapid screening diagnostic test kit for field investigations of hepatitis C viral infection

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Abstract

Background: Hepatitis C virus (HCV) screening, case finding and treatment is an urgent public health issue and a national priority on health, social and economic levels. However, the high cost of the gold standard diagnostic test, polymerase chain reaction (PCR), and the inaccessibility of trustful laboratories in many areas pushed the epidemiologists to search for easier, cheaper and field-applicable screening tools to detect infected individuals and then to confirm only the positive cases by the expensive PCR testing. **Methodology**: A total of 558 blood samples were confirmed for diagnosis of HCV by qualitative and quantitative PCR as a reference test. Those samples, 178 positive cases of HCV and 380 negative samples were further, examined by the enzyme-linked immunosorbant assay (ELISA) and the InTec rapid diagnostic testing kit to detect and compare their sensitivity, specificity, accuracy and efficiency as mass screening tests. **Results**: Compared with the reference PCR test, the InTec rapid diagnostic test showed a sensitivity of 99.4%, a specificity of 98.7%, a positive predictive value of 97.3%, a negative predictive value of 99.7%, an accuracy percent of 98.9% and an error percent of 1.1%. These results were comparable to that of ELISA. **Conclusions**: The InTec rapid diagnostic testing kit is a simple, cheap, sensitive, specific and efficient test that can be used in the field for simple and rapid diagnosis of HCV infection. The accuracy of the InTec kits has implications for its use in clinical and outreach settings Such field diagnosis of HCV infection is essential for case finding, screening and surveillance of HCV and for all the efforts that are exerted to treat, control and combat the disease.

Key words: HCV, Screening, Case finding, InTec test, Rapid detection, Validity

Introduction

Hepatitis C virus (HCV) infection is a worldwide public health problem with a sero-prevalence of about 3% of the world's population. More than 135 Million infections are living in China, Egypt, India, Pakistan, Nigeria and Russia[1-3]. Nearly, up to 500,000 deaths are reported annually as a consequence of HCV-related complications[4].

In Egypt, the prevalence of HCV infection is still to be accurately determined, whereas many small scale studies have been performed[5,6].

However, HCV infection shows no symptoms in about 80% of cases, leading to rapid spread of the infection especially among the house-holds of the patients in a form of familial clustering[6,7].

Thus, it is important to detect the infected cases via a valid screening test that can be used in the field in order to control the increasing spread of HCV infection, especially after the introduction of the oral effective drugs for treatment of HCV that were developed and replaced the injections.

Developing an effective surveillance system for diagnosis and eradication of HCV has gradually risen as a top priority on public health agendas. Therefore, experts have begun to recognize the impact of "test and treat" initiatives on reducing future healthcare burden[8].

The introduction of such direct acting antiviral drugs, with short durations, minimum side effects, and over 90% cure rates, has revolutionnized the treatment of HCV[9].

While these new medications are an exciting improvement, at present, only a small percentage of people have been tested and are sure of their status regarding HCV, and access to affordable, valid and simple tests is vital to the success of these surveillance, "test and treat" initiatives[10].

Egypt is in a terrible need to speed up HCV screening and treatment to cope with the targets of the World Health Organization (WHO) global viral hepatitis strategy (2016-2021)[11]. Hence, we designed the present study to examine the validity of the InTec HCV rapid diagnostic test kit and compare its sensitivity and specificity with ELISA test for further use as in-field mass screening test while using the PCR as a golden reference test.

Methodology

Study samples

A total of 558 (304 males and 254 females) blood samples confirmed diagnosis for HCV by qualitative and quantitative PCR as a golden reference test; 178 positive cases of HCV (96 males and 82 females) and 380 negative samples (208 males and 172 females), were examined by ELISA and InTec rapid diagnositic test to detect and compare their sensitivity and specificity for further use as a field screening tests. The age of the study participants ranged from 7–68y with a mean±SD of 36.3±23.8 years. These samples were collected from randomly selected individuals from different geographical areas from Bani Suef governorate during the period from June 2012 to May 2013. The collected samples comprised urban and rural areas while considering representation of high, middle and low social class population.

Laboratory testing

Blood samples were collected in plain vacutainer tubes and were tested for HCV by both ELISA and PCR. Moreover, all samples were tested by the InTec rapid Anti-HCV testing kit for validation of its results.

Determination of HCV RNA level.

Blood samples that were centrifuged for serum collection were stored at −20°C or −80°C until usage. RNA extraction from stored frozen samples was performed using QIAamp viral RNA Mini kit (QIAGEN) according to the manufacturer's instructions supplied with the kit. Extracted RNA was measured and quantitated with UV spectro- photometer at 260–280 wave length. An RT- PCR assay using TaqMan (fluorescence-based real-time PCR), and probes was designed for the quantitative determination of HCV RNA in the clinical blood samples. Absolute quantitation of the concentration of HCV RNA was based on an external standard curve (HCV Standards IU/mL) in the presence of an internal positive control (IPC). IPC was added to mixture of lysis buffer and sample material during RNA extraction of clinical blood samples. Calculation of RNA viral load was made by using the standard curve[12].

Determination of HCV by ELISA:

Anti-HCV was tested by a third generation enzyme immunoassay (ELISA; Murex anti-HCV; version 4.0; USA) that utilizes microwells coated with a combination of recombinant HCV-encoded antigens (c22-3, c200, and NS5). The assay was performed according to the manufacturer's instructions as previously described [13].

Determination of HCV by InTec rapid Anti-HCV testing kit:

The InTec rapid Anti-HCV testing kit is a colloidal gold enhanced, rapid immune-chromatographic assay for the qualitative detection of antibodies to HCV. It is manufactured by InTec Products, Inc.[14]

Intended use: According to the manufacturer "Rapid Anti-HCV Test is a colloidal gold enhanced, rapid immunochromatographic assay for qualitative detection of antibodies to hepatitis C virus (HCV) in human whole blood

(venous and fingerstick), serum or plasma specimens in adults. The test is intended for healthcare professionals and trained healthcare workers to use as an aid for diagnosis of HCV infection".

Assay description: According to the manufacturer "Recombinant HCV antigen (containing Core, NS2, NS3, NS4, NS5 segments) and mouse anti-human IgG antibody conjugated to colloidal gold are embedded in the sample pad.

If the specimen is positive, the HCV antibody in whole blood, serum or plasma specimen will combine with the colloidal gold conjugated recombinant HCV antigen and generate a complex. As the mixture moves along the test strip, the complex will be captured by the recombinant HCV antigen (containing Core, NS2, NS3, NS4, NS5 segments) immobilized on the membrane, forming a purplish red test band in the test region.

A negative specimen will not form any test band due to the absence of colloidal gold conjugate/HCV antibody complex. Regardless if HCV antibodies exist in a specimen, the unbound gold marked protein will bind to the sheep anti-mouse IgG in the control band region and form a purplish red band.
The assay is only valid when the control band appears".

Result interpretation Negative: Purplish red band only appears on control band region indicates a negative result. Positive: Purplish red bands appear at both the test band region (even very weak) and the control band region indicates a positive result. Invalid 1: A purplish red band appears only at the test band region of the cassette. Repeat the test. Contact the supplier if the control band remains invisible.

Invalid 2: Purplish red band appears at neither the control band region nor the test band region of the cassette. Repeat the test. Contact the supplier if the control band remains invisible[14].

Ethical consideration

The study was approved by the ethical committee of Bani Suef Faculty of Medicine, Bani Suef University, and written informed consents were obtained from participants prior to sample collection. All data of the examined individuals were dealt with complete confidentiality.

Statistical analysis

Data analysis was done on SPSS package version 21.0 (SPSS Inc., Chicago, IL, USA). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy percent of the InTec rapid HCV diagnostic test were calculated and compared with ELISA in relation with the golden reference test which is RNA detection by PCR.

Results

A total of 558 individuals (304 males and 254 females) whose age ranged from 7–68y with a mean±SD of 36.3±23.8 years participated in this study. Their blood samples were tested by PCR as a golden reference test for diagnosis of HCV. PCR results showed that 178 samples were HCV positive, while 380 samples were confirmed negative for HCV. The same samples were further examined by both ELISA test and InTec rapid Anti-HCV testing kit. Table 1 shows the frequency distribution of the true positive, true negative, false positive and false negative results of both InTec and ELISA tests compared with the golden reference PCR test for diagnosis of HCV. The mean age of the positive males was 38.2±17.6 years, while that of females was 31.5±14.9 years.

Table 1: frequency distribution of the true positive, true negative, false positive and false negative results of both InTec and ELISA testes compared with the golden reference PCR test for diagnosis of HCV.

	PCR HCV +ve	PCR HCV -ve	Total (Row)
InTec HCV +ve	177	5	182
InTec HCV –ve	1	375	376
Total (column)	178	380	558
ELISA HCV +ve	177	9	186
ELISA HCV –ve	1	371	372

Compared with the reference PCR test, the InTec rapid diagnostic test showed a sensitivity of 99.4%, a specificity of 98.7%, a positive predictive value of 97.3%, a negative predictive value of 99.7%, an accuracy percent of 98.9% and an error percent of 1.1%. These results were comparable to that of ELISA (Table 2).

Table 2: Comparison between InTec and ELISA testes regarding their sensitivity, specificity, positive predictive value and negative predictive value in diagnosis of HCV.

Test	InTec test		ELISA test	
Sensitivity	177/178	99.4%	177/178	99.4%
Specificity	375/380	98.7%	371/380	97.6%
Positive predictive value	177/182	97.3%	177/186	95.2%
Negative predictive value	375/376	99.7%	371/372	99.7%
Accuracy percent	552/558	98.9%	548/558	98.2%
Error percent	6/558	1.1%	10/558	1.8%

Discussion

Regarding the HCV status, the at-risk populations face social, structural, and economic barriers, such as limited access to testing[15] and lapses in health insurance[16], which hinder early screening and timely engagement with care.

The situation is worse in global low-resource settings, where standardized laboratory tests are expensive and often not covered by public health systems—and thus are rarely performed or offered on-site or in time, leading to suboptimal care and screening.

Worldwide, the increasingly dominant model of laboratory testing is the centralized laboratory, in which automation of analytical processes increases, enabling the analysis of large numbers of samples at a relatively low cost[17].

However, with launching a national program for control of HCV by mass screening and treatment of infected cases, there is an urgent need for developing rapid, cheap and field applicable HCV diagnostics. Scaling up HCV screening efforts with such rapid, cheap and field applicable kits can greatly help to identify HCV infected individuals and link them to the national program against HCV for confirmation of their HCV status, treatment and follow-up[18-20].

In the present study, we aimed to evaluate the diagnostic performance of the rapid screening testing kits in diagnosis of HCV infected individuals taking the InTec diagnostic kit as a model.

Our results showed that the InTec advanced quality rapid screening test has excellent sesitivity (99.4%) and specificity (98.7%) and demonstrated sufficient diagnostic accuracy (98.9%) with a PPV of 97.3% and a NPV of 99.7%.

Our results are comparable to that of a recent study that was performed by Waheed et al., 2019, whose results of the Intec product showed 98.56% sensitivity, 98.91% specificity, 99.51% PPV and 96.80% NPV[21].

Furthermore, InTec rapid diagnostic test had comparable results to that of the laboratory-

based results of ELISA test (a sensitivity of 99.4%, specificity of 97.6% and accuracy percent of 98.2%). However, the InTec rapid HCV-diagnostic kit had the advantages of being feasible, rapid, and cheaper and can be applied in the field without the need for special laboratory equipment, such as centrifuges, refrigerators or large-sized electrical machines and expertise of technicians thus offering additional opportunities to expand screening.

Using a random subsample of 161 patients, the InTec kit results have not been affected by the variations of the sample type, whether, it was whole venipuncture blood, capillary (finger-stick) blood, and serum or plasma samples (data not shown).Moreover, the results of the InTec kits have not been affected by the age and gender.

One of the limitations of the present study is that we have not examined the effect of the presence of co-infections such as hepatitis B viral (HBV) infection or human immune-deficiency viral (HIV) infection as well as the different genotypes of the HCV on the results of the InTec rapid HCV diagnostic testing kits.

Both ELISA and the rapid InTec HCV-diagnostic kit are anti-body based diagnostic tests and can not differentiate between the active current infection or the old infection and the completely cured individuals harboring the HCV antibodies. Therefore, additional conventional laboratory-based confirmatory testing should be considered for resolving such false-positive results.

In the United States, the Centers for Disease Control and Prevention (CDC) recommends using an enzyme immunoassay (EIA) and either recombinant immunoblot assay or HCV nucleic acid testing for RNA to diagnose hepatitis C infection[22]. Although this algorithm effectively detects active infection, the tests are expensive and have long turnaround times. Convenient, quality-assured, antibody-based rapid diagnostic tests could facilitate preliminary screening, although they cannot differentiate between acute and chronic infections[17].

In conclusion, our study provides valuable information that helps making decision of using the feasible InTec rapid diagnostic testing kits in the field for mass screening of HCV infections in Egypt. The rapid InTec diagnostic testing kits evaluated in this study can be used as one of the important and essential tools for HCV screening on much larger scales facilitating the efforts of the national program against HCV.

Disclosures and Potential conflicts of interest: All authors have no conflicts of interest regarding the work reported in this paper.

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