# Evaluation of some available HCV antibody detection tests (ELISA, Chemiluminescence, Immune Assay) and RT-PCR assay in the diagnosis of Hepatitis C virus infection

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## ABSTRACT

**Background:** The purpose of diagnosis of viral infection is to allow the infected persons to be identified & treated and to prevent blood-transfusion infection. Majority of primary HCV-infected patients are asymptomatic, thus, symptoms could not be used as specific indicators for HCV infection. HCV viremia could still exist despite a normal serum alanine aminotransferase (ALT) level. Therefore, virological methods rather than ALT levels are used to diagnose HCV infection. The diagnosis of HCV infection is mainly based on the detection of anti-HCV antibodies by the enzyme immunoassay(EIA) or Chemiluminescence immunoassay (CIA) of serum samples. These anti-HCV assays are used as a screening test, while PCR is essential for detection of screening test falsity. The presence of HCV-RNA in the serum is a reliable marker of viremia. Universal standardization for HCV-RNA titer is important for diagnosis and follow up.

**Objective:** This study aimed to evaluate the commercially available antibody tests for diagnosis of hepatitis c virus infection in comparison to RT-PCR in Egyptian blood donors.

**Materials and Methods:** This study Included 456 serum samples from blood donor at Al-Hussien hospital blood bank (Al-Azhar University, Cairo) from June 2016 to June 2018. Serum samples subjected to routine laboratory tests (CBC, liver and renal function) to exclude organs affection. Also they are subjected to HCV antibody detection by ELISA and Chemiluminescence tests and HCV–RNA detection by RT-PCR assay.

**Results:** We considered PCR as a standard test to evaluate ELISA and Chemiluminescence. The detected percentage of infectivity of donors in this study was 9% by ELISA, 13% by Chemiluminescence and 8 % by PCR. The percentage of false negativity of HCV antibody by ELISA and CIA when compared with PCR assay were 0.96% and 1.5% respectively. The false positivity of HCV –Ab by ELISA and CIA as compared PCR was 14-6% (6 out of 41) and 26.6% (16 out of 60).

**Conclusion:** Generally, ELISA is more sensitive and specific than Chemiluminescence for blood transfusion screening. But, at gray zone results, PCR should be used as confirmatory method. And so it is very important to screen blood donors using RT–PCR to avoid false positive and false negative results.

**Keywords:** HCV, PCR, ELISA, Chemiluminescence.

## INTRODUCTION

The safety of the blood products is one of the major issues in the area of transfusion medicine. Screening of the blood donors plays a major role to decrease the risk of transfusion of infected units. HCV affected around 3% of world population while it is > 10%for Egyptian population [1,2].

Diagnostic procedures of hepatitis C infection in laboratory is based on detection of IgG antibodies against recombinant HCV-polypeptides by two main methods; Enzyme Linked Immunoassay (ELISA) and Chemiluminescence immunoassay (CIA) [3]. Immunoassay detects antibodies against virus that takes an interval between donor's exposure to a virus and production of antibodies known as window period may last 2-3 months after infection. During this period the risk of donated blood can be missed with negative serology tests [4, 2].

Using PCR for detection and quantitation of HCV-RNA before seroconversion in blood and detect viral load in antibody-positive chronic cases is the best. PCR has the ability to detect immune activation in patients with residual hepatitis C virus after treatment with IFN and ribavirin [5]. Quantitative real-time PCR is the gold standard test in diagnosis of HCV [6].

## AIM OF WORK

The main objective of our study is to compare the results of HCV-infection by serological tests (ELISA and Chemiluminescence) with the gold standard test (till now) PCR. Also to select the most sensitive, specific and least cost test for screening blood donors in Egypt.

## MATERIALS AND METHODS

This study was conducted in blood bank and molecular biology units at AL-Hussien hospital, Al-Azhar University (Cairo), from April 2016 to May 2018. We use appropriate consent to participate in this study after explanation to the donors how much it is helpful in accurate diagnosis of HCV to decrease HCV transfusion infection. **The study was approved by the Ethics Board of Al-Azhar University.** The study group was 456 apparently healthy blood donors highly selected after complete history. All within the age group of 18-60 years, 342 males, 114 females. All were allowed to complete a donor health check questionnaire which is an important step insuring the safest possible blood for recipient. Blood samples from donors were subjected to: Routine laboratory investigation (CBC, liver and renal function tests) to exclude any affection of organs. Serological tests: to detect HCV- Abs by: Enzyme linked immunosorbent assay (ELISA) according to **Laperch *et al.* [7] and Lambert [8]** using commercial kits of Monolisa Bio-RAD. It is an antigen-antibody assay. Chemiluminescence assay (CIA).

The Elecsys anti-HCV assay is a 3rd generation test [9]. The assay uses peptides and recombinant antigens representing core, NS3 and NS4proteins for determination of Anti-HCV antibodies.

Principle: sandwich principle [10]. Results are determined automatically by the Elecsys software. PCR assay:

HCV-RNA is reverse transcribed and a specific fragment of RNA is amplified with specific primers in a one-step RT-PCR. The products are detected by using a specific Tagman-MGB probe. This probe is labeled at the 5-end with fluorescent dye and the 3-end by Non-fluorescent quencher. An internal control is supplied [11].

### Statistical Analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0. Qualitative data were expressed as frequency and percentage.

**The following test was done**: **Chi-square test:** was used when comparing between non-parametric data: P-value >0.05 was considered insignificant. P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant.

## RESULTS

Our results were illustrated in tables (1-8) and figure (1). Also, we take PCR as a standard test to evaluate ELISA and Chemiluminescence.

1- The rate of infectivity of blood donors by different methods was 9%, 13%, and 8% for ELISA, Chemiluminescence, and PCR respectively table (1) and Figure (1). Comparison between studied methods (ELISA, Chemiluminescence & PCR) each for another showed significant statistical differences between them except comparison between ELISA and PCR table (2).

**Table (1):** Percentage of infectivity of ELISA, Chemiluminescense and PCR.

| | ELISA | | Chem | | PCR | |
|---|---|---|---|---|---|---|
| | +ve Positive | -ve Negitive | Positive | Negitive | Positive | Negitive |
| Number of donors (N = 456) | 41 | 415 | 60 | 396 | 36 | 420 |
| Percentage of infectivity | (9%) | | (13%) | | (8%) | |
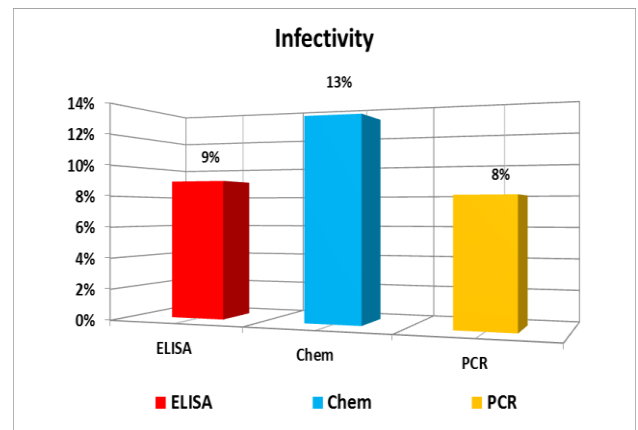


**Figure (1):** Percentage of infectivity of ELISA, Chemiluminescense and PCR.

**Table (2):** Comparison between studied methods (ELISA, Chemiluminescense & PCR) for HCV detection.

| Variables | Groups | ELISA (N = 456) | Chem (N = 456) | PCR (N = 456) |
|---|---|---|---|---|
| **HCV** | Negative | 415 (91%) | 396 (87%) | 420 (92%) |
| | Positive | 41 (9%) | 60 (13%) | 36 (8%) |
| **p-value** | P1 | 0.02* | | |
| | P2 | 0.04* | | |
| | P3 | 0.5 | | |
| | P4 | | 0.01* | |

*: p-value < 0.05 is considered significant.
p1: ELISA vs Chem vs PCR.
p2: ELISA vs Chem.
p3: ELISA vs PCR.
p4: Chem vs PCR.

2- The percentage of false negativity of HCV-antibody ELISA screening was 0.96% (4 out of 415). For CIA was 1.5% (6 out of 396) table (3).

**Table (3):** Percentage of false negative of ELISA and Chemiluminescense.

|  | ELISA | Chem. |
|---|---|---|
| Number of negative donors | 415 | 396 |
| Percentage of false negative | 4 (0.9 %) | (1.5 %) |

3- The percentage of the false positivity of HCV-Ab ELISA test was 14.6% (6out of 41) and for CIA was 26.6% (16 out of 60) table (4).

**Table (4):** Percentage of false positive of ELISA and Chemiluminescense.

|  | ELISA | Chem |
|---|---|---|
| Number of Positive donors | 41 | 60 |
| Percentage of false positive | 6 (14.6%) | (26.6%) |

4- Sensitivity of ELISA was 98.5% and CIAwas 97% comparing with RT-PCR sensitivity.

5- Specificity of ELISA was 99% and CIA was 94% compering with RT-PCR specificity.

6- Regarding to donors' age about 80% was 30-50 years old showed high positivity. Also males to females were 3-1 for sample studied.

7- Lastly we can suggest a simple calculation of S/C (signal of the sample / cut off of run) to detect the ratio S/C (Tables 5-8) to select most apparently positive and negative samples as:

**A- For negativity**

**S/C of true negative cases table (5)**

- ELISA: 0.2-0.89

- CIA: 0.12 – 0.98

**S/C of false negative cases table (6)**

- ELISA: 0.27-0.63

- CIAL: 1-6

So any cases done by ELISA with S/C <=0.6 must confirmed by PCR.

Also cases done by CIA with S/C <=6 should be confirmed by PCR assay.

**Table (5):** Range of S/C for true negative cases.

| Test | Range of S/C |
|---|---|
| ELISA | 0.2 – 0.89 |
| Chem | 0.12 – 0.98 |

**Table (6):** Range of S/C for false negative cases.

| Test | Range of S/C |
|---|---|
| ELISA | 0.27 – 0.63 |
| Chem | 1 – 6 |

**B- For positivity:**

**S/C of true positive cases table (7)**

- ELISA: 1.5-4.5

- CIA:>=6

**S/C of false positive cases table (8)**

- ELISA: 1.02-1.17

- CIA:1-6.1

So any case with S/C 1.02 -1.5 by ELISA should be estimated by RT-PCR also case with S/C <6.1 by CIA should be confirmed by RT-PCR.

**Table (7):** Range of S/C for true positive cases.

| Test | Range of S/C |
|---|---|
| ELISA | 1.5 – 4.5 |
| Chem | ≥ 6 |

**Table (8):** Range of S/C for false positive cases

| Test | Range of S/C |
|---|---|
| ELISA | 1.02 – 1.17 |
| Chem | 1 – 6.1 |

## DISCUSSION

Blood safety is an integral part of every country's national health care policy [13]. The developing countries are more likely to use contaminated blood. This may be due to higher disease prevalence or there is inadequate serology based screening. HCV is one of many blood borne viruses. HCV contaminated blood supply was a big problem before 1992 since there was no laboratory method to test for HCV. Egypt has a high prevalence (>10%) of HCV infection among persons 15-59 years. The diagnostic screening blood tests are the antibody tests (ELISA and CIA), a confirmatory test (RIBA) and the most sensitive test for RNA detection is polymerase chain reaction [14,1].

The presence of HCV antibody suggests prior exposure to HCV but doesn't confirm immunity while positive PCR means detectable level of HCV virus in the blood. PCR can detect HCV-RNA after five days of exposure to infection while antibodies appear up to 2-3 months after infection at this window period there is high risk in donated blood due to false negative serological tests [4].

In 2008, Kamel and Nasser [15] found that the prevalence of chronic HCV infection in Egypt is higher in men than women (12% and 8%, respectively), increase with age reaching > 25 % among person aged > 50 years. Mohamed *et al.* [2] reported that blood donor

prevalence of 4% using NAT assay with 3.67% males and 0.33% females. In this study, regarding infectivity of blood donors, the prevalence of HCV infection among blood donors by ELISA screening was (9%) (41out of 456). While the CIA showed prevalence of (13 %) (60 out of 456) RT-PCR assayInfectivity was lower (8%) (36 out 456) **(table 2)**.

In 2007, **TashKandy** *et al.* [16] found that Saudi Arabian blood donors have 2.44 % infectivity by ELISA and RT-PCR assay. The results of HCV antibodies reactivity were 4.04% in a study done by Abdel Meguid E. and Ebeid M. [17] in the national cancer institute blood bank in Egypt.

In **2011 wasfi and sadek** [18] by their study in Alexandria – Egypt showed that the prevalence of infectivity by ELISA screening was 3.5%.Also our results were slightly different from the study done by **Zaki** [19]in regional blood transfused center Minia in Egypt showed prevalence of HCV by ELISA among blood donors was (6.4%) while in the same year **2009 Ismail** *et al.* [20], in Egypt showed that HCV prevalence was 18% for blood donors while **Tashkandy** *et al.* [16] reported infectivity in blood donors was 19.8% ELISA,20.8% (RIBA) and 23.6%(PCR).

This discrepancy may be due to insufficient applied questionnaire that miss some cases. Also may be due to using 3$^{rd}$ generation of ELISA kit without antigen-antibody combination within 4$^{th}$ generation kit used in our study and differences in nationality.

The false negativity of HCV-Ab by ELISA screening test compared to CIA in blood donors was 0.96% (4 out of 415),1.5% (6 out of 396) **(table 3)**. This false negativity may be due to low level of HCV antibodies (despite viremia) during the HCV window period. So the test should be repeated after six months from exposure [21]. The false positivity of ELISA screens test for HCV-Ab as compared with CIA was respectively 14.6% (6 out of 41) and 26.6%(16 out of 60) **(table4).** This suggests high specificity of RT-PCR to exclude HCV Viremia in spite of non-specific antibodies [12] using 2$^{nd}$ generation ELISA reported that HCV viremia was present in 76.6% of anti-HCV positive blood donors 23.4%false positive in central Brazil.

Also in **2007 Tashkandy** *et al.* [16]reported a false positivity of ELISA compared with RT-PCR to be 10% in Saudi Arabian blood donors. However, the presence of false positivity compared by RT-PCR may be due to the presence of non-specific antibodies

detected by ELISA screening test [1,5] therefore the high degree of false negativity associated with ELISA and CIA screening techniques for HCV–AB among blood donor leads to increase risk of transfusing contaminated blood. On the other hand, false positivity leads to increase wastage of otherwise safe units of blood, especially these who belong to rare blood groups, apart from causing anxiety to the donor.

Thus it is important to screen blood donors by using RT-PCR for HCV-RNA to avoid occurrence of false negative results of HCV-AB detection test. To avoid false positive results, we must repeat RT-PCR every 6 month for 2 years and if still negative this means that the donor is non infected and free from viremia. This agree with **Mohamed** *et al.* [2] when compares ELISA, CIA and NAT (nucleic acid test) for Egyptian blood donor. From our view CIA is completely away from discussion since it is higher false positivity (26.6%)When compared to ELISA 14.6% that may be due to less specificity of CIA and also due to presence of antigen antibody combination in ELISA kits we used in our study (4$^{th}$ generation) ELISA kits HCV-AB test.

## CONCLUSION AND RECOMMENDATION

We concluded that effectiveness of PCR testing for blood donors screening is a debating area in transfusion medicine. Thus it is very important to screen blood donors using RT-PCR to avoid false negative results of antibody testing. To avoid false positive results, we should repeat RT-PCR twice every year and if it remains negative means that the case is free from viremia. This fact strengthens the support for the use of PCR technique for blood donors despite its cost factor.

Also we recommended the use of 4thgeneration of ELISA (combined AG-AB reagents) for HCV-AB screening and calculates S/C ratio to select those within the grey zone for PCR confirmation. IT is important that each laboratory has to calculate S/C value.

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