IRAQI Academic Scientific Journals
Al-Wataniya Journal of Medical Sciences
AL-WATANIYA JOURNAL OF MEDICAL SCIENCES
pISSN: 3105-3165
eISSN: 3105-3173
WJMS Issued by The National University of Science and Technology wjms.nust.edu.iq info.wjms@nust.edu.iq
AL-WATANIYA JOURNAL OF MEDICAL SCIENCES N.U.S.T 2025

ARTICLE

Comparative Study of ELISA and RT-PCR in the Detection of Hepatitis C Virus in Southern Iraq

Mohammed Ali Naser Alasadi*[1], Mohaimen Abd Oun Saod[2]

[1]College of Health and Medical Technologies, National University of Science and Technology, Nasiriyah, Iraq

[2]College of Nursing, National University of Science and Technology, Nasiriyah, Iraq

***Corresponding author. Email:** mohammed.a.naser@nust.edu.iq

How to cite

Alasadi M, Saod M. Comparative Study of ELISA and RT-PCR in the Detection of Hepatitis C Virus in Southern Iraq. Al-Wataniya Journal of Medical Sciences. Sciences. 2025;1(3):29–35.

(Received: Nov 25,2025; Accepted: Dec 30,2025; Published: Dec 30, 2025)

Access Article Online

Abstract

This study aimed to diagnose HCV infection and estimate its prevalence among patients attending healthcare facilities in Dhi Qar Governorate, Iraq, using both serological and molecular tests. Hepatitis C virus (HCV) is one of the most important causes of chronic liver disease, causing hundreds of thousands of deaths worldwide annually. Eighty patients (48 male and 32 female) aged 1-74 years were included in the study from January,5th 2022 to December,12th 2022. HCV antibody was determined by ELISA, viral nucleic acid was determined by real-time PCR.

Fifty samples (62.5%) were positive by ELISA, while only 23 (46% from the positives) had detectable RNA levels upon RT-PCR examination. A higher prevalence was observed in males compared to females and the age group with the highest prevalence was between 40-49 years. The results confirm that ELISA is useful as an initial screening tool, but RT-PCR is a more accurate method for confirming active infection and determining viral load. This study highlights the need for routine integration of RT-PCR into diagnostic workflows to improve detection and management of HCV in southern Iraq.

Keywords: HCV, Pathogenesis, ELISA, RT-PCR, Hepatitis.

1. Introduction

Hepatitis C virus (HCV) is a major global health problem, causing chronic liver inflammation that can progress to cirrhosis or liver cancer. It is a leading cause of liver transplantation in many countries. The World Health Organization reports that approximately 130-150 million people worldwide are chronically infected with the virus, with between 350,000 and 500,000 deaths annually associated with its complications.

The virus is most commonly transmitted through blood transfusions, shared needles, or accidental exposure to contaminated blood. Less common routes, such as sexual transmission, have also been reported. Although similar to other viruses such as HIV, HCV is more transmissible through blood.

In Iraq, HCV infection represents a growing challenge to the public health sector. Prevalence rates vary across geographic regions, with few studies shedding light on the epidemiological situation in the southern governorates, including Dhi Qar Governorate.

Accordingly, this study aims to diagnose hepatitis C virus (HCV) infection among a sample of visitors to healthcare facilities in Dhi Qar Governorate using serological tests (ELISA) and molecular techniques (RT-PCR), and to compare the effectiveness of these methods in detecting active infection.

2. LITERATURE REVIEW

2.1 History of Hepatitis C Virus

Hepatitis C virus (HCV) was first identified in the 1980s as a major cause of non-A, non-B hepatitis. Despite significant progress in research, the exact origins of the virus remain uncertain. Experimental studies have shown that HCV can infect chimpanzees under laboratory conditions, but in nature, humans remain the only natural hosts [1,2]. Before 1995, the discovery of GB virus B (GBV-B) in tamarins, a small primate species, did not reveal any closely related viruses until further characterization studies were conducted [3,4].

2.2 Structure and Genotypic Diversity

Globally, an estimated 170 million individuals are chronically infected with HCV, with 3-4 million new cases occurring annually [5]. The virus shows high genetic diversity with important clinical and epidemiological implications. HCV is categorized into at least seven genotypes, with several subtypes (e.g., 1a, 1b, 2a) sharing varied geographical preference-specific distributions [6,7]. Genotype 1 has the highest rate of prevalence, which accounts for over 50% of all cases around the world, especially in Europe and the Americas. In comparison, genotypes 2 and 4 are less prevalent and infect a little over 10% of the world's population [8,9]. The distribution of genotype is important to design antiviral therapy and predict the outcomes after treatment.

2.3 Epidemiology of HCV

The worldwide prevalence for HCV infection is still high, with 3-4 million new cases annually. Studies seroprevalence confirmed that there are great disparities between regions. The circulation of anti-HCV antibodies is higher in certain areas of Africa and Asia than it is in industrialized countries, like the United States, Western Europe, and Australia, which have lower rates [5]. The differences are mostly due to varying health care infrastructures, infection control practices, and blood safety. The large genetic diversity of HCV is another element that determines its complexity as a virus from an epidemiological point of view, to be controlled and eradicated.

2.4 Pathogenesis of HCV

Unlike other hepatotropic viruses such as hepatitis B virus (HBV), HCV replicates exclusively through an RNA-dependent mechanism without a DNA intermediate, preventing the establishment of a latent nuclear phase [10,11]. Viral entry into host cells occurs via receptor-mediated endocytosis, triggering the activation of innate immune responses. Recognition of viral RNA patterns in the cytoplasm induces the production of interferons and pro-inflammatory cytokines, leading to the recruitment of adaptive immune responses [11]. Effective viral clearance during acute infection requires a coordinated response by CD4+ helper T cells and cytotoxic CD8+ T lymphocytes. However, viral escape mutations and persistent antigenic stimulation often result in T-cell exhaustion, marked by the upregulation of inhibitory receptors. This immunological dysfunction is a key factor in the progression from acute to chronic infection.

3. Materials and Methods

3.1 Study Design and Population

This cross-sectional study was conducted in Dhi Qar Governorate, Iraq, between January 5, 2022, and December 12, 2022. A total of 80 patients (48 males and 32 females), aged 1-74 years, were enrolled. All participants were clinically suspected of having hepatitis C virus (HCV) infection and attended public healthcare facilities. Patients were stratified according to age groups, gender, and residency (urban vs. rural) to assess demographic correlations.

3.2 Sample Collection

Venous blood samples were collected under aseptic conditions. Serum and plasma were separated by centrifugation at 3000 rpm for 10 minutes and stored at -20 °C until further testing.

3.3 Serological Detection of Anti-HCV Antibodies

The presence of anti-HCV antibodies was screened using the Enzyme-Linked Immunosorbent Assay (ELISA) (Foresight, USA). The test is based on the binding of HCV-specific antibodies in patient serum to antigens coated on microtiter wells, followed by conjugation with enzyme-labeled antibodies. After substrate addition, a colorimetric change was measured at 450 nm using an ELISA reader. The intensity of color was proportional to the antibody concentration. Positive and negative controls were included in each run to ensure assay reliability.

3.4 Molecular Detection of HCV RNA

3.4.1 RNA Extraction

Viral RNA was extracted from plasma samples using the Ribo Virus Kit (RIBA) according to the manufacturer's instructions. Briefly, guanidinium thiocyanate-based lysis buffer was used to disrupt viral particles, followed by RNA binding to silica membrane columns. After washing steps to remove contaminants, purified RNA was eluted and stored at -70 °C until amplification.

3.4.2 Real-Time PCR Amplification

Quantitative detection of HCV RNA was performed using a Real-Time Polymerase Chain Reaction (RT-PCR) kit (Sacsce Biotechnologies, Italy). Reverse transcription of viral RNA into complementary DNA (cDNA) was followed by amplification using specific primers and fluorescent probes. An internal control was included to detect potential inhibitors and verify the amplification process. The viral load was determined based on standard curves generated from serial dilutions of HCV quantitation standards, expressed in International Units per milliliter (IU/mL).

3.5 Data Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 25). Results were expressed as frequencies, percentages, and mean ± standard deviation where applicable. Associations between categorical variables (e.g., gender, age group, residency) and HCV infection status were assessed using the Chi-square test. A p-value of <0.05 was considered statistically significant.

4. Results

4.1 Serological Detection of Anti-HCV Antibodies

A total of 80 patients suspected of having HCV infection were tested using ELISA. Out of these, 50 samples (62.5%) were seropositive, while 30 samples (37.5%) tested negative as shown in Table 1. When stratified by gender, 37 out of 48 males (77.1%) were positive, compared to 13 out of 32 females (40.6%). This difference was statistically significant ($p < 0.05$), indicating a higher prevalence of infection among males.

Table 1. Results of serological tests using ELISA technology.

Study totals	No. of samples	No. of positive samples
Positive	23	46%
Male	48	37
Female	32	13
Totals	80	50

4.2 Gender Distribution of Study Population

Of the total study participants, 48 (60%) were males and 32 (40%) were females as shown in Table 2. The higher infection rate observed among males supports the finding that gender may play a role in exposure risk.

Table 2. Distribution of study population by gender.

GENDER	No.	%
Male	48	60.0
Female	32	40.0
Total	80	100

4.3 Age Distribution of HCV-Infected Patients

The patients' ages ranged from 1 to 74 years, with a mean of 32.17 ± 15.02 years. The age group 40-49 years represented the highest infection prevalence (26.3%), followed by the 50-59-year group (21.2%). Children under 10 years accounted for only 7.5% of cases, and patients over 70 years represented 3.7% as shown in Table 3 and Figure 1. These findings suggest that middle-aged adults are at the highest risk of active infection.

Table 3. Distribution of HCV infection according to age groups.

AGE GROUPS	No	%
1-9	6	7.5
10-19	7	8.8
20-29	10	12.5
30-39	11	13.7
40-49	21	26.3
50-59	17	21.2
60-69	5	6.3
≥70	3	3.7

Total	80	100

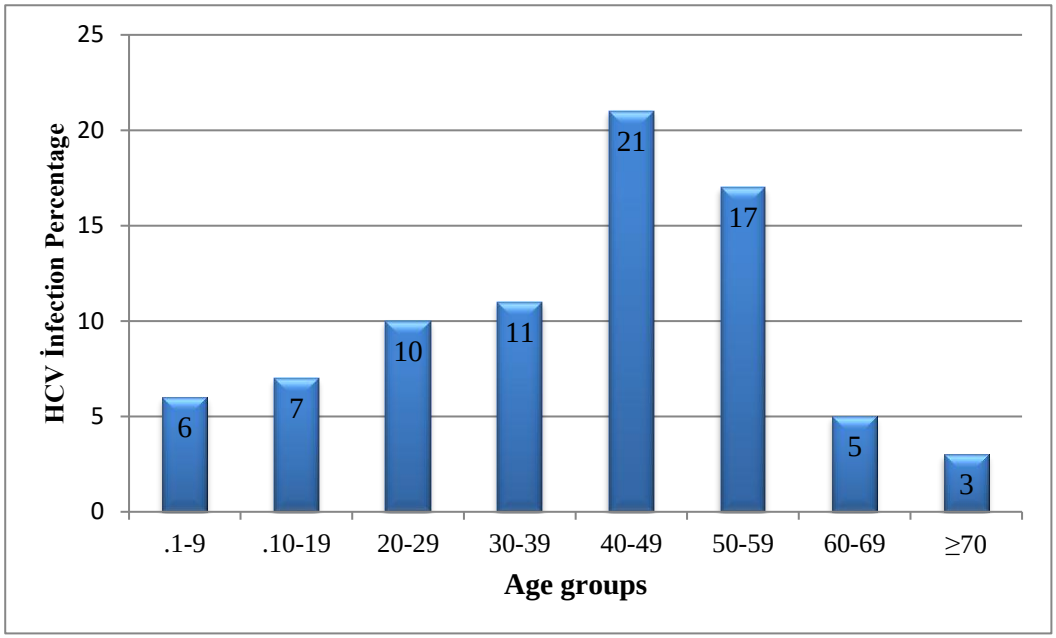

Figure 1. Age group distribution of HCV infection

4.4 Residency Distribution

Analysis of residency revealed that 59 patients (73.7%) were from urban areas, whereas 21 patients (26.3%) resided in rural regions as shown in Table 4 and Figure 2. This indicates a higher detection rate in urban areas, which may reflect greater access to healthcare services and diagnostic facilities.

Table 4. Distribution of patients according to residency.

RESIDENCE	No.	%
Urban	59	73.7
Rural	21	26.3
Total	80	100

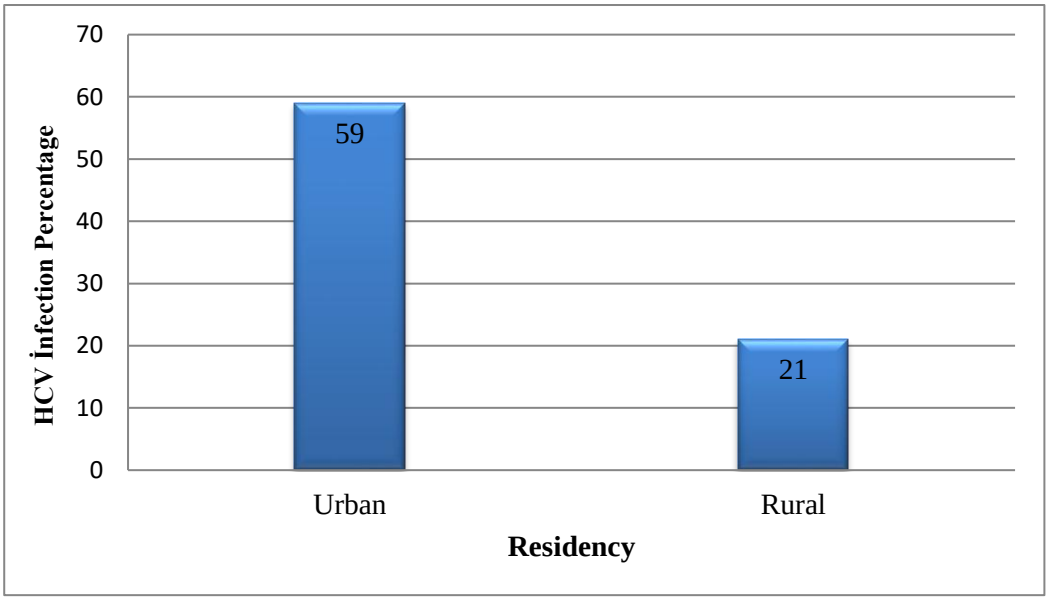

Figure 2. Urban vs. rural distribution of HCV infection

4.5 Molecular Detection of HCV RNA by RT-PCR

Of the 50 ELISA-positive samples, 23 (46%) were confirmed positive for HCV RNA using RT-PCR, while 27 (54%)

were negative. This corresponds to 28.8% of the total study population (23/80). The viral load among PCR-positive samples ranged between 1.8×10^3 IU/mL and 9.12×10^4 IU/mL. This demonstrates that ELISA may overestimate the prevalence of active infection and underscores the importance of molecular confirmation. As shown in Figure 3,4 and Table 5.

Table 5. Detection of HCV RNA among ELISA-positive samples by RT-PCR.

Result	Number of Samples	%
Positive	23	46%
Negative	27	54%
Total	50	100%

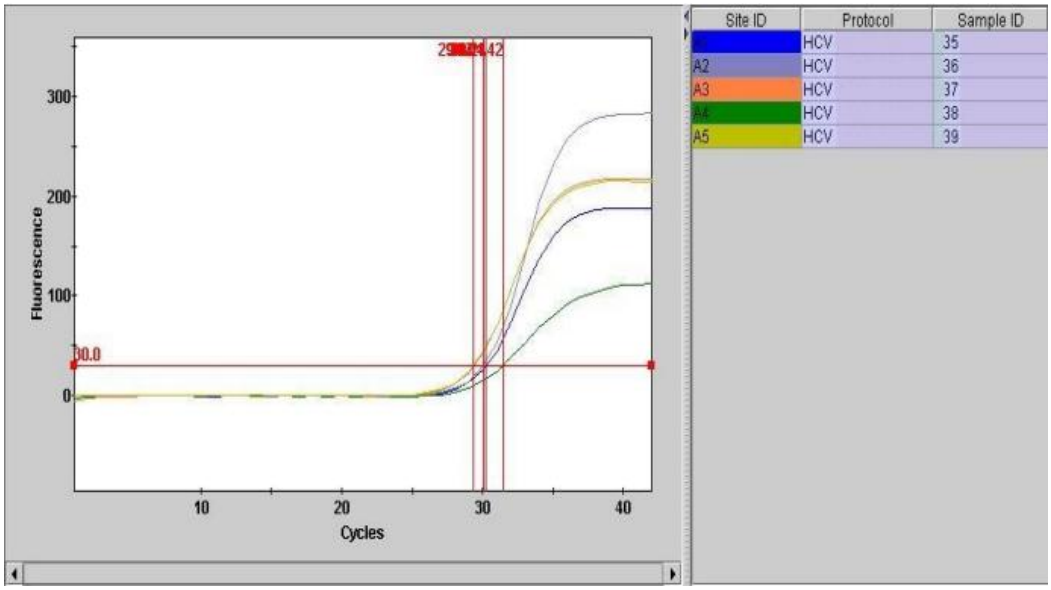

Figure 3. RT-PCR amplification curves for standard parameters

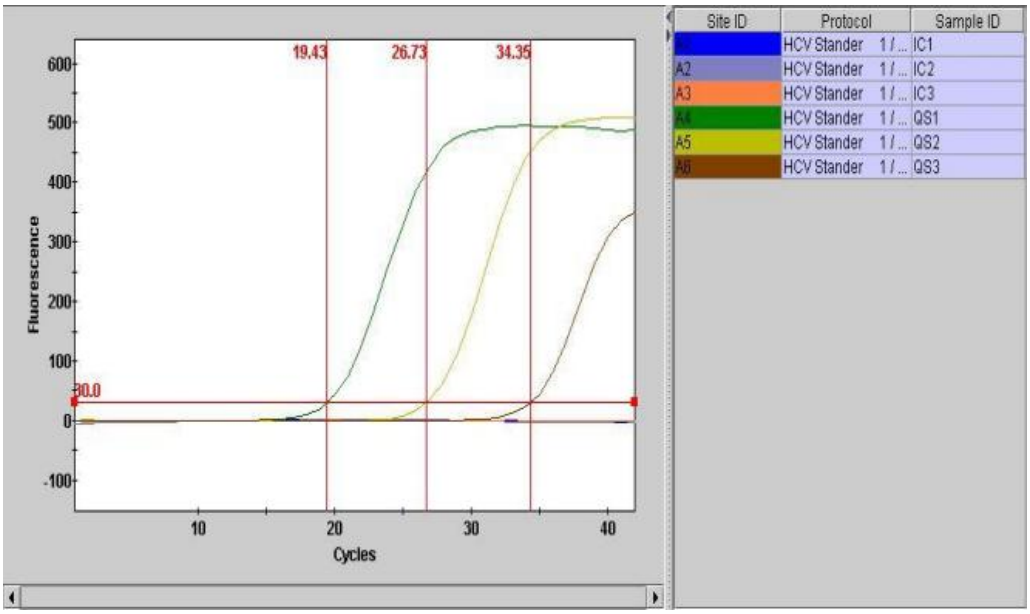

Figure 4. Graphical representation of RNA levels in PCR-positive samples

5. Discussion

In the current study, we investigated the seroprevalence and diagnosis confirmation of HCV infection among patients visiting hospitals in Dhi Qar Governorate. By combining ELISA and RT-PCR, the research presented an overview of seroprevalence according to demographic characteristics and assessment about the accuracy of diagnostic tools available.

5.1 Serological vs. Molecular Diagnosis

The results of which indicated that 62.5% were positive for anti-HCV antibodies using ELISA and only 46% could be confirmed by RT-PCR. This inconsistency emphasizes the weakness of serological examination, revealing past exposure or false positives. By contrast, RT-PCR offers evidence on the active state of infection and determination of viral load; these are material for clinical judgements. These data are in line with the findings of AL-Sayed & AL- Adrosy [12], and highlighted those results generated by molecular tools were more sensitive for detecting infections at early stage.

5.2 Gender Distribution

Males tested had a significantly higher rate of infection (77.1%) than females (40.6%). The same results have been observed in Iraq and other countries, as shown in a study by Al-Sweedan et al in Jordan [13]. The higher prevalence among men compared to women can be due to increased exposure to risk factors like blood transfusion, occupational accidents, IV drug use or unsafe medical practices. The lower incidence of the nosocomial transmission among female patients could also be justified by decreased exposure, both in perihospital areas or outside.

5.3 Age Distribution

The highest infection burden was observed in the 40-49 age group (26.3%), followed by 50-59 years (21.2%). This pattern aligns with the findings of Alter [14], who reported increasing prevalence of HCV with age due to cumulative lifetime exposure. The predominance in middle-aged adults may also reflect the chronic, silent nature of HCV, which remains asymptomatic for years before manifesting as advanced liver disease.

5.4 Residency Differences

Urban residents accounted for nearly three-quarters (73.7%) of the infected population. While this could suggest higher urban prevalence, it is more likely related to better healthcare access and diagnostic testing in urban centers. Rural residents may be underdiagnosed due to limited facilities and lower awareness, as noted in other Iraqi epidemiological studies. Expanding diagnostic services in rural areas is therefore crucial for accurate disease surveillance.

5.5 Comparison with Previous Studies

The prevalence observed in this study is comparable to earlier reports from Iraq. Al-Jubory et al [15] found similar rates among blood donors in Babylon Governorate, while AL-Haris [16] reported comparable infection rates among thalassemia patients. However, the lower RT-PCR confirmation rate in this study compared to Al-Mola et al. [17] may be due to differences in patient populations, sample size, and diagnostic approaches.

5.6 Strengths and Limitations

The main strength of this study lies in its use of both serological and molecular techniques, allowing differentiation between past exposure and active infection. Furthermore, it provides updated local data from Dhi Qar Governorate, a region with limited prior research on HCV. Nonetheless, several limitations should be acknowledged. The relatively small sample size (n=80) restricts the generalizability of the findings. Additionally, HCV genotyping was not performed, which could have provided important insights into circulating strains in southern Iraq. Finally, the absence of detailed risk factor analysis (e.g., transfusions, surgeries, or drug use) limits the ability to identify key modes of transmission.

5.7 Implications

The findings underscore the importance of integrating RT-PCR into routine diagnostic protocols in Iraq to improve diagnostic accuracy, guide treatment strategies, and prevent unnecessary interventions for false-positive ELISA cases. Moreover, the demographic patterns observed, particularly the predominance among males and middle-aged individuals—highlight the need for targeted public health interventions, including education, early detection, and risk reduction strategies.

6. Conclusion

The method for determining anti-HCV is a medium-sensitive and specific method, while the molecular method (PCR) is more specific and sensitive.

The use of molecular methods is necessary to diagnose the virus for the early detection of infections and to prevent its spread.

The results showed that the highest percentage of infection was for the male group (77.1%), while the lowest percentage was for females (46.6%), as the current study showed a significant difference at the level of probability (P 0.005).

Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. Adoption of Molecular Diagnostics: Real-time PCR should be routinely used alongside ELISA to confirm active HCV infection and to measure viral load, particularly in patients with positive serological results.
2. Genotyping and Sequencing: Conduct molecular sequencing of HCV RNA to determine prevalent genotypes and sub genotypes in Dhi Qar Governorate. This will support more targeted treatment strategies.

3. Public Health Measures:
    - Expand HCV diagnostic services in rural healthcare facilities to ensure early detection in underserved populations.
    - Implement regular screening programs for high-risk groups such as blood donors, dialysis patients, and individuals with a history of transfusions.
4. Clinical Management: Infected individuals should undergo periodic monitoring of viral load and liver function to assess disease progression and treatment response.
5. Immunological Studies: Conduct comprehensive immunological studies on infected patients to better understand host immune responses and inform vaccine development efforts.
6. Awareness and Prevention Programs: Strengthen community-based awareness campaigns focusing on transmission routes, safe medical practices, and preventive behaviors to reduce new infections.
7. Integration of Modern Techniques: Encourage healthcare institutions to adopt advanced molecular and immunological methods for the diagnosis and management of viral infections beyond HCV.

References

1. Bradley DW, Maynard JE, Popper H, Cook EH, Ebert JW, McCaustland KA, Posttransfusion non-A, non-B hepatitis in chimpanzees: physicochemical evidence that the tubule-forming agent is a small, enveloped virus. Gastroenterology. 1985;88(3):773-9. 10.1016/0016-5085(85)90150-7; DOI: 10.1016/0016-5085(85)90150-7.
2. Kolykhalov AA, Agapov EV, Blight KJ, Mihalik K, Feinstone SM, Rice CM. Transmission of hepatitis C by intrahepatic inoculation with transcribed RNA. Science. 1997; 277:570-4; DOI: 10.1126/science.277.5325.57 .
3. Muerhoff AS, Simons JN, Leary TP, Erker JC, Chalmers ML, Pilot-Matias TJ, Genomic organization of GB viruses A and B: two new members of the Flaviviridae associated with GB agent hepatitis. J Virol. 1995;69(9):5621-30; DOI: 10.1128/jvi.69.9.5621-5630.1995.
4. Simons JN, Pilot-Matias TJ, Leary TP, Dawson GJ, Desai SM, Schlauder GG. Identification of two flavivirus-like genomes in the GB hepatitis agent. Proc Natl Acad Sci U S A. 1995;92(8):3401-5; DOI: 10.1073/pnas.92.8.340.
5. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333-42; DOI: 10.1002/hep.26141.
6. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005;42(4):962-73; DOI: 10.1002/hep.20819.
7. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318-27; DOI: 10.1002/hep.26744.
8. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014;61(1 Suppl):S45-57; DOI: 10.1016/j.jhep.2014.07.027.
9. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61(1):77-87; DOI: 10.1002/hep.27259.
10. Chung RT, Baumert TF. Curing chronic hepatitis C- the arc of a medical triumph. N Engl J Med. 2014;370(17):1576-8; DOI: 10.1056/NEJMp1400986.
11. Rosen HR. Clinical practice. Chronic hepatitis C infection. N Engl J Med. 2011;364(25):2429-38; DOI: 10.1056/NEJMcp1006613 .
12. Al-Sayed KM, Al-Adrosy H. Recent approaches for early diagnostic detection of HCV infection. Egypt J Immunol. 2004;11(1):9-23.
13. Al-Sweedan SA, Jaradat S, Ameer K, Hayajneh W, Haddad H. Seroprevalence and genotyping of hepatitis C virus in multiple-transfused Jordanian patients with β-thalassemia major. Turk J Hematol. 2011;28(1):47-51; DOI: 10.5152/tjh.2011.05.
14. Alter MJ. Epidemiology of hepatitis C. Hepatology. 1997;26(3):62-5; DOI: 10.1002/hep.510260711.
15. Al-Jubory AWF, Salih HALM, Al-Assaadi MK, Ali AM. Seroprevalence of hepatitis B and C among blood donors in Babylon Governorate, Iraq. Med J Babylon. 2010;7(1-2):1-7.
16. Al-Haris FM. Study of HCV genotypes and viral blood load in thalassemia children in Mid-Euphrates area, Iraq. Int J Adv Res. 2015;3(5):1165-75.
17. Al-Mola GA, Tarish HR, Abdulsada KM, Lateef RH. Investigation of the major HCV genotype in asymptomatic patients in Iraq by the use of reverse-transcription PCR. Int J Sci Eng Res. 2013;4(12):1306-13.

How to cite

Alasadi M, Saod M. Comparative Study of ELISA and RT-PCR in the Detection of Hepatitis C Virus in Southern Iraq. Al-Wataniya Journal of Medical Sciences. Sciences. 2025;1(3):29–35.

(Received: Nov 25,2025; Accepted: Dec 30,2025; Published: Dec 30, 2025)

Access Article Online