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Original Research Article

Comparison of Manual and Automated Nucleic Acid Extraction Methods for HCV- RNA Assays

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ABSTRACT

Background: Hepatitis C Virus (HCV) infection is the most leading cause of chronic hepatitis in world and it is more prone to cause cirrhosis and carcinoma of liver. Hence, early diagnosis of this virus infection may be helpful to prevent these complications. First step in diagnosis of HCV infection is Enzyme Linked Immunosorbent Assay (ELISA), but it cannot differentiate people who are chronically infected from people who had spontaneous resolution, so molecular method by HCV-RNA detection is more helpful. HCV-RNA extraction is done by two methods, manual and automated method.

Material and methods: In present study, we compared amplification results of HCV-RNA which were extracted by both methods. Automated method is more sensitive than manual method as manual method is time consuming, more cumbersome and has high risk of contamination.

Results: Out of 100 samples, amplification result of 16 samples were found to be positive by HCV-RNA which were extracted by automated method but negative by manual method. The data was analysed using Microsoft Excel.

Conclusion: Automated method has high sensitivity and less chance of contamination.

Keywords: HCV-RNA, Molecular Technique, Automated method, Manual method, Comparison, Extraction

INTRODUCTION

One of the leading cause of chronic hepatitis in world is Hepatitis C virus infection and it is major risk factor for cirrhosis and carcinoma of liver after infection progression to advanced entities. [1-3]

There are an estimated 58 million people have chronic hepatitis C virus infection globally and 1.5 million people present with hepatitis C virus infection every year globally. WHO stated that in 2019, approximately 2,90,000 people died from Hepatitis C virus infection. The HCV is a critical viral infection. [3] There are an estimated 3.2 million children & adolescents with chronic hepatitis C virus infection.[4] The prevalence in India is estimated between 0.5-1.5 %. In India there are 12.5 million carriers of chronic hepatitis C virus infection are found. About 12-20% of chronic liver disease and 12-30% of hepatocellular carcinoma cases are due to chronic HCV infection. There are very few drugs such as sofosbuvir, velpatasvir, voxilaprevir, elbasvir, grazoprevir and ledipasvir are approved yet to combat the HCV. [4,5]

HCV infection is prone to develop chronic hepatitis in 60-70%, cirrhosis in 5-20% and hepatocellular carcinoma in 1-5%.[6] Early detection of HCV infection may help to prevent complications which occur due to chronicity of HCV infection. Diagnosis of Hepatitis C virus infection is done by HCV antibody detection Assay by Enzyme Linked Immunosorbent Assay (ELISA), Recombinant Immunoblot Antibody Assay (RIBA), HCV core Antigen Assay, molecular method like PCR detecting HCV RNA. [7-9]

1st and 2nd generation ELISA are now obsolete as they are less sensitive and become positive only after 10 weeks of infection. 3rd generation ELISA has more sensitivity and specificity and become positive in 5 weeks of infection. Hence, Serological test is the First step for the diagnosis of Hepatitis C virus infection. 3RD generation Enzyme Linked Immunosorbent Assay (ELISA) test is used, as it has increased sensitivity and more specificity. [7,8]

Enzyme Linked Immunosorbent Assay (ELISA), cannot differentiate people who are chronically infected from people who had spontaneous resolution, for that it is always necessary to determine presence of virus in the circulation, so for confirmation of active infection and quantification of HCV-RNA and for monitoring the response to treatment, Molecular diagnostic techniques are most sensitive and specific tests. Molecular method for viral load testing is an important diagnostic technique prior to initiation of treatment and for monitoring the prognosis. [8,9]

For HCV Viral load testing, Viral RNA extraction is a key component, as it affects both the reliability and reproducibility of target amplifications. Nucleic acid extraction is done by two methods, Manual extraction system and automated extraction method. [10,11]

Manual extraction system is less sensitive as it is time consuming and requires meticulous technical skills. There is more chance of contamination in manual method. Automated nucleic acid extraction system is beneficial as it reduces the working time, labour cost and risk of contamination. Disadvantage of automated method is its high cost. [12-15]

MATERIAL AND METHODS

The evaluation was carried out from patient samples submitted to department of Microbiology during June 2022-November 2022. The study includes 100 serum samples for HCV-RNA assays.

RNA extraction from these samples was performed using automated isolation system QiA Symphony DSP Viral Pathogen Mini Kit and the manual isolation was performed using Gsure Viral RNA Isolation Kit. The amplification process of the nucleic acid obtained through both methods was conducted with the PCR method using the TRU-PCR Kit.

Manual Extraction Method

RNA isolation from the serum samples was performed by using the Gsure Viral RNA Isolation Kit following the manufacturer's recommendations.

1) Pipet 300 μl GVL Buffer-Carrier-RNA-EB mix into a QAmp Mini Column. (in a 2ml collection tube)
2) 200μl of sample was added to QAmp Mini Column, incubation was done at room temperature for 10 minutes and centrifuged at 8000 rpm for 1 minute. The supernatant was aspirated in another tube.
3) 400 μl chilled ethanol was added to the supernatant and centrifuged at 8000 rpm for 1 minute and flow through was discarded from collection tube.
4) 500μl of buffer AW-1 was added to each QAmp mini column and it centrifuged at 8000 rpm for 1 minute.
5) The QAmp Mini column was placed into another clean 2 ml collection tube and tube containing filtrate was discarded in autoclavable beaker.
6) 500μl of buffer AW-2 was added to each QAmp Mini column and then it was centrifuged at 14000 rpm for 2 minutes. It was again Centrifuged at 8000 rpm for 1 minute. (Dry run)
7) The QAmp Mini column was placed into the another clean 2 ml collection tube and tube containing filtrate was discarded in autoclavable beaker.
8) 50μl of Buffer AVE (Elusion buffer) was added to QAmp mini Column and then it was incubated at room temperature for 2 minutes

9) It was then centrifuged at 1000 rpm for 1 minute.
10) The QAmp mini Column was discarded into autoclavable beaker.
11) RNA extract was kept in 2 ml PCR tube at 2 to 8ºC till plating. (maximum 30 minutes)

Automated Extraction Method

Reagents Preparation:
carrier RNA: 1350µl of buffer AVE was added in a Vial of 1350µg Carrier RNA to obtain solution of 1µg/µl. Dissolve this Carrier RNA thoroughly and divide it into conveniently sized aliquots, and then store it at 2-8˚C for up to 4 weeks.
Internal controls must be added with Carrier RNA-buffer AVE mixture, and the total volume of the internal control-Carrier RNA-buffer AVE mixture should be remained 120 µl.

1) 500 µl of AVL buffer was added into 1.5ml specimen tube.
2) 200 µl of the specimen transferred to AVL Buffer tube, mixed well, vortexed and then add 5.6 µl of carrier RNA and proteinase K and then incubation was done for 10 minutes at the ambient temperature.
3) 500 µl of ethyl alcohol was added to the Sample.
4) Place sample into the appropriate sample carrier, and load them into the sample drawer in automated machine. All other drawer of reagents and consumables are loaded properly in a particular drawer.
5) Using touch screen and enter the required information for each batch of sample processed and press the "Run" button to start purification procedure.
6) After lysis, binding Buffer and magnetic particles were transferred to the sample prep cartridge by magnetic rod protected by rod cover that enter the well and attract magnetic particles.
7) Viral nucleic acid binds to these magnetic particles and then these particles were released in to another well.
8) After that in machine there was wash buffer QSL and QSB.
9) Again, magnetic particles separation occurred and magnetic particles transferred to elute plate and we got the purified nucleic acid.

Amplification process

The amplification process of RNA obtained through both methods was conducted with PCR method using TRU-PCR Kit. For the test quantitative detection range for HCV-RNA was 20 IU/ml to $1* 10^8$ IU/ml.

Statistical analysis

The amplification results from the automated and manual extraction Methods were compared using fisher's exact test. P-values less than 0.05 were considered statistically significant.

RESULTS

Out of 100 samples tested for amplification process for HCV- RNA assay, no amplification was found by the two methods in 47 (47%) samples.

Table-1: Samples tested for amplification process

Total Samples tested for HCV RNA assay	Positive amplification by Automated/Manual Method	No amplification by both Methods
100	53	47

Out of 53 positive samples, all 53 samples were found to be positive by automated method, whereas only 37 samples were found to be positive by manual method.

Table-2: Amplification result

Total positive samples	Amplification result of Automated Method	Amplification result of Manual Method
	53 samples - Positive amplification	37 samples – Positive amplification
	00 sample - Negative amplification	16 samples - Negative amplification
Total Samples	53	53

16 samples were found to be positive by automated method but negative by manual extraction method. These 16 samples had positive value near borderline (<1000 IU/ml) when tested through automated method.

Table-3: Result of 16 samples

Result of 16 Samples	Automated Isolation Method	Manual Isolation Method
<100 IU/ml	09	No amplification found
>100 - <1000 IU/ml	07	No amplification found

Out of 100 total samples, 53 tested positive by at least one method. Out of these 53 positive samples, 53 were positive by the automated method while only 37 were positive by the manual method (Table-2). The automated method detected 16 more positive samples than the manual method. Using fisher's exact test, this difference was statistically significant (P=0.0018).

DISCUSSION

Nucleic acid extraction is the first and important step in molecular method of HCV detection. So, it affects the final result and prognosis of patients.

Table-4: Comparison of Results in Different Studies

Study	Year of study	Result
Present Study	2023	16 %
Julia H Knepp et al[12]	2003	15 %
Alpaslan Alp et al[13]	2004	09 %
Nicola Dundas et al[15]	2008	12 %
Gulhan Yagmur et al[16]	2015	30 %

In present study, there is 16% variation in amplification results of nucleic acid which were extracted by automated and manual extraction method. Similar study was done by Julia H Knepp , Melissa A Geahr, Michael S Forman, Alexandra Valsamakis,[12] Department of microbiology and pathology, Johns Hopkins Medical Institutions, Baltimore, Maryland, in 2003, which shows 15% variation in results by both methods.

Another similar study was done by Nicola Dundas, N. KristineLeos, Midori Mitui, Paula Revell and Beverly Barton Rogers,[15] Department of pathology, Dallas, Texas and Department of children's medical centre Dallas, Dallas, and The University of Texas southwestern Medical Centre, Dallas, Texas, in 2008, which shows 12% variation in results by manual and automated methods.

Another similar study was done by Alpaslan Alp, Dürdal Us, Gülşen Hasçelik,[13] Department of Microbiology, Anabilim Dali, Ankara, Turkey in 2004, which shows 09% variation in results by both methods.

Yet another similar study was done by Gulhan Yagmur and Ela Basok[16] Department of Microbiology, Turkey, in 2015, which shows 30% variation in results by both methods.

This little variation in different studies may be due to the different platform used by different laboratory.

Nucleic acid extraction, if performed by manually, it takes a long time and requires cautions due to high risk of contamination. There is less manual handling in automated extraction method. Thus, it requires less time and minimal manpower and avoid technical errors. Accurate and consistent pipetting helps to achieve uniformity in most crucial sample processing steps such as lysis, washing and elution. [8-11,17]

Manual method is less preferable as it involves many lengthy steps, which are time consuming, more cumbersome and more prone to impurities. It needs heavy manpower and has high risk of cross contamination.

The automated extraction method detected significantly more positive samples than the manual extraction method (P=0.0018). This indicates the automated method is more sensitive for HCV RNA detection, consistent with other published studies. The higher sensitivity of automated extraction is likely due to minimal manual handling, reducing the risk of sample loss or degradation during processing. Our statistical analysis strengthens the conclusion that automated extraction is superior to manual methods for HCV viral load testing.

CONCLUSIONS

Automated Nucleic acid extraction method is more sensitive and less cumbersome as compared to manual extraction method. Few manual handlings reduce the threat of external contamination. Large sample numbers with greater speed can be processed in automated extraction method.

A very high cost of automated nucleic acid extraction system restricts its use in routine clinical laboratories but for better result and better prognosis of patients automated extraction system is advisable and feasible.

REFERENCES

1. T. Poynard, P. Bedossa, and P. Opolon, “Natural history of liver fibrosis progression in patients with chronic hepatitis C,” The Lancet, vol.349,no.9055, pp.825-832, 1997.

2. J.F. Perz, G.L. Armstrong, L.A Farrington, Y.J.F. Hutin, and B.P. Bell, “The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide,” Journal of Hepatology, vol. 45, no. 4, pp. 529-538, 2006

3. Lauer GM, Walker BD: Hepatitis C virus infection, *N Engl J Med* 345: 41-52, 2001.

4. World Health Organization. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. Available at:https://www.who.imt/publications/i/item/9789240027077external icon

5. Liang TJ, Rehermann B, Seeff L, et al.: Pathogenesis, natural history, treatment, and prevention of hepatitis C *Ann Intern Med* 132: 296-305, 2000.

6. National Viral Hepatitis Control program (NVHCP) guideline-2018.

7. Apurba S Sastry, Sandhya B. Essential of medical Microbiology, Edition 2nd. 2019; 559-561.

8. Nakatsuji Y, Matsumoto A, Tanaka E, et al. Detection of chronic hepatitis C virus infection by four diagnostic systems: first-generation and second generation recombinant immunoblot assay and nested polymerase chain reaction analysis. Hepatology. 1992;16:300-305.

9. Cockerill FR: Application of rapid-cycle real time polymerase chain reaction for diagnostic testing in the clinical microbiology laboratory, *Arch Pathol Lab Med* 127: 1112-1120, 2003.

10. Beuselinck K., Ranst M.V., Eldere J.V. Automated extraction of viral pathogen RNA and DNA for high-throughput quantitative Real-time PCR. J. Clin. Micro-biol. 43, 11, 5541-5546, 2005.

11. Fiebelkorn K.R., Lee B.G., Hill C.E., Caliendo A.M., Nolte F.S. Clinical evaluation of an automated nucleic acid isolation system. Clin. Chem. 48, 9, 1613-1615, 2002.

12. Knepp J.H., Geahr M.A., Forman M.S., Valsamiks A. Comparison of automated and manual nucleic acid extraction methods for detection of enterovirus RNA. J. Clin. Microbiol. 41, 8, 3532-3536, 2003.

13. Alp A., Us D., Hascelik G. Comparison of manual and automated (Magna Pure) nucleic acid isolation methods in molecular diagnosis of HIV

infections. Mik-robiyol. Bul. 38, 1-2, 77-83, 2004.

14. Lee A.V., Atkinson C., Manuel R.J., Clark D.A. Comparative evaluation of the QIAGEN QIAsympho-ny® SP system and bioMérieux NucliSens easyMAG automated extraction platforms in a clinical virology laboratory. J. Clin. Virol. 52, 4, 339-343, 2011.

15. Dundas N., Leos N.K., Mitui M., Revell P., Rogers B.B. Comparison of automated nucleic acid extraction methods with manual extraction. J. Mol. Diagn. 10, 4, 311-316, 2008

16. Yagmur G, Altun HU, Gokahmetoglu S, Basok E. Comparison of manual and automated nucleic acid isolation methods for HBV-DNA and HCV-RNA assays. Infez med. 2015 Sep;23(3):247-52.

17. H. H. Kessler, A. M. K. Clarici, E. Stelzl et al., "Fully automated detection of hepatitis C virus RNA in serum and whole -blood samples," *Clinical and Diagnostic Laboratory Immunology*, vol.9, no. 6, pp. 1385-1388, 2002.

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