A Complete method of Detection of HCV Either Positive or Negative

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ABSTRACT

Several studies show that acute infections with hepatitis C virus (HCV) frequently progress to chronic diseases, which eventually can lead to liver cirrhosis and hepatocellular carcinoma. Thus, the development of simple and reliable HCV detection methods. In this research paper, I discuss the detection of HCV by PCR based thermal cycler in the CAP/CTM 48 Analyzer.

Keywords: Infection, Analyzer, Amplification, Serum, Transcription.

Abbreviation:

(HCV): Hepatitis C Virus, (PCR): Polymerase Chain Reaction, (CHC): Chronic hepatitis C, (PWID): People Who Inject Drug, (LMICs): low and Middle-Income Countries, (ALT): Amino Transferase Level: (EDTA) Ethylene-Diamine-Tetra-Acetic acid.; (dNTPs): Deoxynucleotide Tri Phosphates, (IC): Internal Control, (sUTP): Deoxy Uridine Triphosphate.

Introduction

Hepatitis C is an international public health problem. It has been estimated that further than 170 million persons globally have chronic hepatitis C (CHC) infections, and about 10% to 20% of them will grow severe liver diseases such as cirrhosis and hepatocellular cancer (Perz and Alter 2006). Its causal agent, hepatitis C virus (HCV), is a small, positive-sense, single-stranded RNA virus (Kim and Chang 2013). HCV endemic is compounded by lacking public health resources and a lack of knowledge of Hepatitis C mostly between the at-risk population health care providers, and policymakers (Mitchell, Colvin, and Beasley 1992).

In high-income countries, most cases of hepatitis C virus (HCV) infection are attributable to non-sterile drug injection (Mehta et al. 2011; Shepard, Finelli, and Alter 2005). The incubation period of HCV may last periods with symptoms only appearing after permanent liver damage has happened. The asymptomatic nature of HCV hampers early diagnosis, especially among those with limited or inconsistent access to health care, those uninsured or partly protected, and relegated peoples including PWID (Gowda 2019; Munoz-plaza et al. 2008). Healthcare-associated transmission, through unsterilized needles or transfusion with contaminated blood, remains a major route of HCV infection, particularly in low- and middle-income countries (LMICs) (Eze et al. 2014; Frank et al. 2000; Lanini et al. 2016).

Disease progression after HCV infection depends on factors including gender, coinfection with HIV, alcohol consumption, and duration of chronic infection (Hajarizadeh, Grebely, and Dore 2013; Wasitthanasem, Vichaiwattana, and Auphimai 2017). Another advantage of the HCV Agassay is that it can often be performed on the same instrument and simultaneously with the anti-HCV assay, an added value when determining the HCV prevalence in the community (Mixson-hayden et al. 2015; Wasitthanasem, Vichaiwattana, and Auphimai 2017).

Materials and Methods

Hepatitis C Virus is considered to be the principal etiological agent responsible for 90 to 95% of the cases of post-transfusion hepatitis C is a single-stranded, positive-sense RNA virus with a genome of approximately 9,500 nucleotides coding for 3,000 amino acids. As a blood-borne virus, HCV can be transmitted by blood and blood products. Widespread adoption of HCV blood screening measures has markedly loaded the risk of transfusion-associated hepatitis. The incidence of HCV infection is highest in associations with intravenous drug abuse and to a lesser extent with other percutaneous exposures. The global prevalence of HCV infection, as determined by immunoserology, ranges from 0.6% in Canada to 1.5% in Japan. Spontaneous viral clearance rates in the exposed individual are highly variable; between 10 and 60% have been reported as measured clinically by normalization of liver enzymes and clearance of plasma HCV RNA.

HCV virus particles cannot be cultured from infected blood samples hence the presence of anti-HCV antibodies in patients infected with HCV has led to the development of immunobiological assays that are specific for these antibodies. The presence of anti-HCV antibodies, however, is a measure of prior exposure to HCV infection, but cannot be considered a marker for current infection. The measurement of alanine aminotransferase levels (ALT) is considered to be a surrogate indicator of HCV infection but is not a direct measure of viremia.

Alternatively, the detection of HCV RNA by nucleic acid tests may provide evidence for current infection. Using nucleic acid tests, it is possible to detect HCV viremia before immunological seroconversion. Since nucleic acid tests can detect HCV RNA directly, i.e. independent of the immunological status of the patient, a nucleic acid-based test is valuable in detecting HCV RNA in immunocompromised patients.

Principles

The CAP/CTM 48 Analyzer is a nucleic acid amplification test for the detection of hepatitis C virus (HCV) RNA in human serum or EDTA plasma. Specimen preparation is automated using the CAP instruments with amplification and detection automated using the CTM analyzer or CTM 48 analyzer.

This is based on three major processes: (1) specimen preparation to isolate HCV RNA, (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probes specific to the target.

Sample Preparation

The sample input volume is 650µL, whereas the procedure processes 500µL of EDTA plasma or serum. The HCV virus particles are lysed by incubation at elevated temperature with protease and chaotropic lysis/binding buffer that release nucleic acids and protect the release of HCV RNA from RNases in serum or EDTA plasma. Protease and a known number HCV Internal Control (IC) RNA molecules are introduced into each specimen along with the lysis reagent and magnetic glass particles. Subsequently, the mixture is incubated, and the HCV RNA and HCV IC RNA are bound to the surface of magnetic glass particles. Unbound substances, such as salts, proteins, and other cellular impurities, are removed by washing the magnetic glass particles. After separating the beads and completing the washing steps, the absorbed nucleic acids are eluted at elevated temperature with an aqueous solution. The

processed specimen, containing the released HCV RNA and HCV IC RNA is added to the amplification mixture and transferred to the CTM Analyzer.

Reverse Transcription and PCR Amplification

During this procedure, reverse transcription of HCV RNA to complementary DNA (cDNA) and PCR amplification of cDNA using primers that define a sequence within the highly conserved region of the 5'-untranslated region of the HCV genome. The nucleotide sequence of the primers has been optimized to yield comparable amplification of HCV genotypes 1 to 6. The reverse transcription and PCR amplification reactions are performed with an optimized blend of thermostable recombinant enzymes and DNA Polymerase. In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, have both reverse transcriptase and DNA polymerase activity. This allows both reverse transcription and PCR amplification to occur together with real-time detection of the amplicon. Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which both reverse transcription and PCR amplification occur. The reaction mixture is heated to allow a downstream primer to anneal specifically to the HCV target RNA and the HCV IC RNA. In the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, and deoxyuridine triphosphates, polymerases extend the annealed primers forming a DNA strand complementary to an RNA target.

Target Amplification

Following reverse transcription of the HCV target RNA and the HCV IC RNA, the thermal cycler in the CAP/CTM 48 Analyzer heats the reaction mixture to denature the RNA: cDNA hybrid and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target cDNA. The thermostable DNA polymerase (Z05 and Z05D) in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), extend the annealed primers along the target template to produce a double-stranded DNA molecule termed an amplicon. CAP/CTM 48 analyzer doubling the amount of amplicon DNA. The required number of cycles is preprogrammed into the CAP/CTM 48 analyzer. Amplification occurs only in the region of the HCV genome between the primers; the entire HCV genome is not amplified.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the CAP/CTM 48 analyzer using AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strand containing deoxyuridine, but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, the only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme before amplification of the target DNA.

Also, the nonspecific product formed after the initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position.

When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period once exposed to temperatures above 55°C, i.e. throughout the thermal cycling steps, and therefore does not destroy the target amplicon formed throughout the PCR reaction.

Detection of cleaved dual-labeled probes and HCV RNA

The CAP/CTM 48 analyzer utilizes real-time PCR technology. The use of dual-labeled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring the emission intensity of fluorescent reporter dyes released during the amplification process. The probe consists of HCV and HCV IC-specific oligonucleotide probes with a reporter dye and a quencher dye. In the CAP/CTM, the HCV and HCV IC probes are labeled with different fluorescent reporter dyes. When these probes are intact, the fluorescent of the reporter dye is suppressed by the proximity of the quencher dye due to Forster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' 3' nuclease activity of the thermostable DNA polymerases. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of HCV RNA and HCV IC RNA is measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of HCV RNA and HCV IC RNA.

In nucleic acid amplification processes, efficiency can be reduced by inhibitors that may be present in the specimen. The HCV IC has been added to the CAP/CTM 48 analyzer to permit the identification of processed specimens containing substances that may interfere with PCR amplification. The HCV IC is a non-infectious armored RNA (aRNA) construct that contains fragments of HCV sequences with identical primer binding sites as the HCV target RNA and a unique probe binding region that allows HCV IC amplicon to be distinguished from HCV target amplicon. It serves as an extraction and amplification control for each independently processed specimen.

During the extraction phase of the PCR on the CAP/CTM 48 analyzer, the specimens are illuminated and excited by filtered light and the filters emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. This fluorescence reading is sent by the instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the HCV IC RNA data represent valid sets, and flags are generated when the data lie outside the present limits. After all, Pre-Checks are completed and passed, the fluorescence readings are processed to generate Ct values for the HCV RNA and HCV IC RNA. Results are reported as either positive or negative.

Conclusion

Results are reported as either positive or negative HCV.

Conflict of Interest

There is no conflict of Interest.

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