The suitability of using a modified real-time reverse transcriptase PCR method in the detection of Enterovirus in respiratory samples from Iraq

Anwar Salih Saihood¹, Areej Salih Saihood², Ahmed Raheem Rayshan³

1,2 University of Al-Qadisiyah, College of Medicine, Department of Microbiology, Iraq
3 University of Al-Qadisiyah, College of Biotechnologies, Department of Medical, Iraq

*Corresponding author: anwarsalih264@yahoo.com (Saihood)

Abstract

The Enterovirus is an important infectious agent that should focus on for better recognition and control. The current work was initiated to use a modified real-time reverse transcriptase PCR (RT-PCR) technique. For this aim, clinical specimens of 300 human septum samples from patients with respiratory symptom were obtained different regions in Al-Diwaniyah province from Iraq. The study was aimed at using VP1 gene based target with a specific primer set. The results of the RT-PCR method uncovered the presence of Enterovirus in 96(32%) samples. The method findings demonstrated successful rates with sensitivity (100%) and specificity (95%). The results provide evidence about the probability of the existence of the virus in those samples plus give critical importance about the use of this method in the identifying the target virus alone with high accuracy.

Keyword: Enterovirus, RT-PCR, VP1 gene

How to cite this article: Saihood AS, Saihood AS, Rayshan AR(2021): The suitability of using a modified real-time reverse transcriptase PCR method in the detection of Enterovirus in respiratory samples from Iraq, Ann Trop Med & Public Health; 24(S2): SP24237. DOI: http://doi.org/10.36295/ASRO.2021.24237

Introduction

Enterovirus is one of the most common human pathogenic microorganisms, causing 10 to 15 million new infections in the United States per year. Therefore, both men have several EV diseases during their lives. There are 116 EV types in all four Enterovirus variants (A, B, C, D). Enteroviruses may induce either asymptomatic infections or acute diseases like diarrhea to encephalitis. Enterovirus disease, during early life years, offers lifetime immunity toward
post-homologous virus exposure and can also help to prohibit the occurrence of autoimmune diseases. Human Enterovirus was first identified from children with respiratory infections. The Fermon strain was referred to as the variant type obtained from samples of those children. However, till the beginning of the 2000s, there had not yet been a significant number of the virus occurrences. Since 2008 to 2012, nevertheless, the virus has been identified as an evolving microorganism sufficient to cause serious respiratory diseases in some of Asian, European, America countries such as the Philippines, Japan, and the Netherlands and the United States\(^{(1-4)}\).

Initially categorized via serotyping, the genetic relatedness of enterovirus types which cause human infections is now categorized into 4 species (EV-A to EV-D). The group comprises three rhinovirus members (RV-A to RV-C, common cold triggers) and five animal affecting organisms. Most recently, Picornaviridae has produced a new family, the Parechoviruses, which included identical yet genetic variations, which were historically known as enteroviruses. Such viruses may trigger Enterovirus-like clinical diseases, although a large scale is not yet studied in conjunction with more chronic diseases\(^{(5-7)}\).

The genomes of the Enterovirus are positive-sense RNA-single stranded with total length of 7.4 kilobases. It consist of a 5” (5’UTR) untranslated region, a single polyprotein coding area and 3” a small untranslated area with a 3” polyadenylated tail. The 5UTR is fairly well-maintained and is typically utilized for PCR research of medical sample identification. In the RNA replication regulation and polyprotein translation both 5’UTR and 3’UTR are engaged. This is then cleaved into eleven mature proteins, using proteases, (VP1toVP-4) which are the viral capsid, using an RNA polymerase manner throughout post-translation refining. The genes that encode functional proteins produce a high level of biodiversity and contribute to a wide range of different serotypes. The VP1 region's sequence demonstrates the most highly correlated methods of conventional serotyping, which are utilized most frequently in Enterovirus detection. Respiratory (upper respiratory Tract, URT) and Faecal-oral (gastrointestinal tract, GIT) routes play a major role in spreading enteroviruses. Incubation is usually only a few days, with replication happening primarily in the URT or in the GIT (via inhaling or exposure to oral or nasal mucosa)\(^{(8)}\).

The Enterovirus is an important infectious agent that should focus on for better recognition and control. The current work was initiated to use a modified RT-PCR technique.
Materials and methods

Patients

For inducing the current work, 300 septum specimens were collected from children age at 8-12 years old from various regions of Al-Diwaniyah province, Iraq. Samples were obtained from the children under the acceptance of their parents or guardians. Samples were placed in dry-sterilized containers. Directly, the samples were exposed to steps of genome-based processing.

RNA extraction

The nucleic acid was sputum-extracted with a QIAamp Viral Mini Kit (Qiagen, Inc., USA) with focusing on the company protocol.

VP1 based RT-PCR

The primer set was followed from (9), and the primers were; F: TCGCAGTGAATCAGCA(T/C)GG (modified with inserting degenerate options of bases for broader identification) and R: CCAAAGCTGCTCTACTGAGAAA with 6-carboxyfluorescein probing. Here, the samples were tested utilizing an Applied Biosystems 7500 Real-Time PCR method for the AgPath-ID RT-PCR package and suggested cycling conditions. For the test to be carried out in the 5' untranslated EV-D68 region, we used a real-time PCR system Applied Biosystems 7500 to obey the suggested methods and cycle requirements by (9).

Results

The study was aimed at using VP1 gene based target with a specific primer set. The results of the RT-PCR method uncovered the presence of Enterovirus in 96(32%) samples. High detection rates were identified using this method with accuracy of one-hundred percent of success rates. Figure 1 displays this successful procedure.
Figure 2 recognizes the real time PCR results of the Enterovirus from septum samples of children in which it demonstrates the sensitivity (100%) and specificity (95%) of the method.

Discussion

It is surprising that Enterovirus uncovers accelerated, powerful influence of the cell translation machinery. The Enterovirus nucleic acid is a functional mRNA, which has been shown to be expressed as the first move of the
pathogenic cycle. Cell translation, transcription, and DNA replication throughout disease are suppressed by 
*Enterovirus* but firstly, the suppression of translations is most dramatic that occurs as the beginning process. 
Because the translation units rapidly reacts to stress and changed environmental conditions, various elements of the 
translation machine must be regulated by the virus to encourage not only the expression of its own genes, but to 
limit those cell reaction measures of activated innate immunity\(^{10-12}\).

The current investigation was conducted for the first time to detect *Enterovirus* from sputum samples of children in 
South of Iraq using a modified RT-PCR technique with a modified set of identifying primers. The findings unveiled 
successful recognition of the virus in those samples at an incidence rate of 32\% of the total examined samples. The 
number of detected positive samples is relatively high, and this agrees with the data obtained by work literatures of 
the identification of the *Enterovirus* from various regions of the world \(^{9}\). The rate of incidence is considerably 
higher than those revealed by some studies in healthy populations as it was recognized as 10\%, and this could be due 
to that some cases from which the samples were taken could have been in the recovery periods or simply a high 
sensitivity rate of unveiling the virus even in relatively low-concentrated samples of the virus presence due to 
exposure to the virus from the environment but not from infection processes \(^{13}\).

This method is completely useful to detect epidemiological occurrence of the infections by this virus especially 
when compared with present commercial techniques. This fact was highly confirmed when take a brief look on the 
available studies performed on the current virus. (Wylie *et al.* identified that using such methods may increase the 
successful rates of detecting the virus using considerably limited periods of time\(^{9}\). The samples that were detected 
as negative specimens even with presence of respiratory infections in the patients that those samples were collected 
from may have been infected with different infectious agents or with allergic respiratory conditions The samples that 
were detected as negative specimens even with presence of respiratory infections in the patients that those samples 
were collected from may have been infected with different infectious agents or with allergic respiratory conditions 
\(^{13-15}\).

The results provide evidence about the probability of the existence of the virus in those samples plus give critical 
importance about the use of this method in the identifying the target virus alone with high accuracy.
References


