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Comparison of sandwich ELISA and conventional PCR assay for enterovirus detection in children with acute gastroenteritis in Mosul/ Iraq

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Abstract
Acute gastroenteritis (AGE) is a gastrointestinal infection that most usually affects young children. Recently, the Enterovirus (EV) was added to the list of pathogens responsible for acute gastroenteritis. No previous studies in Iraq on the propagation of EV infection. This study's objective was to use Sandwich Enzyme-linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) techniques to determine the prevalence of EV in children with AGE. To accomplish this, 120 stool samples were taken from Mosul hospitals in the period from October 2021 to January 2022 and the tested patients were within the age group of less than five years for both sexes. The results showed that the EV was detected in 16(13.33%) by ELISA and 26(21.67%) by PCR out of 120 stool samples. The sensitivity of ELISA was 53.85%, while the specificity was 97.87% when compared with PCR results. The ELISA and PCR both had a kappa value of (0.814) and an accuracy rate of (88.33%).

Conclusions: Compared to PCR, ELISA has the advantage of being faster, less expensive, easier to perform, and appropriate for analyzing a large number of samples. It was very close to the PCR results. Therefore, it is possible to conclude that epidemiological studies and routine diagnostic procedures can both use ELISA to detect EV. The PCR technique is regarded as the gold standard for EV detection due to its high sensitivity and specificity.

Keywords: ELISA, enterovirus, gastroenteritis, PCR

Introduction

Gastroenteritis is a watery diarrhea illness that is self-limiting, usually lasts less than a week, and is characterized by signs and symptoms like nausea, vomiting, appetite loss, diarrhea, fever, and abdominal discomfort. Due to the illness's severity, a child may become severely dehydrated and necessitate hospitalization, or maybe even result in a child's death (Khan *et al*., 2022) [13]. Globally, high mortality and morbidity rates in babies and young children are caused by AGE. According to estimates, this illness causes the deaths of about 2 million children worldwide each year (Mokomane *et al*., 2018) [17]. The most common causes of AGE are viruses such as Rotavirus, Norovirus, Adenovirus, and Astrovirus (González-Serrano *et al*., 2020; Rojjanadumrongkul *et al*., 2020) [10, 20]. However, EV has recently been linked to acute gastroenteritis (Tatte and Gopalkrishna, 2019; Alcalá *et al*., 2018; Bányai *et al*., 2018) [22, 1, 3].

Human Enteroviruses belong to the EV genus within the Picornaviridae family, which includes four species of EV (A to D) and three species of rhinovirus (A to C). Human Enteroviruses (A to D) also represent a wide range of serotypes such as Poliovirus, Coxsackievirus A, Coxsackievirus B, Echovirus, and numbered EVs. Most EV transmission occurs via the fecal-oral and respiratory pathways (Rojjanadumrongkul *et al*., 2020; Kumthip *et al*., 2017) [20, 15]. EVs are regarded as a significant contributor to a wide variety of diseases, including, paralysis, cardiomyopathy, meningitis, gastroenteritis, pneumonia, and infections of the central nervous system. Even though the majority of infections are asymptomatic, approximately 1% of EV infections cause serious illnesses with high mortality and morbidity rates in babies and young children (Macaya and Felipe-Rucián, 2017; Zhou *et al*., 2016) [16, 24].

The Sandwich ELISA test confirms EV infection by detecting the viral protein presence (Jin *et al*., 2021) [12]. On the other hand, all members of the EV genus can be detected using molecular diagnostics targeting highly conserved sites located in the five untranslated regions (5UTR) (Harvala *et al*., 2018) [11].

Materials and Methods

One hundred twenty stool samples were collected from diarrheal children at three main Mosul hospitals (Mosul General Hospital, Al-Salam Teaching Hospital, and Ibn Al-Atheer Teaching Hospital for children) beginning in October 2021 and ending in January 2022. These patients are under five years of age. Stool samples were divided into two parts and placed in an Eppendorf tube. It was numbered and labeled with information. The first

part was used for the ELISA test and the second for the PCR test. It was immediately frozen at -20°C until tests were performed on it.

Sandwich Enzyme-linked Immunosorbent Assay (ELISA)
The test was run utilizing the SunLong Biotech Co., LTD "Human Enterovirus ELISA Kit"

Polymerase Chain Reaction (PCR)
RNA extraction and cDNA preparation: RNA was extracted using Wiz Prep ™ Viral DNA/RNA Mini Kit (V2) (Wiz ™ Solutions, South Korea), and cDNA was synthesized by using Accu Power ® RT Pre Mix (Bioneer Inc, South Korea).

Primers: The conventional PCR was conducted to detect the 5'UTR region of EVs by using specific primers published previously (Robinson *et al*., 2002) [19].
(EV-F: CAAGCACTTCTGTTTCCCCGG)
(EV-R: ATTGTCACCATAAGCAGCCA)

PCR reaction mixture: The PCR Master mix tube (Accu Power® Gold Hot start Taq PCR Pre Mix & Master Mix) (Bioneer Inc, South Korea) was filled with 7µl of cDNA template, 1µl of each primer, and 11µl of nuclease-free water to make the amplification mixture (20µl)

PCR conditions: include 35 cycles, each cycle consisting of denaturation at 94°C for 45 sec, annealing at 54°C for 45 sec, extension at 72°C for 45 sec, and final extension at 72°C for 10 min.

Interpretation of the results
The formulas below were used to calculate the sensitivity, specificity, kappa value, and accuracy rate.
Sensitivity = $A/(A+C) \times 100\%$, specificity = $D/(D+B) \times 100\%$, kappa value $=2\times(A\times D - C\times B)/(A+B)\times(B+D)+(A+C)\times(C+D)$, accuracy rate = $(A+D)/(A+B+C+D) \times 100\%$. Where: A= true positive, B= false positive, C= false negative, D= true negative

Results
Enterovirus detection using ELISA and PCR
The ELISA test on stool samples revealed a prevalence of EV of 16(13.33%) out of 120 stool samples. Whereas, 26 (21.67%) of these samples showed the expected band size for EV when amplification of the 5'UTR region by PCR (Table 1), (Figure 1).

Table 1: Enterovirus detection rate in stool samples

Results	ELISA	PCR	Total
Positive	16(13.33%)	26(21.67%)	
Negative	104(86.67%)	94(78.33%)	120 (100%)

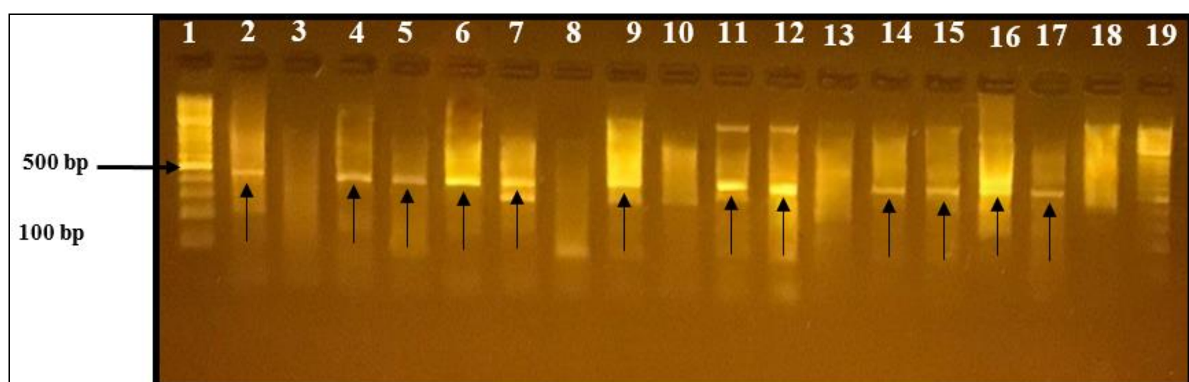

Fig 1: Detection of EV gene (430-450 bp) in Enterovirus. Line1and 19, DNA markers (100 bp). Lines 2,4,5,6,7,9,11,12,14,15,16 and 17 represent positive results. Lines 3,8,10,13 and 18 represent negative results. Electrophoresis conditions are 5v /cm^2.

Comparison between the results of ELISA and PCR methods for Enterovirus
Table 2 shows that the sensitivity, specificity, accuracy rate, and kappa values were (53.85%, 97.87%, 88.33%, and 0.814) respectively.

Table 2: Comparison between ELISA and PCR methods for the detection of Enterovirus

ELISA	PCR		
	Positive	**Negative**	**Total**
Positive	14(87.5%)	2(12.5%)	16
Negative	12(11.54%)	92(88.46)	104
Total	26	94	120

Discussion

According to our information, our study is the first to detect EV in the stools of diarrheal children using the ELISA method, so there are no previous results for comparison. Contrarily, the scientists employed molecular methods to identify the EV that causes diarrhea in some nations, including Nigeria, Iran, Venezuela, Japan, Thailand, and India with a prevalence rate ranging between (2.5% and 40%) (Efunshile *et al*., 2022; Fazelipour *et al*., 2019; Alcalá *et al* 2018; Pham *et al*., 2018; Chaimongkol *et al*., 2012; Chitambar *et al*., 2012) [7, 9, 1, 18, 5, 6]. These differences may be due to variations in a geographical area, community hygiene, specimens and patient issues, and variation in examination equipment, kits, and methods (Khan *et al*., 2022; Al-Tabtabai *et al*., 2020) [13, 2].

When comparing the results of ELISA and PCR tests, we note that there is a discordant in the number of positive and negative samples in both tests. samples that test positive by ELISA but negative by PCR this might be because the viral genetic material is present in low concentration, PCR inhibitors present in stool samples, and the false positive ELISA results may result from antibodies' non-specific binding to virus antigens (Kramme *et al*., 2022; Cardona-Ospina *et al*., 2019) [14, 4]. Whereas, the presence of samples that tested negative by ELISA but positive by PCR might be because of low viral titers in stool samples which prevents the ELISA test from detecting antigens. This result was also noticed by (Fan *et al*., 2020; Sozzi *et al*., 2010) [8, 21].

The sandwich ELISA and PCR methods appear to have high consistency and perfect agreement overall, as indicated by the accuracy rate and kappa values of these two methods, which were respectively 88.33% and 0.814. This percentage is consistent with that recorded by (Wang *et al*., 2021) [23].

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