

# Development of a Multiplex PCR Assay to Detect Gastroenteric Pathogens in the Feces of Mexican Children

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**Abstract** Acute gastroenteritis (AGE) is a major cause of childhood morbidity and mortality worldwide; the etiology of AGE includes viruses, bacteria, and parasites. A multiplex PCR assay to simultaneously identify human Astrovirus (HAstV), Calicivirus (HuCVs), *Entamoeba histolytica* (*E. histolytica*), and enteroinvasive *Escherichia coli* (EIEC) in stool samples is described. A total of 103 samples were individually analyzed by ELISA (enzyme-linked immunosorbent assays) and RT-PCR/PCR. HAstV and HuCVs were detected in four out of 103 samples (3.8 %) by RT-PCR, but ELISAs found only one sample as positive for HuCVs (2.5 %). *E. histolytica* was identified in two out of 19 samples (10.5 %) and EIEC in 13 out of 20 samples (70 %) by PCR, and all PCR products were sequenced to verify their identities. Our multiplex PCR results demonstrate the simultaneous amplification of different pathogens such as HAstV, EIEC, and *E. histolytica* in the same reaction, though the HuCVs signal was weak in every replicate. Regardless, this multiplex PCR protocol represents a novel tool for the

identification of distinct pathogens and may provide support for the diagnosis of AGE in children.

## Introduction

Acute gastroenteritis (AGE) of infectious origin is a health problem in both developing and developed countries. AGE is responsible for approximately 3 million deaths per year, mainly affecting children under 5 years old [23, 33]. The causes of diarrhea include a wide range of viruses, bacteria, and parasites. Several epidemiological studies have shown that up to 50 % of AGE is caused by bacteria or parasites alone [25], whereas some estimates consider that almost 80 % of cases of AGE are due to viruses [4, 22]. The rotavirus is the most important agent responsible for diarrhea in children under 2 years of age. Currently, depending on the region, the human Astrovirus (HAstV) is the second or the third most common virus involved in AGE worldwide, as shown by ELISAs or RT-PCR in samples from different geographic regions in México or hospital samples collected in Madrid, Spain [24, 34]. The human Calicivirus (HuCV) is considered to be the most common cause of non-bacterial gastroenteritis outbreaks in all age groups [4, 37]. HuCV is highly infectious and frequently occurs in both developing and developed countries. Recently, it has been reported that the Norovirus (NoV) causes approximately 10 % of intestinal infections in developed countries, e.g., Holland and England, in a prospective population-based cohort study [9, 40].

HAstV and HuCVs are both single-stranded positive-sense RNA viruses with a similar genomic structure consisting of three open reading frames (ORFs) [3, 11, 23]. The NoV and *Sapovirus* genera belong to *Caliciviridae*, although *Sapovirus* is mainly associated with pediatric

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