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# Integrated molecular and epidemiological assessment of pediatric viral gastroenteritis using qRT-PCR versus rapid immunochromatographic diagnostics

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**ABSTRACT**

**Background:** Viral gastroenteritis represents a major cause of pediatric morbidity, being one of the leading acute conditions requiring emergency care or hospitalization. Enteric viruses such as rotavirus, norovirus, adenovirus, astrovirus, and sapovirus are frequently implicated, with prevalence varying by age and season. The objective of this study was to assess the distribution of viral agents in children and to compare the performance of two diagnostic methods: immunochromatographic (ICT) and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR).

**Materials and methods:** In a retrospective study, a total of 164 children hospitalized with acute gastroenteritis between February 2023 and March 2025 were investigated using qRT-PCR and ICT methods.

**Results:** The positivity rate for rotavirus, norovirus and adenovirus was 58.5% by qRT-PCR compared to 25.0% by ICT, with slightly higher rates in urban areas (62/103) and in females (54/73). The most affected age group was 1-3 years, with increased incidence during warm seasons. rotavirus was the predominant pathogen (66 cases by qRT-PCR; 32 by ICT), followed by norovirus (41 cases vs. 11), while viral co-infections accounted for 11% of cases. These findings highlight the importance of rapid and accurate etiological diagnosis for appropriate management, prevention of complications, and reduction of unnecessary antibiotic use.

**Conclusions:** Our findings demonstrated a higher diagnostic performance of qRT-PCR compared with ICT, particularly for the detection of norovirus and rotavirus. Although rapid ICT tests may serve as an initial diagnostic tool in settings with limited access to molecular testing, qRT-PCR provides more reliable pathogen detection and may improve the accuracy of etiological diagnosis. However, the clinical impact of molecular testing on treatment decisions, antibiotic prescriptions, hospitalization duration, and patient outcomes was not assessed in this study.

**Keywords:** viral gastroenteritis, qRT-PCR, ICT

## INTRODUCTION

Acute gastroenteritis remains an important cause of morbidity in children and contributes substantially to healthcare utilization worldwide. Viral pathogens, particularly rotavirus, norovirus, and adenovirus, are important causes of acute diarrheal illness in the pediatric population. The clinical presentation may range from self-limiting diarrhea and vomiting to more severe illness associated with dehydration and the need for hospital care. Because symptoms caused by different enteric viruses can overlap, clinical assessment alone may not reliably establish the etiological diagnosis. Accurate pathogen identification can therefore support clinical evaluation, epidemiological surveillance, and appropriate infection-control measures, particularly in healthcare settings where transmission between patients is a concern [1, 2].

The risk of viral gastroenteritis in children is influenced by several demographic, environmental, and behavioral factors. Young age, immature immune responses, close contact with other children, attendance at daycare or school, inadequate hand hygiene, and exposure to contaminated food, water, or

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surfaces may facilitate the transmission of enteric viruses. Household crowding and contact with infected family members can also contribute to the spread of infection. In addition, rotavirus vaccination status is relevant when assessing the risk of rotavirus-associated disease, particularly in settings where vaccination is available. The relative contribution of these factors may vary according to local epidemiology, socioeconomic conditions, and circulating viral strains. In the present study, the analysis focused on pathogen detection and diagnostic performance; therefore, these factors are discussed as established epidemiological considerations rather than as variables directly assessed in our patient cohort.

Infectious diarrhea is caused by a wide range of viral, bacterial, and parasitic pathogens. Among viral agents, rotavirus, norovirus, and adenovirus are most frequently detected, whereas astrovirus and sapovirus are less commonly identified. Each virus exhibits distinct clinical presentations and epidemiological patterns [3].

Rotavirus belongs to the Reoviridae family and is characterized by a double-stranded ribonucleic acid (RNA) genome [4]. At the national level, rotavirus-associated viral gastroenteritis represents a significant public health concern. In Romania, the 2024 summer season saw a significant increase in acute diarrheal diseases, with 50,081 cases reported between June and October (weeks 23-44), exceeding the average of the 2021-2023 surveillance seasons and highlighting the need for rapid identification of pathogens (26.4% of all cases were with rotavirus) [5]. By November 2024, a total of 126 countries had introduced the rotavirus vaccine, thus contributing to a significant reduction in mortality associated with these infections [6, 7]. In Romania, the rotavirus vaccine is not yet included in the national mandatory immunization schedule and is available only on an optional basis.

Norovirus, a single-stranded RNA virus of the Caliciviridae family, divided into genogroups GI-GX (of which GI, GII, and GIV infect humans), is the second leading cause of viral gastroenteritis, responsible for 16-18% of cases of acute diarrhea in children under five years of age and approximately 200,000 deaths annually, with the GII.4 genotype being the most frequently involved [8, 9].

Enteric adenoviruses belong to the genus Mastadenovirus in the family Adenovidae, are double-stranded, non-enveloped deoxyribonucleic acid (DNA) viruses, associated mainly with gastroenteritis in children. Transmission is mainly by the fecal-oral route, through ingestion of contaminated water or food, direct contact with infected persons or through contaminated surfaces [10, 11]. The most commonly involved are types 40 and 41 [12].

Regardless of the causative viral agent, acute gastroenteritis is primarily evaluated and managed based on disease severity. Dehydration reflects the severity of the disease, and the mainstay of treatment is oral rehydration with hypoosmolar solutions, initiated early, with maintenance of breastfeeding and normal nutrition. Probiotics, diosmectite, racecadotril, and ondansetron may be useful as adjuvants, with hospitalization only required when enteral or parenteral rehydration is indicated [13], and rapid identification of the etiological agent is essential for proper patient management and reduction of unnecessary antibiotic use [14].

While the initial diagnosis of acute gastroenteritis is still based on classical methods with limited sensitivity and coverage, the present study evaluates the performance of the gastrointestinal panels performed by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), which simultaneously detect five enteric viruses. By rapidly and accurately identifying viral DNA/RNA, these molecular methods can significantly increase patient management and prognosis [15].

Recent studies have continued to evaluate the diagnostic performance of rapid antigen-based assays and molecular methods for the detection of enteric viruses. Evidence from systematic reviews indicates that the sensitivity of immunochromatographic (ICT) tests may vary according to the viral target and assay used, particularly for norovirus detection [16]. More recent evaluations of rapid ICT assays and multiplex molecular diagnostic panels have further highlighted the importance of balancing diagnostic accuracy, turnaround time, and clinical requirements [17-19]. In this context, comparing rapid tests with molecular detection in a local pediatric hospital population may provide useful evidence for interpreting diagnostic results and selecting appropriate testing strategies [16-19].

The aim of this study was to assess the prevalence and distribution of viral pathogens detected by qRT-PCR in a pediatric population, as well as to analyze epidemiological patterns in relation to patient age and seasonal distribution. In addition, we compared the diagnostic performance of qRT-PCR and ICT tests for pathogens with overlapping targets, namely rotavirus, norovirus, and adenovirus. Finally, the results were integrated with existing literature to highlight the applicability and epidemiological relevance of molecular diagnostic methods.

Despite the availability of rapid ICT tests for the detection of common enteric viruses, their diagnostic performance may vary according to the pathogen and the characteristics of the tested population. Molecular methods, such as qRT-PCR, offer increased analytical sensitivity but may require more specialized laboratory infrastructure and resources. In pediatric patients, where timely identification of the causative pathogen can support clinical assessment and infection-control decisions, understanding the diagnostic agreement and differences between these methods is particularly important. Therefore, this study compared ICT results with qRT-PCR findings for rotavirus, norovirus, and adenovirus in children hospitalized with acute gastroenteritis, with the aim of evaluating the diagnostic contribution of molecular testing in this clinical setting.

## MATERIALS AND METHODS

### Sample Collection

The retrospective study was conducted on 164 pediatric patients with acute gastrointestinal symptoms (diarrhea, vomiting, abdominal cramps, and fever), aged between 0 and 16 years, admitted to the “Sf. Ioan” Children’s Emergency Clinical Hospital in Galati, between February 2023 and March 2025.

Children aged 0 to 16 years presenting with gastrointestinal symptoms, including diarrhea, vomiting, or abdominal pain, were eligible for inclusion in the study. Patients were identified through a retrospective review of hospital clinical and laboratory records. Only patients who underwent both rapid antigen testing and multiplex real-time reverse-transcription

polymerase chain reaction (qRT-PCR) testing using the same stool sample were included in the analysis.

Patients with chronic diseases that could interfere with the interpretation of gastrointestinal symptoms or diagnostic findings, including inflammatory bowel disease, celiac disease, and malabsorption syndromes, were excluded. Patients with incomplete clinical or laboratory data, insufficient stool sample volume, or stool samples that did not meet the laboratory requirements for testing were also excluded. The final study population comprised patients who met all inclusion criteria and none of the exclusion criteria.

### *Sampling and processing procedure*

Stool samples were collected in sterile containers and transported immediately to the laboratory. Portions of each sample were homogenized in a universal lysis buffer to facilitate viral particle disruption and release of viral RNA. The homogenates were clarified by centrifugation to remove debris and potential PCR inhibitors, ensuring optimal sample quality for downstream molecular analysis. All 164 stool samples were transported to the laboratory within two hours of collection and initially tested immediately by ICT, then stored at -80 °C until PCR analysis. Extracted RNA was immediately processed for reverse transcription into complementary DNA using a semi-automated system, which also included internal controls to monitor the efficiency of extraction and detect potential inhibition.

### qRT-PCR Analysis

Molecular diagnosis was performed using the Allplex™ GI-Virus Assay (Seegene Inc., Republic of Korea), a multiplex real-time one-step RT-PCR assay designed for the simultaneous detection of six major enteric viruses: norovirus GI and GII, rotavirus, adenovirus F (serotypes 40/41), astrovirus, and sapovirus (genogroups GI, GII, GIV, and GV).

Total nucleic acids, including viral RNA and DNA, were extracted from stool specimens using the STARMag 96 X 4 Universal Cartridge Kit (Seegene Inc., Republic of Korea) on the automated Nimbus system (Seegene Inc., Republic of Korea). The extracted nucleic acids were subsequently analyzed using the Allplex™ GI-Virus Assay. During the one-step RT-PCR procedure, RNA from norovirus GI/GII, rotavirus, astrovirus, and sapovirus underwent reverse transcription into complementary DNA, followed by amplification. In contrast, adenovirus F DNA was amplified directly by PCR, without requiring a reverse transcription step. Amplification and fluorescent signal detection were performed using the CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories Inc., USA).

The reaction mixture was prepared according to the manufacturer's instructions. The master mix had a total volume of 20 µL per reaction and consisted of 5 µL of GI-Virus MuDT Oligo Mix, 8 µL of RNase-free water, 5 µL of real-time one-step buffer, and 2 µL of real-time one-step enzyme. Subsequently, 5 µL of extracted nucleic acid was added to each reaction, resulting in a final reaction volume of 25 µL.

The thermal cycling protocol consisted of an initial reverse transcription step at 50 °C for 20 minutes, followed by initial denaturation at 95 °C for 15 minutes. This was followed by 45 amplification cycles, each comprising denaturation at 95 °C for 10 seconds, annealing at 60 °C for 1 minute, and extension at 72 °C for 30 seconds. Fluorescence acquisition was performed at both 60 °C and 72 °C during each amplification cycle.

The assay included internal and external controls to monitor the validity of the amplification process and detect potential contamination or PCR inhibition. Fluorescence was detected using the FAM, HEX, Cal Red 610, and Quasar 670 channels. Data interpretation was performed using Seegene Viewer v3 software, according to the manufacturer's instructions. Test validity was confirmed by the appropriate performance of the controls, and samples were retested whenever the control criteria were not met. The assay was performed according to the manufacturer's protocol (Allplex™ GI-Virus Assay, Seegene Inc., version 3.00, May 2022).

### Rapid Immunochromatographic Tests

In parallel, fecal samples were also analyzed by rapid ICT tests (ICTs, ACRO BIOTECH, Inc. USA), used for the qualitative detection of norovirus, rotavirus and adenovirus.

The principle of the method is based on the use of specific antibodies, fixed on the membrane, which allow the selective identification of viral antigens. The result is available in approximately 15 minutes.

Interpretation of the test: a positive result indicates the presence of viral antigen in the sample; a negative result may indicate either the absence of infection or a late phase of the disease, in which the viral load falls below the detection limit of the test. According to the data provided in the kit insert, the test has a relative sensitivity of 99.9% for norovirus rapid test cassette, 97.3% for rotavirus and 95.2% for adenovirus; a relative specificity of 98.1% for norovirus, 97.1% for rotavirus and 97.7% for adenovirus, as well as a relative accuracy of 98.5% for norovirus, 97.2% for Rotavirus and 96.8% for adenovirus.

Also, fecal samples were investigated using traditional methods – coprocytogram – which involves microscopic examination to highlight cellular elements (leukocytes, erythrocytes) and the presence of mucus, the guiding parameters in differentiating infectious from inflammatory etiology.

### Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software, version 26, with a significance level set at 0.05. The Chi-square ($\chi^2$) test and the p-value were used for statistical analysis to assess the significance of differences between variables. Where possible, positive and negative predictive values were calculated to assess the diagnostic performance of the tests. Cohen's kappa coefficient was also used to determine the degree of agreement between diagnostic methods and the McNemar test was used to compare the accuracy of two methods ( qRT-PCR vs. ICT) in detecting a pathogen when each patient is tested by both methods.

## RESULTS

Between February 2023 and March 2025, rapid viral tests from feces were performed on 923 pediatric patients aged 0 to 16 years, admitted to the "SfantulIoan" Children's Emergency Hospital in Galati, who presented with digestive symptoms, including diarrhea, vomiting, fever and abdominal pain.

In 164 (17.7%) of them, two methods of viral detection from feces were performed in parallel: rapid ICT tests and real-time PCR (qRT-PCR) tests. Analysis of the distribution by age group

**Table 1.** Distribution of patients by gender, area of origin, age groups, diagnosis at admission, and season

| Patient characteristics | | Total number (n = 164) | Patients (%) |
|---|---|---|---|
| Gender of the patients | Female | 73 | 44.5 |
| | Male | 91 | 55.5 |
| The environment of origin | Urban | 103 | 63 |
| | Rural | 61 | 37 |
| Age (years) | 0-2 | 111 | 68 |
| | 3-5 | 33 | 20 |
| | 6-9 | 14 | 8 |
| | 10-16 | 6 | 4 |
| Diagnosis at admission | Gastroenteritis/acute enteritis | 137 | 83 |
| | Dyspepsia/unspecified abdominal pain | 20 | 12 |
| | Diarrhea/dehydration | 6 | 4 |
| | Nausea and vomiting | 1 | 1 |
| Season | Winter | 28 | 17 |
| | Spring | 57 | 35 |
| | Summer | 56 | 34 |
| | Autumn | 23 | 14 |

**Table 2.** Distribution of patients according to reported symptoms

| Symptom | Number of patients (n) | Percentage (%) |
|---|---|---|
| Diarrhea | 114 | 69.5 |
| Vomiting | 83 | 50.6 |
| Fever | 75 | 45.7 |
| Abdominal pain | 11 | 6.7 |

Note. Patients may be presented with more than one symptom

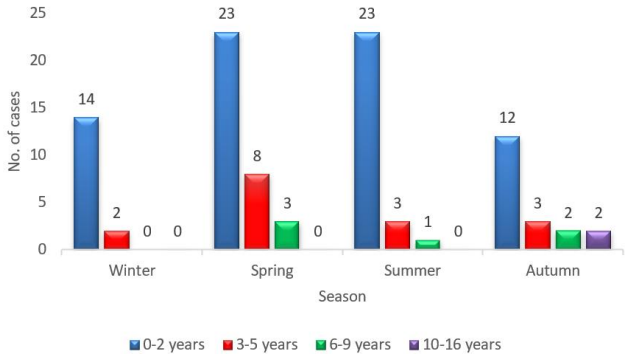



**Figure 1.** Distribution of positive patients by age and season (qRT-PCR method) (the authors' own elaboration)

showed that the 0-5 year old group (84%, 138/164 patients) was the most frequently encountered, with a peak incidence in children between 1-3 years old (43%, 70/164), followed by patients under 1 year old (25%, 41/164). A decrease in incidence was observed with increasing age.

Regarding the area of origin, a predominance of patients from urban areas (63%, 103/164) was found, compared to those from rural areas (37%, 61/164).

The distribution by gender of patients revealed a higher percentage of male patients (55%, 91/164) compared to female patients (45%, 73/164). The most frequently encountered diagnosis was acute gastroenteritis/enteritis (83%, 136/164) (**Table 1**).

At admission, diarrhea, vomiting, and fever were the most frequently reported symptoms among pediatric patients (**Table 2**).

An increased frequency of hospitalizations was observed during the spring seasons 20.7% (34/164) and summer 16.4% (27/164) and especially in children 0-2 years (67.6% [23/34] and 85.1% [23/27], respectively). The age groups 3-5 and 6-9

**Table 3.** Positive cases by age category and viral type

| Virus identified | Age (years) | | | |
|---|---|---|---|---|
| | 0-2 (n = 72) | 3-5 (n = 16) | 6-9 (n = 6) | 10-16 (n = 2) |
| Rotavirus | 40 | 9 | 3 | 1 |
| Norovirus | 18 | 3 | 3 | 1 |
| Adenovirus | 0 | 0 | 0 | 0 |
| Norovirus + adenovirus | 4 | 1 | 0 | 0 |
| Norovirus + rotavirus | 9 | 2 | 0 | 0 |
| Rotavirus + adenovirus | 1 | 1 | 0 | 0 |

showed moderate fluctuations, with peak values recorded in the spring season. In autumn, cases were observed exclusively in the 10-16-year age group, suggesting a diversification during this season (**Figure 1**).

Regarding etiology, rotavirus was the most frequently identified agent, especially in spring (22/48) and summer (16/48). Coinfections were present throughout the year, but with a higher frequency in spring and summer. Norovirus was consistently detected, reaching a peak in the autumn season (10/23). Astrovirus and sapovirus were detected sporadically, only in winter and autumn.

Clinical data analysis performed in parallel by the two identification methods (quantitative real time reverse transcription and rapid ICT tests) revealed that pediatric patients under 5 years of age constituted the most frequently affected group (PCR/ICT = 88/96 (~92%) vs. 39/40 (~98%), with one of the highest incidences observed in the 0-2 year age range (PCR/ICT = 72/96 (75%), 27/40 (~68%).

Viral monoinfections were identified in 78/164 (48%) patients tested by the qRT-PCR method and in 48/164 (29%) patients tested by the ICT method. By the qRT-PCR method, coinfections with two viruses were observed in 18/164 (11%). The most frequent coinfections were norovirus with rotavirus (11/164, 6,7%), followed by norovirus with adenovirus (5/164, 3,0%) and rotavirus with adenovirus (2/164, 1,2%) (**Table 3**).

Rotavirus detected by qRT-PCR was the most frequently encountered (66/164, 40.2%). Of these, by concurrent testing with the ICT method, 27/66 (41%) stool samples confirmed the presence of rotavirus, 1/66 (1.5%) identified norovirus, 1/66 (1.5%) adenovirus and 3/66 (4.5%) norovirus/adenovirus coinfection. The remaining 34 stool samples were negative although the patients presented with diarrhea, vomiting and fever.

**Table 4.** Rotavirus positive/negative with qRT-PCR and ICT detection

| Number of cases | qRT-PCR positive | qRT-PCR negative | Total |
|---|---|---|---|
| ICT positive | 27 | 3 | 30 |
| ICT negative | 39 | 95 | 134 |
| Total | 66 | 98 | 164 |

Note. TP: True positive: Positive results by both ICT and qRT-PCR; FP: False positive: Positive results by ICT but negative by qRT-PCR; FN: False negative: Negative results by ICT but positive by qRT-PCR; & TN: True negative: Negative results by both ICT and qRT-PCR

**Table 5.** Norovirus positive/negative with qRT-PCR and ICT detection

| Number of cases | qRT-PCR positive | qRT-PCR negative | Total |
|---|---|---|---|
| ICT positive | 7 | 4 | 11 |
| ICT negative | 34 | 119 | 153 |
| Total | 41 | 123 | 164 |

Note. TP: True positive: Positive results by both ICT and qRT-PCR; FP: False positive: Positive results by ICT but negative by qRT-PCR; FN: False negative: Negative results by ICT but positive by qRT-PCR; & TN: True negative: Negative results by both ICT and qRT-PCR

The comparison of the ICT test with the qRT-PCR method for the detection of rotavirus revealed a sensitivity of 40.9% and a specificity of 96.9%. The positive percent agreement was 40.9% and the negative percent agreement of 70.9%, with an overall accuracy of 74.4%. The Cohen's kappa coefficient (0.415) was 0.41, indicating a moderate level of agreement between ICT and qRT-PCR. The McNemar test was applied to evaluate the differences between discordant results, based on the 3 ICT positive but qRT-PCR negative cases and the 39 ICT negative but qRT-PCR positive cases. The uncorrected $\chi^2$ value was 30.86 and the corrected value was 29.17, well above the critical threshold of 3.84 for one degree of freedom. The associated statistical significance was extremely high ($p < 0.0001$), confirming the existence of a significant difference between the methods. This result indicates that ICT generates a very high number of false negatives compared to qRT-PCR. ICT still maintains its usefulness in excluding infection, but the overall performance indicates the need for confirmation by qRT-PCR in suspicious or critical cases (**Table 4**).

Regarding norovirus identification, 41/164 patients (25%)were positive by qRT-PCR, while only 7/41 (17%) of them were identified by concurrent testing with the ICT method. Of the remaining 34/41 samples (83%), the ICT method identified 2/41 patients (5%) with rotavirus and 1/41 (2.4%) patient with adenovirus.

The sensitivity of ICT dropped to only 17.1%, indicating a very low capacity to detect real cases, while the specificity remained high (~96.8%). The positive percent agreement decreased to 63.6%, meaning that only 47 out of 1,011 positive ICT results were confirmed by qRT-PCR. The negative predictive value remained relatively good (~77.8%), but was insufficient to reliably exclude infection, and the overall accuracy was approximately 76.8%. The Cohen's kappa coefficient (0.18) indicated a poor agreement between ICT and qRT-PCR, and the McNemar test showed a statistically significant difference between the results ($\chi^2 = 23.68$, $p < 0.0001$). Thus, ICT and qRT-PCR were not equivalent in the detection of norovirus , as ICT missed significantly more cases compared to qRT-PCR (**Table 5**).

Adenovirus was identified by qRT-PCR only in coinfections with rotavirus in 2/164 pacients (1.2%) and norovirus, in 5/164 patients (3%), and by ICT 5/164 positive samples (3%) were identified, of which 2 in coinfections with rotavirus and norovirus, respectively (**Table 4** and **Table 5**).

As for astrovirusand sapovirus, they were identified exclusively by qRT-PCR, in a total of 12/164 patients (7.3%): 2/164 patients (1.2%) with astrovirus, 2/164 patients (1.2%) with sapovirus, 8/164 patients (5%) with mixed infections with norovirus and rotavirus. These viruses could not be detected by ICT, as they are currently not included in the rapid ICT tests available on the market, therefore the data were not compared.

## DISCUSSION

The present study compared rapid ICT testing with qRT-PCR for the detection of rotavirus, norovirus, and adenovirus in children hospitalized with acute gastroenteritis. The higher proportion of positive results obtained by qRT-PCR than by ICT suggests that the diagnostic method can substantially influence the identification of viral pathogens in this clinical setting. Rotavirus was the predominant detected pathogen, and viral coinfections were also observed. These findings emphasize the importance of interpreting rapid-test results in conjunction with the clinical presentation and the limitations of the diagnostic method used. The results also provide a basis for considering the complementary roles of rapid antigen testing and molecular diagnostics, while recognizing that the retrospective design and the availability of qRT-PCR results for only a subset of children limit broader generalization.

While rapid ICT tests may still serve as an initial diagnostic option in settings with limited resources or restricted access to molecular testing, a positive result allows for rapid clinical decision-making; however, a negative ICT result does not exclude infection, particularly in cases with high clinical suspicion or increased epidemiological risk [15]. In such situations, confirmatory testing by qRT-PCR is recommended to ensure accurate etiological diagnosis. These findings provide original data from a pediatric hospital in southeastern Romania, where routine diagnostics currently rely mainly on rapid tests, and support the integration of molecular methods to improve diagnostic accuracy and epidemiological assessment.

Our results are consistent with previous reports [20-22], which show that qRT-PCR offers clear advantages over rapid ICT tests for the identification of gastrointestinal viruses [23, 24]. The extremely low sensitivity of the ICT test for norovirus observed in our study can be explained by the high antigenic diversity of norovirus and by variability in viral load, which may limit detection by rapid antigen-based assays.

Recent evidence provides additional context for interpreting the diagnostic performance observed in our study. A systematic review and meta-analysis of ICT tests for

norovirus reported pooled sensitivity of approximately 61% and specificity of 97%, illustrating that rapid antigen tests may have high specificity but variable sensitivity [16]. In a 2024 evaluation of a triplex ICT assay, sensitivity also differed according to the viral target, reaching 86.7% for rotavirus, 70.6% for norovirus, and 76.2% for adenovirus [17]. These findings support the need to interpret rapid-test performance in relation to the specific assay and pathogen being investigated. In our cohort, the observed sensitivities of ICT were lower, particularly for norovirus (17.1%) and rotavirus (40.9%). Differences in assay design, study population, specimen characteristics, and testing conditions may contribute to the variation between studies. Accordingly, our findings should be interpreted as evidence of limited sensitivity of the ICT assays evaluated in this particular setting, rather than as a general estimate of the performance of all ICT tests [17, 18].

The estimated mean age in our study of patients with acute gastroenteritis is approximately 2.16 years, similar data being observed in other studies such as the one from Ontario, Canada, PHO laboratory where the mean age was 3.8 years, all under 5 years old. In the same study, Korea [25], a similarity was observed in the distribution of patients by gender with our study (42.3% vs. 44.5% for females and 57.6% vs. 55.5% for males). In Croatia [20], females predominated 67% compared to males 33%, but patients under 5 years of age were also present in greater numbers here 32% compared to the 5-18 age group with 17%.

Norovirus was found to be present throughout the year, with a peak incidence in December, while rotavirus showed a marked increase in March-April. Similar data were reported in a study from the Korea [26], where norovirus also showed an increased incidence in the winter season, rotavirus was detected throughout the year, adenovirus caused epidemics in the summer season, astrovirusin the winter season, and sapovirus in the spring. In our study, most patients presented in the spring season (35%) and summer (34%), followed by those in the winter (17%) and autumn (14%). rotavirus was found, as in previous studies, in the spring, and norovirus throughout the year, with a peak incidence also in the cold season, but especially in autumn. In Korea [26], the seasonal distribution was different, with the highest proportion of cases in winter (56%), followed by summer (31.3%), spring (6.6%), and autumn (6.0%).

In Shanghai, China [27], a higher prevalence of rotavirus infection (43.3%), followed by norovirus (28.9%), was reported in children with acute gastroenteritis. Similar results were obtained in a study from Korea [26], where rotavirus was the most frequently detected (17.1%), followed by norovirus(5.0%). Our findings are consistent with these observations, with rotavirus being identified in 29.2% of monoinfection cases and norovirusin 14.0%. In contrast, a study conducted in Japan [28] reported a predominance of norovirus (51.6%) over rotavirus (21.6%), a difference explained by the inclusion of two live attenuated rotavirus vaccines in the national immunization program.

The data obtained confirm that rotavirus remains the main etiological agent involved in viral gastroenteritis in children, especially at young ages (0-3 years) [15, 29, 30]. This observation is consistent with the specialized literature, which emphasizes the major impact of rotavirus on pediatric public health, especially in the absence of vaccination [7, 29, 31-33]. Global epidemiology consistently documents that rotavirus infections are common among children under 5 years of age, while the prevalence in older age groups is much reduced, reflecting the immunity acquired in childhood [32].

Norovirus is the second most common cause [1, 26, 29, 34, 35], with a significant number of cases detected in infants and young children. Several cases of viral coinfections have also been identified, the most common being the association between norovirus and rotavirus, which have been reported in previous studies, although their clinical significance and potential association with disease severity remain variable [25].

In Korea [28], monoinfections accounted for 39.0% of cases, coinfections with two pathogens 14.8%, and those with three pathogens 3.3%. In our study, the proportion of monoinfections was higher (47%), and coinfections represented 11%, of which 6% were norovirus with rotavirus. In Burkina Faso [20], multiple infections accounted for 35.7% of cases, with the most frequent associations being rotavirus with adenovirus(45.7%) and rotavirus with norovirus (18.1%); two cases of coinfection with four viruses were also reported. In other regions, the prevalence of mixed infections was lower: 15.2% in Tunisia [36], 9% in East Anglia region of England [37] and 5.2% in China [38].

Adenoviruses, astroviruses, and sapoviruses were rarely identified as mono-infections, accounting for only four cases (6.7%), and were more frequently detected in mixed infections (15 cases, 25%), most commonly in association with norovirus and rotavirus. This distribution may indicate either a synergistic contribution to the pathogenesis of gastroenteritis or a limited replicative capacity of these viruses within the gastrointestinal tract, as previously suggested [39, 40].

The relationship between viral infection and disease severity could not be assessed due to the lack of systematically collected data on disease severity.

A major strength of this study is the comparison of rapid ICT testing with qRT-PCR for three clinically relevant enteric viruses—rotavirus, norovirus, and adenovirus—in a pediatric hospital setting. By examining results obtained using both diagnostic approaches, the study provides practical information on the differences in pathogen detection that may occur when rapid tests are used in routine clinical practice. The inclusion of multiple viral targets also allows the diagnostic findings to be considered across pathogens rather than focusing on a single virus. Furthermore, the study contributes local epidemiological data from hospitalized children in southeastern Romania, a setting for which institution-specific diagnostic data may be useful for clinical and laboratory planning. These strengths should be interpreted in light of the retrospective, single-center design and the fact that only one subset of the children tested by ICT also underwent qRT-PCR.

Although qRT-PCR demonstrated superior diagnostic performance compared with ICT, the present study was not designed to evaluate the clinical impact of molecular testing. Specifically, we did not assess whether the availability of qRT-PCR results influenced antibiotic prescribing, treatment decisions, length of hospitalization, the occurrence of complications, or clinical outcomes. Therefore, any potential benefits of improved pathogen detection for patient management should be interpreted cautiously and cannot be directly established from our findings. Further prospective studies are needed to determine whether the implementation of molecular diagnostic methods translates into measurable

improvements in clinical management and patient outcomes. The potential impact of qRT-PCR on treatment decisions and clinical outcomes requires further investigation.

### Study Limitations

Several limitations should be considered when interpreting these findings. First, the retrospective, single-center design limits the generalizability of the results to other pediatric populations and healthcare settings. Second, although 923 children underwent rapid ICT testing, qRT-PCR results were available for only a subset of 164 children. This selective availability of molecular results may have introduced selection bias and limits the extent to which the diagnostic findings can be extrapolated to the entire tested population. Third, epidemiological and clinical variables such as vaccination status, daycare attendance, household exposure, hygiene practices, and relevant socioeconomic factors were not systematically collected; consequently, their associations with viral detection could not be evaluated. Fourth, the study focused on diagnostic findings and did not assess whether the use of qRT-PCR changed treatment decisions, infection-control practices, length of hospitalization, or patient outcomes. Finally, the results reflect the local epidemiological context and the diagnostic methods used during the study period. These limitations should be considered when interpreting the observed differences between ICT and qRT-PCR and when planning future studies.

Sample size – The study included a limited number of patients and was conducted exclusively on hospitalized patients. Data were collected from a single medical center, located in a single geographic region, which limits the representativeness of the results for the entire pediatric population nationwide.

Lack of comprehensive testing for all viruses – ICT does not allow the detection of astrovirusand sapovirus, which limits direct comparison between methods for these agents.

Absence of viral load monitoring – Viral load values were not correlated with symptom severity, which could have provided a more nuanced understanding of diagnostic performance.

Cross-sectional design – The study captured only a single moment of infection; longitudinal follow-up would have allowed the assessment of the duration of infection and immune response.

While ICT may remain useful as an initial diagnostic approach, molecular testing provides more reliable etiological identification. Lack of assessment of clinical impact – The study focused on the diagnostic performance of qRT-PCR and ICT and did not evaluate the influence of molecular test results on antibiotic prescribing, treatment modifications, hospitalization duration, complications, or patient outcomes. Consequently, the potential clinical benefits of improved pathogen detection could not be assessed.

### Future Perspectives and Recommendations

Future studies should include larger, prospective, and preferably multicenter pediatric cohorts to validate the diagnostic performance of ICT and qRT-PCR across different clinical and epidemiological settings. The systematic collection of relevant demographic, clinical, vaccination, and exposure-related variables would help clarify the factors associated with viral detection and disease burden. Further research should also evaluate the diagnostic performance of each method using clearly defined reference standards and appropriate statistical measures, including sensitivity, specificity, and agreement where the study design permits. In addition, studies incorporating clinical outcomes, turnaround time, laboratory costs, and the consequences of diagnostic results for infection-control decisions would help determine the most efficient testing strategy for routine pediatric practice. Until such evidence is available, the choice between rapid testing and molecular confirmation should be guided by the clinical context, local laboratory capacity, and applicable diagnostic protocols.

Expanding the sample and study period - Multicenter studies over longer periods could confirm the seasonal distribution and prevalence of viral coinfections.

Evaluation of multiplex molecular methods - Integration of multiplex PCR tests that simultaneously detect multiple viruses could increase the accuracy of rapid diagnosis.

Correlating viral load with clinical severity - Quantitative monitoring of viral RNA could help in risk stratification and patient management.

Implementation of combined diagnostic algorithms - Use of ICT as an initial rapid test, followed by PCR in symptomatic or high epidemiological risk cases, could optimize costs and diagnostic times.

Immune response studies - Investigation of inflammatory and immunological biomarkers in correlation with viral infections could clarify pathogenic mechanisms and severity factors.

## CONCLUSIONS

This study highlights important differences between rapid ICT tests and qRT-PCR in the detection of major viral pathogens associated with pediatric acute gastroenteritis. In the evaluated cohort, qRT-PCR identified viral infections more frequently than ICT, emphasizing the importance of considering the diagnostic limitations of rapid tests when interpreting negative results. Rotavirus was the predominant detected pathogen, while viral coinfections were also identified. These findings support the potential complementary role of rapid testing and molecular diagnostics: ICT may facilitate timely initial assessment, whereas qRT-PCR can provide additional diagnostic information when clinical suspicion persists or pathogen identification is particularly important. However, because molecular testing was available for only a subset of the children tested by ICT and the study was retrospective and single-center, the findings should be interpreted within this context. Larger prospective studies are warranted to determine how diagnostic strategies influence clinical decision-making, infection-control practices, and healthcare resource utilization in pediatric gastroenteritis.

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