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Evaluation of non-invasive diagnostic tools for diarrhea: a systematic review of point-of-care tests and biomarkers

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Background: Diarrhea is a prevalent condition affecting millions worldwide. However, current standard diagnostic methods have many drawbacks. This review examines various non-invasive point-of-care (POC) tests and biomarkers aiding rapid diagnosis of diarrhea from different causes.

Methods: PubMed, PubMed Central, ScienceDirect, Cochrane Library, and Google Scholar were searched from 2013 to present for relevant literature. Two reviewers independently assessed included studies' quality using the Critical Appraisal Skills Programme (CASP) checklist.

Results: The search yielded 1453 studies, of which 39 were included after screening and applying eligibility criteria. Polymerase chain reaction (PCR) was the POC test in 25 studies, providing consistent sensitivity and specificity. For biomarkers, C-reactive protein (CRP), fecal calprotectin, and procalcitonin offered high sensitivity and specificity for conditions like acute pediatric diarrhea, microscopic colitis, and inflammatory diarrhea, respectively.

Conclusion: PCR proved the ideal POC test for rapid diarrhea diagnosis, while the procalcitonin biomarker helps differentiate inflammatory from non-inflammatory diarrhea. Other reviewed tools also demonstrated promising diagnostic performance, though improvements in sensitivity, specificity, and usability are still needed.

Keywords: biomarkers, diarrhea, point-of-care tests

Introduction

Diarrheal diseases significantly burden public health infrastructure worldwide, particularly in low and middle-income countries, affecting all age groups. Despite medical advancement and timely healthcare provision, diarrhea remains a leading cause of morbidity and mortality, especially among children under five (the second leading cause of death in this age group, accounting for 15% of all deaths)[1]. Diarrhea results in 1.6 million annual deaths mainly in developing nations, presenting severe implications needing diagnostic and treatment improvements[2]. Impact extends beyond individual outcomes with economic implications, straining limited resources and impeding universal quality healthcare access[3]. Additionally, treatment costs burden affected households, exacerbating poverty[4].

Presentation varies by pathophysiology (osmotic, secretory, inflammatory, motility), duration (acute/chronic), and cause (pathogen, treatment, disease)[5]. Accurate, timely diagnosis of underlying cause is crucial for intervention and treatment. However, standard methods like stool cultures and biopsied

HIGHLIGHTS

- Diarrheal diseases significantly burden public health infrastructure worldwide, particularly in low and middle-income countries, affecting all age groups. Despite medical advancement and timely healthcare provision, diarrhea remains a leading cause of morbidity and mortality, especially among children under five (the second leading cause of death in this age group, accounting for 15% of all deaths).
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sample histology are expensive, operator-dependent, time-consuming with prolonged time-to-result (TTR). Traditional approaches like history, examination, and basic laboratory tests have accuracy, speed, and cost-effectiveness limitations[2,6].

Reliance on self-reported symptoms and subjective assessment risks errors and etiology identification delays[7]. Available tools, like stool/blood cultures while helping identify bacterial/parasitic causes, may not provide comprehensive pathogen understanding or distinguish etiologies[7].

Additionally, effective methods like colonoscopy require highly trained physicians and facilities, limiting accessibility[8], contributing to delays, and hampering timely intervention.

To address inadequacy of current methods and improve outcomes, providers must explore accurate, rapid, simple, affordable non-invasive diagnostic tools, increasingly important for diagnostic information without invasive procedures. These encompass biomarkers, imaging, and sensors, offering advantages over invasive techniques[9]. These should apply across settings, including resource-limited ones.

For diarrhea investigation, techniques can enable rapid, accurate diagnosis critical for management and complication prevention[10]. A key benefit is timely results without invasive risks/discomfort. Conventional methods like stool culture are time-consuming, requiring days for growth and delaying diagnosis[11]. Innovations delivering accurate rapid results could significantly improve diagnosis and management. Several emerging non-invasive techniques are alternatives to invasive diagnostics like colonoscopies, including stool/blood biomarker evaluation and imaging modalities[12]. These may enable faster, affordable, lower-risk diagnoses with reduced hospitalization and fewer complications. Research aims to determine if these match/exceed diagnostic accuracy of conventional invasive approaches[13]. Accurate implementation could eliminate delays, expenses, and risks associated with invasive testing.

Objectives

To date, no systematic review has comprehensively evaluated and compared the wide range of emerging point-of-care (POC) and biomarker approaches for non-invasive diarrhea diagnosis. Several rapid diagnostic tests and novel biomarkers show promise for timeliness, accessibility, affordability, and accuracy improvements, but the synthesis of validation data across techniques lacks. This review thoroughly assesses validated and potential POC tests and biomarkers enabling precise, patient-centered diagnosis guiding appropriate therapy.

Findings will inform future guidelines and standardization efforts by summarizing performance characteristics across index tests, identifying accurate, appropriate options for diverse settings. Comparative diagnostic accuracy analysis between non-invasive and conventional tests can facilitate the implementation of new tools replacing/complementing stool culture and microscopy. Assessment of latest validation studies clarifies gaps and priorities for ongoing test optimization.

By evaluating emerging POC and biomarker non-invasive diarrhea diagnosis approaches, this review addresses a critical evidence gap toward patient-centered, precise diagnosis. Our findings will guide adoption of these methods improving over conventional methods accessibility, efficiency, and accuracy. Ultimately, this review promotes diarrhea diagnostic paradigm improvements, especially in limited-resource environments with high morbidity/mortality.

Methodology

Eligibility criteria

The review included primary research studies published in English between 2013 and 2023 that evaluated non-invasive POC tests (POCTs) or biomarkers for diagnosing diarrhea in individuals with diarrhea symptoms. Eligible study designs were RCTs, cohort studies, case–control studies, and cross-sectional studies. Reviews, editorials, and case reports were excluded. There were no restrictions on study population demographics. Eligible index tests included rapid tests, immunoassays, molecular assays, or biomarkers measured non-invasively. Comparator tests were conventional methods like stool culture or microscopy. Outcomes of interest were diagnostic accuracy measures (sensitivity, specificity, predictive values, diagnostic odds ratio) and clinical utility (ease of use, time to result, cost-effectiveness, feasibility). This work has been reported in line with AMSTAR.

Information sources

The systematic review was registered on PROSPERO (Registration ID: CRD42023437285A). A comprehensive search of the literature was conducted by (H.M.P., A.P.) to identify relevant studies on non-invasive POCTs and biomarkers for diagnosing diarrhea. Electronic databases including PubMed, PubMed Central, ScienceDirect, Cochrane Central Register of Controlled Trials, and Google Scholar were searched from 2013 to the present.

Search strategy

The search strategy included a combination of controlled vocabulary terms (e.g. MeSH) and free text keywords related to the key concepts. The MeSH keywords used are: (diarrhea OR diarrhoea) AND ("point-of-care tests" OR "biomarkers") AND ("diagnostic accuracy" OR sensitivity OR specificity).

Selection process

The literature search results were imported into Rayyan[14] where duplicates were removed, followed by article screening according to inclusion and exclusion criteria. Three reviewers (S.H.K., M.K., H.F.) had independently screened the titles/abstracts of retrieved studies, followed by a full-text review of potentially relevant articles. Disagreements were resolved by consensus or consultation with a third reviewer.

Data collection process and data items

Three reviewers (S.H.K., M.K., H.F.) independently extracted data from the included studies using a predefined data extraction form. The following information was extracted: authors, publication year, study title, journal/source, study design, setting, sample size, population characteristics, study objective/purpose, index test(s)/biomarker(s), inclusion/exclusion criteria, reference standard, sample collection/handling procedures, analytical platforms, summary of findings, sensitivity, specificity, predictive values, diagnostic accuracy measures, additional relevant findings, limitations, and conclusions.

Extracted data were cross-checked between reviewers to ensure accuracy and resolve any discrepancies through discussion and consensus. The final extracted data were compiled into a Microsoft Excel spreadsheet.

Study risk of bias assessment

The quality of the included studies was independently assessed by two reviewers (Z.H., A.P.) using the Critical Appraisal Skills Programme (CASP) checklist[15]. Each study was categorized as having a low, moderate, or high risk of bias (RoB) based on the assessment.

Results

Study selection

The study selection process is documented using a PRISMA[16] flow diagram as in Figure 1.

Study characteristics

The systematic review included studies with diverse population characteristics. Many studies focused on pediatric populations, including children under 5 years old with acute diarrhea or gastroenteritis. Other studies enrolled adults, either unspecified age groups or older adults. Both immunocompetent and immunocompromised patients were represented, such as HIV patients and kidney transplant recipients. Regarding sample sizes, they ranged considerably from 40 to over 4000 participants across studies. Most studies included a mix of males and females, while a few focused exclusively on one gender. Participants were drawn from various global regions, including North America, Europe, Asia, Africa, and the Middle East. Settings varied as well, with patients recruited from outpatient clinics, emergency departments, inpatient wards, refugee camps, and community hospitals. While many studies did not specify the clinical severity, some characterized patients as having mild, moderate, or severe diarrhea. Overall, the reviewed studies encompassed heterogeneous populations across age groups, immune status, sample size, gender, geographical region, and healthcare setting. The distribution of studies as per the population characteristics is shown in Table 1 below.

Risk of bias in studies

The RoB assessment of included studies is described in Table 2.

Studies categorized as high risk were interpreted with caution regarding their findings and conclusions. The variable quality of the included studies was considered when synthesizing results and drawing conclusions from the systematic review. No studies were excluded based on the RoB ratings.

Results of syntheses

The results of the included studies are summarized in Tables 3 and 4.

Point-of-care tests (POCTs)

(1) PCR
The 25 PCR studies demonstrated consistently high diagnostic accuracy, with sensitivity ranging 87.5–100% and specificity 93.4–100%.
(2) Cell Culture Cytotoxin Neutralisation Assay (CCNA)
Two studies examined CCNA performance. Study 1 exhibited 51% sensitivity and 99.4% specificity. Study 2 showed lower 33% sensitivity but perfect 100% specificity.
(3) Culture
Three studies utilized culture but did not provide sensitivity or specificity values, using it as a comparator test.
(4) Enzyme-Linked Immunosorbent Assay (ELISA)
Of the 9 ELISA studies, 6 reported relevant metrics spanning wide ranges: 29–95.7% sensitivity, 72.4–97% specificity, 53–100% positive predicted value (PPV), and 84–98% negative predicted value (NPV), indicating variable diagnostic performance.
(5) Immunochromatography
Two study metrics – Study 1: 90.7% sensitivity, 99.5% specificity, 99.2% PPV, and 94.2% NPV. Study 2 exhibited a lower 76% sensitivity and 90.2% specificity.
(6) Isothermal DNA amplification
Both AmpliVue and Illumigene showed high diagnostic potential.
(7) Microscopy
One study assessed two techniques: Trichrome, 100% specific but only 63.8% sensitive; Calcofluor white, 82.2% specific and 79.7% sensitive.
(8) Non-invasive imaging

One study compared bowel sonography (BS) and magnetic resonance (MR) enterography for diagnosing Crohn's disease (CD). BS: sensitivity 94%, specificity 97%, PPV 97%, and NPV 94%. MR: sensitivity 96%, specificity 94%, PPV 94%, and NPV 96%.

Biomarkers

In our examination of diagnostic approaches for diarrhea, we found a diverse array of biomarkers and methodologies employed across a range of studies. Seven of these studies leaned on the cultivation of pathogens as a pivotal biomarker, often complemented by the utilization of polymerase chain reaction (PCR) as the primary POCT for detection. Two separate investigations explored the presence of glutamate dehydrogenase (GDH) as a diagnostic biomarker. Additionally, two studies detected the presence of lipopolysaccharide (LPS) antigens of bacteria, employing Immunochromatography as the preferred detection method.

Furthermore, six studies delved into the analysis of serum and stool proteins as diagnostic indicators, including proteins such as calprotectin, lactoferrin, CRP, fibroblast growth factor 19 (FGF19), total free fecal bile acids (TFFBA), soluble triggering receptor expressed on myeloid cells (sTREM), Procalcitonin (PCT), and serum C4 concentration. In one study that examined FGF19, TFFBA, and calprotectin, FGF19 and TFFBA levels were quantified using ELISA, while calprotectin was measured using an immunochromatographic method.

Moreover, nine studies assessed target genes as biomarkers for diarrhea detection, each delving into specific genes such as the *Clostridium difficile* toxin A and B gene, stx-1, stx-2, eae, and ipaH (invasion plasmid antigen H) gene. Lastly, two studies incorporated the analysis of pathogen toxins as diagnostic biomarkers, with one of these studies focusing specifically on the detection of Shiga toxin.

Discussion

Interpretation of the review findings

A number of POCTs and biomarkers were examined in the systematic review. Of all the tests used in our studies, PCR was the

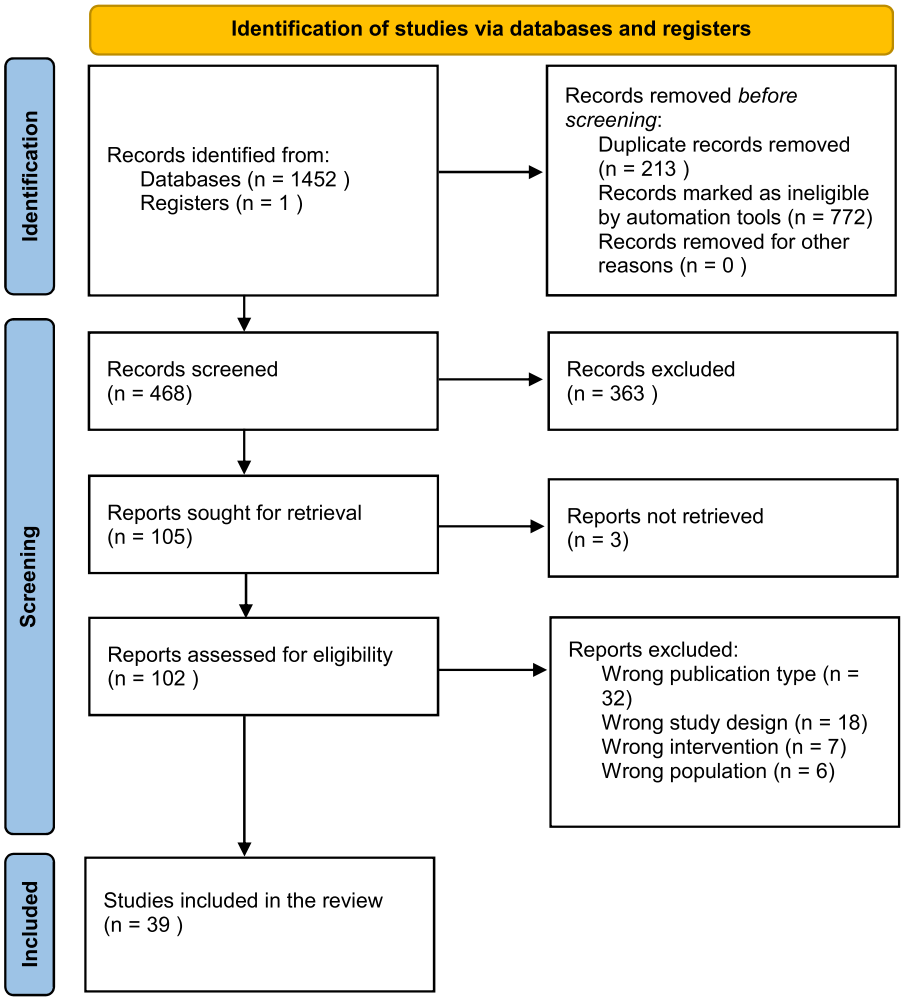

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram for the identification, screening, eligibility, and inclusion of studies.

most utilized. Various diagnostic tools used PCR as their principle and demonstrated high sensitivity and specificity when it came to the detection of the various biomarkers mentioned above. ELISA was also a tool that was widely used in the studies. Even though cultures of the organisms were included in the studies, there was no relevant data available and was mainly used for comparison of other diagnostic tools. Lesser utilized methods such as CCNA, immunochromatography, immunoblotting, and Isothermal DNA amplification were also assessed, and results showed their viability as POCT in the clinical field.

The study also revealed a number of biomarkers that can be examined in a patient with diarrhea. Various biomarkers that correlated with multiple etiologies were determined. These biomarkers can be used to determine the causative agent and aid in the formation of differentials.

In the realm of POCTs, diagnostic performance varies across different methods. Cell Culture Cytotoxin Neutralisation Assay (CCNA) demonstrates an impressive specificity, albeit with variable sensitivity, making it valuable for confirmation rather than initial screening. Culture serves as a comparator without specific sensitivity and specificity values. ELISA exhibits a wide range of sensitivity and specificity, with variable positive and negative predictive values. Immunochromatography, while demonstrating promise, exhibits variable performance. Isothermal DNA amplification methods, AmpliVue and Illumigene, generally exhibit high diagnostic potential. Microscopy provides limited data, with varying specificity and sensitivity. Non-invasive imaging tests like BS and MR enterography offer high sensitivity and specificity for CD diagnosis. PCR emerges as a robust diagnostic tool with consistently high sensitivity and specificity. Ultimately, the choice of POCT should align with specific diagnostic needs and the target pathogen or condition, considering the trade-offs in sensitivity and specificity offered by each method.

In the case of biomarkers, several have emerged as valuable tools for discerning specific etiologies of diarrhea and associated conditions. C-reactive protein (CRP) has proven useful in identifying fever and bacterial causation in acute pediatric diarrhea, with enhanced diagnostic capabilities when assessed with fecal lactoferrin. FGF19 exhibits promise as a screening tool for bile acid malabsorption (BAM) in post-surgery patients with irritable bowel syndrome with diarrhea (IBS-D) and inflammatory bowel disease (IBD), with lower FGF19 levels indicating a higher likelihood of BAM. Fecal calprotectin (FC) demonstrates commendable diagnostic accuracy in active IBD but requires

Table 1

Summary of population characteristics in all 39 included studies.

Authors	Title of the study	Study type	Study setting	Sample size	Diagnostic test/biomarker
Feghaly *et al.* 2013[17]	Intestinal inflammatory biomarkers and outcome in pediatric *Clostridium difficile* infections	Prospective cohort study	St. Louis Children's Hospital (SLCH)	102	Phosphorylated p38
Nazeer *et al.* 2013[18]	Use of multiplex real-time PCR for detection of common diarrhea causing protozoan parasites in Egypt	Case–control study	Cairo and the Egyptian governorates Fayoum and Benha	598	Multiplex real time PCR
Castiglione *et al.* 2013[10]	Non-invasive diagnosis of small bowel Crohn's disease: direct comparison of bowel sonography and magnetic resonance enterography	Prospective non-inferiority diagnostic study	Tertiary care IBD unit	249	Bowel sonography (BS), magnetic resonance (MR) enterography
Sarafraz *et al.* 2013[19]	Detection of *Dientamoeba fragilis* among diarrheal patients referred to Tabriz health care centers by nested PCR	Observational	Tabriz health care centers, Northwest Iran	1000	Nested PCR
Coste *et al.* 2013[20]	Microbiological diagnosis of severe diarrhea in kidney transplant recipients by use of multiplex PCR assays	Retrospective observational study	Nephrology department of the Reims University Hospital in Champagne Ardennes, France	49	PCR
Saigal *et al.* 2013[21]	Comparison of staining techniques and multiplex nested PCR for diagnosis of intestinal microsporidiosis	Observational study	Postgraduate Institute of Medical Education and Research, Chandigarh, a tertiary care center in north India	395	Modified trichrome staining, calcofluor white staining, and PCR
Stellrecht *et al.* 2014[22]	Premarket evaluations of the imdx *C. difficile* for Abbott m2000 Assay and the BD Max Cdiff Assay	Prospective and retrospective analysis	Department of Pathology and Laboratory Medicine, Albany Medical Center, Albany, New York, USA	199 specimens (111 prospectively analyzed and 88 retrospectively analyzed)	Two automated PCR systems (imdx and Max)
Berry *et al.* 2014[23]	Real-time polymerase chain reaction correlates well with clinical diagnosis of *Clostridium difficile* infection	Prospective	Two acute hospitals within ABM UHB	1034 stool specimens	Cell Culture Cytotoxin Neutralization Assay (CCNA), real-time polymerase chain reaction (PCR) using the genexpert, glutamate dehydrogenase (GDH)/toxin enzyme immuno-assay
Eckert *et al.* 2014[11]	Molecular test based on isothermal helicase-dependent amplification for detection of the *Clostridium difficile* toxin A gene	Prospective	National Reference Laboratory for *Clostridium difficile* in Paris, France	308 consecutive diarrheal stool samples	AmpliVue *Clostridium difficile* assay and GDH-Illumigene algorithm
Al-Talib *et al.* 2014[24]	Pentaplex PCR assay for detection of hemorrhagic bacteria from stool samples	Laboratory-based diagnostic study	University laboratory in Malaysia	223 samples	Multiplex PCR assay
Hart *et al.* 2014[25]	*Clostridium difficile* infection diagnosis in a paediatric population: comparison of methodologies	Validation study	Tertiary pediatric hospital in Perth, Western Australia	150 consecutive stools from 75 patients	C. diff Quik Chek Complete, Illumigene *C. difficile*, geneohm Cdiff, cycloserine cefoxitin fructose agar (CCFA) culture, and Cell Culture Cytotoxin Neutralisation Assay (CCNA)
Harrington *et al.* 2015[26]	Multicenter evaluation of the BD max enteric bacterial panel PCR assay for rapid detection of Salmonella spp., Shigella spp., Campylobacter spp. (*C. jejuni and C. coli*), and Shiga toxin 1 and 2 genes	Multicenter clinical study	United States and Canada	4242 stool specimens	BD Max EBP automated PCR assay
Antonara *et al.* 2015[27]	A large-scale clinical evaluation of the AmpliVue and Illumigene molecular tests for the identification of *Clostridium difficile*-associated diarrhea in adult and pediatric patients	Large-scale clinical evaluation and comparative	Three geographically diverse clinical microbiology laboratories (Nationwide Children's Hospital, Columbus, OH, USA; Penn State Hershey Hospital, Hershey, PA, USA; Primary Children's, Salt Lake City, UT, USA)	758 fresh stool specimens	AmpliVue and Illumigene molecular tests for *C. difficile* toxin

Table 1

(*Continued*)

Authors	Title of the study	Study type	Study setting	Sample size	Diagnostic test/biomarker
Zhang *et al.* 2015[28]	A probe-free four-tube real-time PCR assay for simultaneous detection of twelve enteric viruses and bacteria	Experimental	Key Laboratory for Medical Virology, Ministry of Health, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China	122	Multiplex real time PCR assay
Knabl *et al.* 2016[29]	Comparison of the BD MAX® Enteric Bacterial Panel assay with conventional diagnostic procedures in diarrheal stool samples	Comparative study	Division of Hygiene and Medical Microbiology, Medical University Innsbruck	971	Cultivation of pathogens, multiplex PCR assay
De Rauw *et al.* 2016[30]	Detection of Shiga toxin-producing and other diarrheagenic *Escherichia coli* by the biofire filmarray® Gastrointestinal Panel in human fecal samples	Observational study		386	Biofire filmarray® Gastrointestinal (FA GI) Panel
Hanabara *et al.* 2016[31]	A rapid and simple real-time PCR assay for detecting foodborne pathogenic bacteria in human feces	Experimental	Japan	—	Target genes
Al-Asy *et al.* 2017[32]	New diagnostic biomarker in acute diarrhea due to bacterial infection in children	Case control	Pediatric Department at Tanta University Hospital, Tanta, Egypt	110	Strem, PCT, CRP
Thongprachum *et al.* 2017[33]	Multiplex RT-PCR for rapid detection of viruses commonly causing diarrhea in pediatric patients	Evaluation study	Japanese pediatric outpatients	751	Multiplex RT-PCR
Eigner *et al.* 2017[34]	Evaluation of a new real-time PCR assay for the direct detection of diarrheagenic *Escherichia coli* in stool specimens	Evaluation study	University Hospital Regensburg	315	RG real time PCR system
Piralla *et al.* 2017[35]	Filmarray™ GI panel performance for the diagnosis of acute gastroenteritis or hemorrhagic diarrhea	Retrospective observational study	University of Iowa Hospitals and Clinics, Iowa City, Iowa, USA	168	Filmarray™ GI panel
Ope *et al.* 2018[36]	Evaluation of the field performance of immunocard STAT!(®) rapid diagnostic test for Rotavirus in Dadaab Refugee Camp and at the Kenya–Somalia Border	Prospective observational study	The study was conducted in Dadaab Refugee Camp and Liboi Health Center, located at the Kenya–Somalia border	213 participants were enrolled in the study.	Immunocard STAT!® Rotavirus (ICS-RV) rapid diagnostic test
Henrique *et al.* 2018[37]	Large-scale evaluation of a rapid diagnostic test for diarrhea caused by enterotoxigenic *Escherichia coli* targeting the heat-labile toxin	Diagnostic study	Not specified	Not specified	Immunochromatographic (IC) test
Zhuo *et al.* 2018[38]	Identification of enteric viruses in oral swabs from children with acute gastroenteritis	Diagnostic study	Alberta, Canada		Quantitative RT-PCR Gastroenteritis Virus Panel
Sayeed *et al.* 2018[39]	Development of a new dipstick (Cholkit) for rapid detection of *Vibrio cholerae* O1 in acute watery diarrheal stools	Diagnostic test evaluation	ICDDR,B hospital in Dhaka, Bangladesh	76	Cholkit
Huang *et al.* 2018[40]	Detection of common diarrhea-causing pathogens in Northern Taiwan by multiplex polymerase chain reaction	Comparative	Northern Taiwan	217	Luminex xtag Gastrointestinal Pathogen Panel (xtag GPP)
Shin *et al.* 2018[41]	Serum procalcitonin levels can be used to differentiate between inflammatory and non-inflammatory diarrhea in acute infectious diarrhea	Retrospective study	Tertiary hospital in Daejeon, Republic of Korea	514 participants	Procalcitonin levels
Eckert *et al.* 2018[42]	Evaluation of a novel molecular assay to diagnose toxigenic strains of *Clostridium difficile*	Evaluation study	National Reference Laboratory (NRL) for *Clostridium difficile*, Paris, France	309	Amplidiag C. difficile + 027® assay

Schnee *et al.* 2018[43]	Evaluation of two new membrane-based and microtiter plate enzyme-linked immunosorbent assays for detection of *Campylobacter jejuni* in stools of Bangladeshi children	Evaluation study	International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka, Bangladesh	158	Two new membrane-based and microtiter plate EIAs.
Islam *et al.* 2019[44]	Field evaluation of a locally produced rapid diagnostic test for early detection of cholera in Bangladesh	Prospective diagnostic study	Field settings in Bangladesh	7220 participants	Cholkit RDT and Crystal VC RDT
Park *et al.* 2019[45]	Clinical significance of inflammatory biomarkers in acute pediatric diarrhea	Prospective observational study	Incheon St. Mary's Hospital	Incheon St. Mary's Hospital	Fecal biomarkers (calprotectin, lactoferrin, PMN-e) and blood inflammatory biomarkers (CRP, ESR, leukocytes)
Batista *et al.* 2019[46]	Usefulness of fecal calprotectin as a biomarker of microscopic colitis in a cohort of patients with chronic watery diarrhoea of functional characteristics	Retrospective observational study	Hospital Universitari Mútua Terrassa (HUMT), Catalonia region, Spain	94 patients with chronic non-bloody watery diarrhea	Fecal calprotectin concentration
Battat *et al.* 2019[47]	Serum concentrations of 7α-hydroxy-4-cholesten-3-one are associated with bile acid diarrhea in patients with Crohn's disease		University of Calgary in Calgary, Alberta, Canada	127	Serum C4 concentration
Tilmanne *et al.* 2019[48]	Enteropathogens in paediatric gastroenteritis: comparison of routine diagnostic and molecular methods	Observational study	Two university hospitals in Brussels, Belgium	185 cases and 179 controls	Luminex xtag Gastrointestinal Pathogen Panel
Lyutakov *et al.* 2021[49]	Diagnostic accuracy and predictive value of serum fibroblast growth factor 19 (FGF19) and total free fecal bile acids as biomarkers of bile acid malabsorption in patients with chronic diarrhea: a pilot study	Prospective observational study	Clinic of Gastroenterology, University Hospital "Tsaritsa Yoanna," Sofia, Bulgaria	40 participants	FGF19, TFFBA, and FC
Mashock *et al.* 2020[50]	A multicenter study of the Revogene *C. difficile* system for detection of the toxin B gene from unformed stool specimens	Multisite investigational evaluation	Seven geographically distributed clinical centers within Canada and the United States	2461 residual stool specimens	Revogene *C. difficile* assay (real-time PCR-based assay) I
Leli *et al.* 2020[51]	Evaluation of a multiplex gastrointestinal PCR panel for the aetiological diagnosis of infectious diarrhoea	Retrospective analysis	Microbiology laboratory in a community hospital	183 stool samples	Filmarray GI panel (molecular assay) and standard culture
Montasser *et al.* 2022[52]	Multiplex PCR: aid to more-timely and directed therapeutic intervention for patients with infectious gastroenteritis	Comparative study	Helwan, South Valley, and Tanta Universities outpatient clinics	200 stool samples	Multiplex PCR targeting specific genes

Table 2

RoB assessment results.

Authors	RoB
Feghaly *et al.* 2013[17]	Low
Nazeer *et al.* 2013[18]	Low
Castiglione *et al.* 2013[10]	Low
Sarafraz *et al.* 2013[19]	Low
Coste *et al.* 2013[20]	Low
Saigal *et al.* 2013[21]	Low
Stellrecht *et al.* 2014[22]	Low
Berry *et al.* 2014[23]	Low
Eckert *et al.* 2014[11]	Low
Al-Talib *et al.* 2014[24]	Moderate
Hart *et al.* 2014[25]	Low
Harrington *et al.* 2015[26]	Low
Antonara *et al.* 2015[27]	Low
Zhang *et al.* 2015[28]	Low
Knabl *et al.* 2016[29]	Low
De Rauw *et al.* 2016[30]	Low
Hanabara *et al.* 2016[31]	Low
Al-Asy *et al.* 2017[32]	Low
Thongprachum *et al.* 2017[33]	Low
Eigner *et al.* 2017[34]	Low
Piralla *et al.* 2017[35]	Low
Ope *et al.* 2018[36]	Low
Henrique *et al.* 2018[37]	Low
Zhuo *et al.* 2018[38]	Low
Sayeed *et al.* 2018[39]	Low
Huang *et al.* 2018[40]	Low
Shin *et al.* 2018[41]	Low
Eckert *et al.* 2018[42]	Low
Schnee *et al.* 2018[43]	Low
Islam *et al.* 2019[44]	Low
Park *et al.* 2019[45]	Low
Batista *et al.* 2019[46]	Low
Battat *et al.* 2019[47]	Low
Tilmanne *et al.* 2019[48]	Moderate
Lyutakov *et al.* 2021[49]	High
Mashock *et al.* 2020[50]	Low
Leli *et al.* 2020[51]	Low
Montasser *et al.*[52]	Low

supplementary assessment in cases of microscopic colitis (MC). Phospho-p38 (pp38) presents specificity for *C. difficile*-associated injury in pediatric patients. Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) proves valuable in early diagnosis of acute bacterial infection-induced diarrhea in children, exhibiting superior discriminatory power compared to PCT. Serum C4 concentration serves as a robust biomarker for bile acid diarrhea (BAD) in CD patients, offering high sensitivity and specificity. PCT holds promise in distinguishing inflammatory from non-inflammatory diarrhea, although further comprehensive studies are essential to establish its diagnostic worth.

Limitations of the reviewed studies

Key limitations demonstrating variable study quality were small, non-generalizable sample sizes ($n=12$), restricting result validity. Additionally, lacking outcomes assessments ($n=9$) limited clinical interpretation. Spurious results due to deficient methodology ($n=8$) indicate the need for enhanced research quality standards. Other major shortcomings included insufficient data ($n=7$), absent quantitative analyses and strain typing alongside technical limitations ($n=3$), RoB from cost estimate omissions ($n=2$), low assay specificity ($n=2$), incomplete pathogen enrichment culture ($n=2$), and reliance on retrospective designs ($n=2$). This heterogeneity highlights the imperative for more methodologically rigorous investigations using standardized protocols to validate emerging tools' true clinical worth in diarrhea diagnosis. Expanded, high-quality studies are essential to inform clinical guidelines and facilitate adoption of new non-invasive techniques.

Practice, policy, and future research

POCTs and biomarker testing, though, required less invasive measures and low-cost infrastructure, but their applicability varies across multiple settings. Overall, non-invasive rapid tests seemed most feasible for low-resource and primary care settings where access to traditional diagnostics is limited[55]. Another advantageous factor of rapid testing is that it provides actionable results in minutes compared to days for culture, enabling prompt treatment initiation. However, quality control and training on proper use are needed to ensure reliability. Biomarker testing requires equipped

Table 3

Sensitivity, Specificity, PPV and NPV of Non-Invasive Diagnostic Tools and Imaging Tests for Diarrheal Diseases.

Non-invasive tool	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Cell culture cytotoxin neutralisation Assay (CCNA)[23,25]	Study 1: 51	Study 1: 99.4	Study 1: 91.9	Study 1: 94.3
	Study 2: 33	Study 2: 100	Study 2: 100	Study 2: 78
ELISA[17,43]	Range: 29–95.7	Range: 72.4–97	Range: 53–100	Range: 84–98
Immunochromatography[37,39]	Study 1: 90.7	Study 1: 99.5	Study 1: 99.2	Study 1: 94.2
	Study 2: 76	Study 2: 90.2	Study 2: 35.2	Study 2: 98.2
Isothermal DNA amplification[11,25,27]	Study 1: AmpliVue – 91.7	Study 1: AmpliVue – 100	Study 1: AmpliVue – 100	Study 1: AmpliVue – 98.9
	Study 2: AmpliVue – 96.1	Study 2: AmpliVue – 99.2	Study 2: AmpliVue – 96.1	Study 2: AmpliVue – 99.2
	Study 1: Illumigene – 96.1	Study 1: Illumigene – 99.8	Study 1: Illumigene – 99.2	Study 1: Illumigene – 99.2
	Study 2: Illumigene – 89	Study 2: Illumigene – 100	Study 2: Illumigene – 100	Study 2: Illumigene – 95
	Study 3: Illumigene – 91.7	Study 3: Illumigene – 100	Study 3: Illumigene – 100	Study 3: Illumigene – 98.9
Microscopy[21]	Trichrome: 63.8	Trichrome: 100	Trichrome: –	Trichrome: –
	Calcofluor white: 79.7	Calcofluor white: 82.2	Calcofluor white: –	Calcofluor white: –
Non-invasive imaging test[10]	Bowel sonography: 94	Bowel sonography: 97	Bowel sonography: 97	Bowel sonography: 94
	MR enterography: 96	MR enterography: 94	MR enterography: 94	MR enterography: 96
PCR[18–26,28–31,33–35,38–40,42,48,50,51,53,54]	Range: 87.5–100	Range: 93.4–100	–	–

NPV, negative predictive value; PPV, positive predictive value.

Table 4

Performance Metrics of Biomarkers in Diagnosing Various Conditions Associated with Diarrhea.

Biomarker	Conditions	Cut-off level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
C-reactive protein (CRP)[32,41,45]	Acute pediatric diarrhea	13.7 mg/l	Study 1: 83.3	Study 1: 68.2	Study 1: 51.7	Study 1: 90.9
	Acute bacterial diarrhea in children	> 46.00 mg/l	Study 2: 100	Study 2: 80	Study 2: 97.83	Study 2: 100
	Inflammatory diarrhea in acute infectious diarrhea	> 1.5 mg/l	Study 3: 81.08	Study 3: 51.39	Study 3: –	Study 3: –
Fecal lactoferrin[45]	Acute pediatric diarrhea	22.8 µg/ml	77.8	70.5	51.8	88.6
CRP and lactoferrin[45]	Acute pediatric diarrhea	CRP: 13.7 mg/l (Study 1) Lactoferrin: 22.8 µg/ml	72.2	95.5	86.7	89.4
Fibroblast growth factor 19 (FGF19)[49]	Bile acid malabsorption in patients with chronic diarrhea	88.22 pg/ml	72.7	72.4	—	—
Fecal calprotectin (FC)[47,50]	Acute pediatric diarrhea	74.0 µg/g	Study 1: 94.4	Study 1: 38.6	Study 1: 38.6	Study 1: 94.4
	Microscopic colitis	> 100 µg/g	Study 2: 67	Study 2: 75	Study 2: 53	Study 2: 85
Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1)[32]	Acute bacterial diarrhea in children	> 14.5 ng/ml	93.33	93.33	99.21	60.86
Procalcitonin (PCT)[32,41]	Acute bacterial diarrhea in children	> 4.95 ng/ml	66.7	80	96.77	21.05
	Inflammatory diarrhea in acute infectious diarrhea	> 0.08 ng/ml	87.03	68.75	—	—
Serum C4 concentration[47]	Bile acid diarrhea in patients with Crohn's disease	< 48.3 ng/ml	90.9	84.4	50	98.2
		> 48.3 ng/ml	90.9	95.5	90.9	95.5

labs, limiting applicability in remote areas. However, biomarkers may be valuable for monitoring and prognostication in inpatient settings[56]. Regarding age groups, rapid tests and biomarkers are minimally invasive for children compared to stool culture. Still, pediatric-specific cut-offs are needed as adult reference ranges may misdiagnose children[57]. Careful selection and validation are required to implement the optimal non-invasive tool per setting, population, and available infrastructure.

The introduction of accurate, rapid non-invasive diagnostics can transform clinical and public health management of diarrheal diseases. At the patient level, timely definitive diagnosis guides appropriate therapy, reducing inappropriate antibiotic use and risks of complications or death[58]. For healthcare systems, faster turnaround relieves diagnostic delays that prolong hospital stays and costs. At the population level, prompt outbreak detection with scalable rapid tests facilitates monitoring and containment. However, lack of sensitivity for some pathogens and antimicrobial resistance may remain challenges[59]. Implementation must be coupled with training, quality assurance, and affordability measures to truly benefit underserved communities. Overall, non-invasive tools show immense potential to improve individual outcomes and epidemiologic control, advancing diarrhea management.

While several promising options exist, there are opportunities to optimize non-invasive diagnostics for diarrhea through further research and innovation. Development and validation of multiplex platforms enabling the detection of a wide panel of pathogens from single samples could maximize clinical utility[60]. Usability should be enhanced for healthcare workers with minimal training, and costs lowered to increase uptake in resource-limited settings. Exploring stable storage conditions and transport media could broaden access to complex tests relying on equipped labs[61]. As new biomarkers and rapid tests emerge, rigorous evaluation frameworks incorporating clinical outcomes are essential to demonstrate added value over conventional methods. Continued evolution and appropriate application of non-invasive diagnostics have the potential to reduce diarrhea burden worldwide.

Conclusion

Summary of the main findings

In this comprehensive review of non-invasive diagnostic tools and biomarkers for diarrheal diseases, we have synthesized a wealth of data from diverse studies to elucidate their diagnostic accuracy and potential clinical applications. Notably, our findings reveal a landscape of diagnostic approaches that exhibit variable performance characteristics, presenting opportunities and challenges in the realm of diarrhea diagnosis.

Point-of-care tests (POCTs)

Our analysis encompasses a range of POCTs, each with distinct strengths and limitations. PCR emerges as having high sensitivity (range: 87.5–100%) and specificity (range: 93.4–100%). Cell Culture Cytotoxin Neutralization Assay (CCNA) demonstrates high specificity (99.4%) but variable sensitivity (33–51%). Immunochromatography shows promise but variability (sensitivity range: 76–90.7%; specificity range: 90.2–99.5%). Isothermal DNA amplification methods like AmpliVue and

Illumigene exhibit high performance (sensitivity and specificity ≥96%). Non-invasive imaging modalities also showcase high accuracy particularly for CD diagnosis – BS has 94% sensitivity and 97% specificity; MR enterography has 96% sensitivity and 94% specificity.

Biomarkers

A multitude of biomarkers have been explored as valuable tools for identifying specific etiologies of diarrhea and associated conditions. CRP, in conjunction with fecal lactoferrin, proves valuable for detecting fever and bacterial causation in acute pediatric diarrhea. FGF19 shows promise as a screening tool (90.9% sensitivity and 95.5% specificity) for BAM, while FC exhibits commendable diagnostic accuracy in active IBD at cut-offs more than 250 μg/g but requires additional tests for MC diagnosis. Additionally, biomarkers like pp38, sTREM-1, serum C4 concentration, and PCT offer potential diagnostic insights into specific conditions. However, further research is needed to establish their diagnostic worth conclusively.

Reiteration of the importance of non-invasive diagnostic tools

The significance of non-invasive diagnostic tools in the context of diarrheal diseases cannot be overstated. These tools offer several critical advantages:

(1) Timeliness: Non-invasive tests, particularly rapid POCTs, provide actionable results within minutes, enabling prompt initiation of appropriate treatment. This rapidity is especially crucial in cases where delayed diagnosis can lead to severe complications.
(2) Reduced antibiotic misuse: Accurate non-invasive diagnostics help in distinguishing between bacterial and non-bacterial causes of diarrhea, reducing the unnecessary use of antibiotics and mitigating the risk of antimicrobial resistance.
(3) Cost and resource efficiency: Many non-invasive tests are cost-effective, making them suitable for resource-limited settings where access to traditional diagnostic methods may be limited. This efficiency can lead to substantial cost savings in healthcare systems.
(4) Epidemiological control: The ability to rapidly detect specific pathogens using non-invasive tools facilitates the early identification of outbreaks, aiding in containment and preventing the spread of infectious diseases.

Ethical approval

Ethical approval was not required for this article type.

Consent

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Author contribution

R.K., M.H.A., H.M.P., Z.H., A.P., S.H.K., M.K., H.F., Y.G.K., A.A., and M.A.H.: literature search and manuscript preparation; M.A.H.: conceptualization, methodology, and supervision.

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Data availability statement

The data analyzed in the study are original data from the institution and cannot be shared openly to protect study participant privacy.

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