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Performance evaluation of the Biosynex AMPLIQUICK Fecal Bacteriology PCR kit

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Abstract

This study assessed the clinical performance of the Biosynex AMPLIQUICK Fecal Bacteriology kit for detecting enteropathogenic bacteria in cases of acute community-acquired diarrhea. In total, 194 retrospectively collected and 207 prospectively collected stool samples were analyzed. In cases of discordant results between the AMPLIQUICK Fecal Bacteriology kit and initial status of the 401 stools, samples were reanalyzed using the Seegene Gastrointestinal Panel 1 and 2 kits or targeted PCR assays. Among the 401 samples, 190 were expected to be positive for *Campylobacter* spp. (*C. jejuni* or *C. coli*), 48 for *Salmonella* spp., 39 for *Shigella* spp./enteroinvasive *Escherichia coli* (EIEC), 21 for *Yersinia enterocolitica*, and 30 for enterohemorrhagic *E. coli* (EHEC). Additionally, 64 samples were expected to be negative. Nine other samples tested positive for either other enteropathogens (*Aeromonas* spp., *Plesiomonas shigelloides*, *Vibrio* spp., or *Clostridioides difficile*) or co-infections with two pathogens. Only three samples (four discrepancies) yielded discordant results with the AMPLIQUICK Fecal Bacteriology kit: one false positive for enterotoxigenic *E. coli* (ETEC), one false negative for EHEC, and one sample that was both falsely positive for *Salmonella* spp. and falsely negative for EHEC. The analytical performance was calculated using a composite reference standard (CRS) in the absence of a perfect gold standard. Due to the low number or absence of positive cases, the performance of the Biosynex AMPLIQUICK Fecal Bacteriology kit could not be determined for *Vibrio* spp., *Yersinia pseudotuberculosis*, hypervirulent *C. difficile* strains, Cholera toxin, ETEC, 0157 EHEC and *P. shigelloides*. Using this CRS, positive, negative and overall agreement rates ranged from 98.24 to 100% for all the other pathogens. In conclusion, the Biosynex AMPLIQUICK Fecal Bacteriology kit enables comprehensive screening for key bacterial pathogens associated with gastrointestinal infections in a single PCR assay. With excellent clinical performance, it represents a reliable tool for the rapid and accurate diagnosis of bacterial gastroenteritis in routine practice.

Keywords Stools, Syndromic PCR, Enteropathogens

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Introduction

In March 2024, the World Health Organization ranked diarrheal syndrome as the third leading cause of death among children aged 1–59 months, estimating 443,832 deaths in children under five in 2021 (Diarrhoea. https://www.who.int/health-topics/diarrhoea. Retrieved 28 May 2024) [1]. This syndrome is primarily of infectious origin, with bacterial etiology accounting for 10–20% of cases. Efforts to combat these infections focus on both prevention and symptomatic treatment. Preventive measures include hygiene promotion, access to clean drinking water and sanitation, and vaccination campaigns. Symptomatic treatment aims to alleviate symptoms and prevent severe complications through administration of oral rehydration solutions, zinc supplements, and, in certain cases, antibiotics to manage bacterial infections. In some instances, etiological investigations are recommended to optimize patient management, minimize the risk of pathogen transmission, and mitigate antibiotic resistance. Reliability and rapid identification of pathogens are the two most critical factors in etiological research.

Most intestinal infections are self-limiting and resolve spontaneously, requiring neither empirical antibiotic treatment nor laboratory investigation. Symptoms often subside before stool culture results are available, and these results rarely influence patient management. However, accurately identifying the infectious cause is crucial in specific circumstances. These include severe cases in which the infection may disseminate systemically or lead to major complications, chronic or recurrent infections, and when complications are a concern due to the clinical context or patient comorbidities. Furthermore, pathogen identification plays a vital role for health authorities in tracking the epidemiology of gastrointestinal infections and monitoring outbreaks at the regional level.

Various techniques are available for detecting infectious agents, particularly enteropathogenic bacteria. Coproculture remains the gold standard for diagnosing bacterial diarrhea. The primary challenge lies in isolating pathogens from a highly diverse and abundant commensal flora. This requires the use of selective culture media and enrichment techniques to target clinically relevant bacteria specifically. The coproculture process involves multiple steps, including sample receipt and pretreatment, inoculation onto various media, bacterial identification, and antibiotic susceptibility testing. This method is time-consuming and labor-intensive, typically requiring 2–4 days to yield a definitive result. Such delays can hinder timely infection management, potentially leading to diagnostic errors and overuse of empirical antibiotic therapies. Despite these limitations, coproculture remains valuable, as it provides an antibiogram, enabling treatment adjustments based on identified bacterial resistance patterns. It also allows the production of isolates important for public health including identification of foodborne outbreaks.

Antibiotic susceptibility testing is generally not recommended as a routine practice, except in specific cases. These include children under 6 months of age, immunocompromised patients, individuals with severe symptoms, or those with persistent diarrhea that does not resolve spontaneously [2, 3]. These criteria highlight the importance of close communication between the laboratory and the prescribing physician to determine when stool analysis is warranted and whether antibiotic susceptibility testing is appropriate. However, such testing remains crucial for monitoring bacterial resistance and assessing epidemiological trends.

The standard approach for diagnosing community-acquired bacterial diarrhea includes testing for *Campylobacter* spp., *Salmonella* spp., *Shigella* spp./EIEC, and, when indicated, *Yersinia* spp [4]. Depending on the clinical and epidemiological context, specific selective culture media may be required to detect certain pathogens [4], such as enterohemorrhagic *Escherichia coli* (EHEC), *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Klebsiella oxytoca*, or *Plesiomonas shigelloides*. The isolation and identification of enteropathogenic bacteria in a patient with diarrhea are generally considered clinically significant. However, results must always be interpreted in the context of the patient's clinical and epidemiological background.

Although coproculture exhibits excellent specificity, its sensitivity is relatively low, particularly compared to molecular diagnostic methods. Since the early 2010 s, syndromic PCR panels have revolutionized infectious-disease diagnostics, broadening the scope of clinical microbiology [5–13]. These panels allow simultaneous detection of multiple pathogens associated with specific symptoms, significantly reducing diagnostic time due to their multiplexing capability [14–16]. Additionally, they offer excellent analytical sensitivity and specificity. Some syndromic PCR panels require prior DNA extraction using a dedicated platform, followed by amplification on a separate machine, as seen with the Seegene® Allplex Gastrointestinal kit (Seegene, Seoul, South Korea).

Other systems integrate both extraction and amplification within a single cartridge or strip that is designed for use with dedicated automated platforms, such as the BD MAX Enteric Panel (Becton Dickinson, Franklin Lakes, USA) or the Novodiag® Bacterial GE +kit (Mobidiag, Paris, France) [5, 6, 17, 18]. These automated systems align closely with the "point-of-care" concept: standalone analytical modules that enable on-demand testing, unlike kits requiring a separate amplification platform that operates with batches. Point-of-care kits offer several advantages, including simplified operation with minimal staff training, space and ergonomic efficiency through the

use of a single device and reagent system, and reduced contamination risk due to their closed design, which prevents intermediate handling during extraction and amplification.

Additionally, syndromic PCR kits can detect bacteria that are difficult to identify or isolate using conventional methods, such as certain *E. coli* pathovars, thereby expanding the diagnostic coverage of diarrheal syndromes with a more comprehensive detection panel [19]. Some kits are designed to detect a specific single bacterium such as *Clostridioides difficile* in targeted clinical contexts. The growing availability of syndromic PCR kits for diagnosing gastrointestinal infections has raised several important questions [20]. The primary concern is the reliability of these results compared to traditional methods or previously validated kits. It is also essential to assess how integrating these new technologies impacts laboratory workflow, as well as their relevance and clinical utility in relation to local epidemiology and diagnostic needs [21].

The aim of our study was to evaluate the analytical performance of the Biosynex AMPLIQUICK Fecal Bacteriology kit prior to its launch for the diagnosis of bacterial gastrointestinal infections. This evaluation was conducted through both retrospective and prospective studies, comparing the results obtained using this kit with those obtained using other diagnostic techniques.

Materials and methods

Sample selection

Retrospective study: In total,194 stool samples, all preserved in Cary-Blair medium, were collected between November 2018 and May 2023 from patients hospitalized for gastrointestinal symptoms at Bordeaux University Hospital (pediatric and adult emergency departments) (Suppl. Table 1). All samples had tested positive for enteric bacteria using the BD MAX Enteric Bacterial Panel multiplex PCR (standard ± extended). The BD MAX™ Enteric Bacterial Panel detects *Salmonella* spp., *Shigella* spp./enteroinvasive *E. coli* (EIEC), *Campylobacter* spp. (*jejuni* and *coli*) and Shiga toxin producing organisms (STEC, *Shigella dysenteriae*). The BD MAX™ Bacterial Panel Extended Enteric panel detected *Y. enterocolitica*, Enterotoxigenic *E. coli* (ETEC), *P. shigelloides*, *Vibrio* (*V. vulnuficus*, *V. parahaemolyticus* and *V. cholerae*).

With the exception of *E. coli* O157:H7 (EHEC) and enterotoxigenic *E. coli* (ETEC), the detected bacteria were further cultured on selective agar plates. The samples were then aliquoted and stored at −80 °C following BD MAX processing (Suppl. Table 1).

Prospective study: In total, 138 Cary-Blair stool samples from patients hospitalized for gastrointestinal symptoms at Bordeaux University Hospital (pediatric and adult emergency departments) between May 2023 and January 2024 were analyzed (Suppl. Table 2). These samples were tested using the BD MAX™ Enteric Bacterial Panel and subsequently stored at _80 °C. Additionally, 64 stool samples from a private laboratory, Cerballiance (Le Haillan, France), were included between February 2024 and March 2024. These samples were analyzed using the Allplex GI-Bacteria (I) Assay (Seegene) syndromic multiplex PCR, followed by targeted culture, except for *Campylobacter spp.* The samples were then aliquoted and stored at −80 °C (Suppl. Table 2).

A summary of the results for all tested samples is provided in Table 1. The median age for the 401 patients included was 26.6 year old (± 28.4) with a sex ratio of 1.5.

Table 1 Summary of study samples

Enteropathogens	Number of stools in the retrospective study	Number of stools in the prospective study	Total number of stools
Campylobacter spp.	112	78	190
Campylobacter spp. + *Salmonella* spp.	1	0	1
Salmonella spp.	27	21	48
Salmonella spp. + EHEC	1	0	1
Salmonella spp. + *Aeromonas* spp.	0	1	1
Shigella spp./EIEC	7	32	39
Shigella spp./EIEC + EHEC	0	1	1
EHEC	21	9	30
Yersinia enterocolitica	14	7	21
Clostridioides difficile	0	3	3
Vibrio spp.	0	1	1
Plesiomonas spp.	1	0	1
Negative	10	54	64
Total	194	207	401

AMPLIQUICK fecal bacteriology

The AMPLIQUICK Fecal Bacteriology kit is designed as a 96-well plate that is divided into 12 PCR strips, each containing 8 wells. These strips are pre-aliquoted with five reagent mixes in wells A, B, C, D, and E. Each mix contains deoxynucleoside triphosphates, $MgCl_2$, primers, and specific fluorescent probes. The primers used target specific DNA regions of enteropathogens (Suppl. Table 3). Wells A–D each contain primers specific to the target bacteria, along with an internal control (IC) primer. This IC consists of synthetic DNA, which is directly incorporated into the mix and serves to validate the amplification step. Well E, in addition to primers for the target bacteria, includes primers for the internal procedural control (IPC). The IPC, also composed of synthetic DNA, is added directly to the primary sample. Its detection confirms the integrity of the entire sample-processing workflow (extraction and PCR amplification), thereby

minimizing the risk of false-negative results due to extraction failure. If the IC or IPC is not detected, the test is considered invalid, except when an enteropathogen is detected in the same well. This is because competition between DNA amplification of the target pathogen and the internal control may occur. To ensure assay reliability, positive and negative controls are included in the last two wells of the first PCR strip. These controls validate the PCR process, helping to prevent false-positive results due to contamination and false-negative results due to amplification failure.

The AMPLIQUICK Fecal Bacteriology kit requires prior DNA extraction from stool samples. In this study, nucleic acid extraction was performed using a NucleoMag® Dx Pathogen kit (Macherey-Nagel, Hoerdt, France) on 200 µL of FecalSwab™ liquid with the addition of 5 µL of the IPC. DNAs were extracted on MagnetaPure automated system (Dutsher, Bernolsheim, Germany). For PCR preparation, 5 µL of each eluate (200 µL) was dispensed into the first five wells of the pre-aliquoted PCR strips, which had been stored at −20 °C and thawed immediately before use. Following gentle centrifugation, the strips were placed in a CFX96 thermal cycler (Bio-Rad, Marnes-la-Coquette, France) for amplification, following the manufacturer's instructions. Fluorescence from the labeled probes was detected in real-time using optical methods across four detection channels—three for bacterial targets and one for internal controls (IC or IPC). The results were then analyzed using CFX Maestro Software for CFX Real-Time PCR Instruments, based on predefined acceptability criteria (Suppl. Tables 4–8) (Fig. 1).

Data analysis and confirmation methods

The results obtained with the AMPLIQUICK Fecal Bacteriology kit were compared to those from initial qualification methods, including PCR and culture (Table 1; Suppl. Tables 1–2). In cases of discordant results between AMPLIQUICK Fecal Bacteriology and initial qualification, further analysis was performed using the Allplex™ GI-Bacteria (I) Assay (ref. GI9801X) for *Campylobacter spp.*, *Yersinia enterocolitica*, *Salmonella spp.*, *Vibrio spp.*, *Aeromonas spp.*, *C. difficile* Toxin B, and *Shigella spp./E. coli* EIEC. For samples testing positive for *E. coli* EHEC (*stx1/stx2*), E. coli EPEC (*eaeA*), *E. coli* ETEC (*lt/st*), enteroaggregative *E. coli* (EAEC) (*aggR*), *E. coli* O157 serotype, and hypervirulent *C. difficile* strains, a second qualification step was conducted using the Allplex™ GI-Bacteria (II) Assay (ref. GI9702X) (Suppl Table 9).

Testing with the Seegene Allplex™ assays was performed using 5 µL of eluate in a 25-µL total PCR reaction volume, following the manufacturer's instructions. Amplification was carried out on the Bio-Rad Opus CFX96 thermocycler, and data analysis was conducted using CFX Maestro and Seegene Viewer software.

Additional PCRs were performed in case of discordant result between the AMPLIQUICK Fecal Bacteriology kit and the initial status of the stools (Fig. 1). PCR amplifications for sequencing analysis were performed using the following primers: Fw 5'-GCG GTT GGA ACG CAG ATA A-3' and Rv 5'-CCC ATT CGG TTA GAG CAC TAT ATT T-3' for the *aap* gene of *E. coli* EAEC. Targeting both genes allows the detection of typical EAEC (*aggR*-positive) and atypical EAEC strains that can be *aggr*-negative but *aap*-positive. Fw 5'-GGC GAT GAT

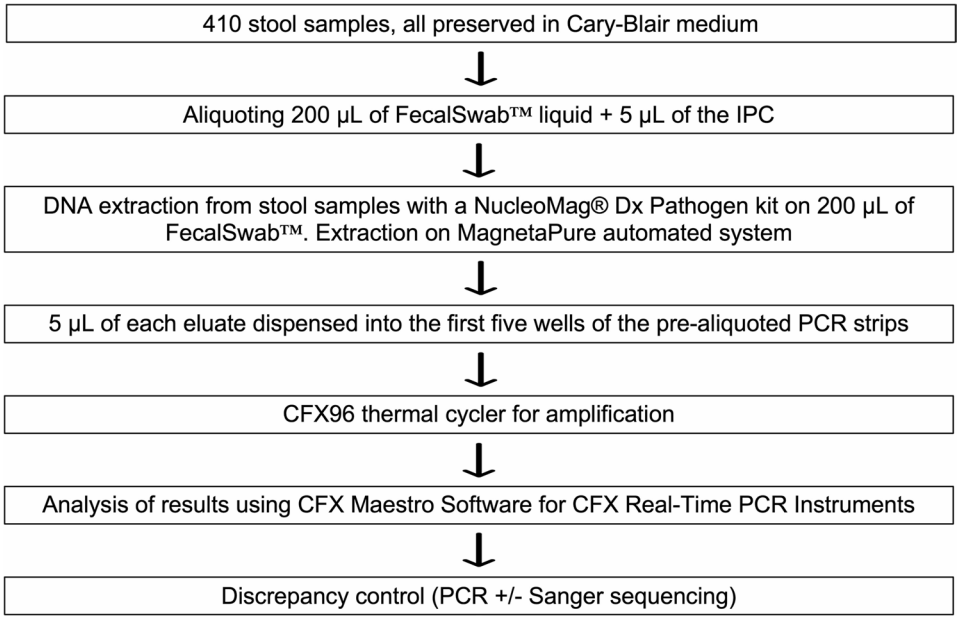

Fig. 1 Experimental procedures of the study

GTG CAA ATT GA-3' and Rv 5'-ACT TCT AAT ACT AGC CCT ATT TCC C-3' for the *lpxA* gene of *C. upsaliensis*; Fw 5'-GCA GAA CAG CGT CGT ACT AT-3' and Rv 5'- CAC CGA AAT ACC GCC AAT AAA G-3' for the *invA* gene of *Salmonella enterica;* Fw 5'- CCT ACC ATC CTG CAC TGG A-3' and Rv 5'-GGA GGA AAC AGA CCA GAC GG-3' for the *sta* gene of *E. coli* ETEC; Fw 5'- TTC AGT TAA TGC GAT TGC TAA GGA-3', Fw2 5'TTC TGT TAA TGT GGT TGC GAA GGA-3', and Rv 5'-AAA GCT TCA GCT GTC ACA GTA AC-3' for the *stx1* gene of *E. coli* EHEC; and Fw 5'-TTA ATG CAA TGG CGG CGG AT-3', Rv 5'- TTA AAC TGC ACT TCA GCA AAT CC-3', and Rv2 5' TTA AAC TTC ACC TGG GCA AAG CC-3' of the *stx2* gene of *E. coli* EHEC. The expected PCR product lengths for the tested genes were 273, 541, 702, 594, 525, and 218 bp, respectively.

For PCR, 5 µL of sample eluate was used in a 20-µL reaction volume. Amplification was performed on a Bio-Rad CFX thermocycler, followed by agarose gel electrophoresis (1.3% Seakem® GTG® agarose gel). The PCR products were then purified using a Monarch Gel Extraction Kit (NEB, ref. T1020L) and sequenced by Sanger sequencing at Eurofins Genomics using the primers listed above. The resulting FASTA sequences were analyzed using the Basic Local Alignment Search Tool (BLAST, NIH).

Statistical analyses and composite reference standard

The analytical performances were calculated using a composite reference standard (CRS) in the absence of a perfect gold standard (lack of sensitivity of culture). The CRS was defined as positive when culture was positive or, in the case of a negative culture (or if conventional culture was not performed or unable to detect the pathogen, i.e., EPEC, EAEC, ETEC), when the result was concordant with the one previously obtained with the BD Max Enteric Bacterial Panel multiplex PCR (standard ± extended) in the restrospective study or Allplex GI-Bacteria (I) Assay in the prospective study.

When a discrepancy was noted, the CRS was defined as positive when at least one independent PCR assay (± Sanger sequencing) used for verification (see above) was concordant with the AMPLIQUICK Fecal Bactariology kit (Suppl Table 9).

Positive, negative and overall agreement rates were calculated manually, 95% confidence intervals were calculated using an on-line tool available at http://www.vassarstats.net/.

Results

Retrospective study

In the retrospective study, among the 194 stool samples tested (Table 2, Suppl Table 1), no amplification signal was detected for any of the targets in seven out of ten samples classified as negative using the AMPLIQUICK Fecal Bacteriology kit. Three samples were positive for 1 *Aeromonas sp* (n°5), 1 *Salmonella spp* (n°200) and 1 *Campylobacter sp* + EAEC. The presence of these pathogens was verified by Allplex TM GI-Bacteria (I) assay. The kit successfully identified *Campylobacter* spp. in all 113 known positive samples and additionally detected its presence in two more samples (n°108 and 205) (Suppl Table 10). The presence of *Campylobacter* spp. in these two samples was verified using the Seegene Gastrointestinal Panel 1 PCR.

For *Salmonella* spp., the AMPLIQUICK kit accurately detected all 29 known positive samples. Additionally, it identified *Salmonella* spp. DNA in six more samples. Among these, five (n°179, 188, 189, 193, and 200) were verified as true positives by the Seegene Gastrointestinal Panel 1, whereas one sample (n°180) was determined to be a false positive.

The kit also demonstrated high accuracy in detecting other bacterial pathogens. It correctly identified *Shigella spp.*/EIEC DNA in six of the seven known positive samples. The only sample classified as negative by the AMPLIQUICK Fecal Bacteriology kit (n°67) was found also negative by the Seegene Gastrointestinal Panel 1 kit, representing a true negative result.

The kit also detected EHEC DNA in 18 of the 22 positive cases. Among the six discordant cases for EHEC, four samples (n°136, 180, 190, and 193) that were expected to be positive were identified as negative by the AMPLIQUICK Fecal Bacteriology kit. Of these, two samples (n°136 and 193) were verified as true negatives by the Seegene Gastrointestinal Panel 2, whereas the remaining two (n°180 and 190) were classified as positive by the Seegene Gastrointestinal Panel 2, indicating false negatives. Finally, the last two discordant EHEC samples (n°135 and 67), which were expected to be negative, were identified as positive by the AMPLIQUICK Fecal Bacteriology kit and subsequently also characterized as true positives by Seegene PCR.

Yersinia DNA was detected in all 14 expected positive samples, and *P. shigelloides* DNA in the only known positive sample (n°171).

For targets not detected during the initial qualification (because they were not included in the initial panel used), the AMPLIQUICK Fecal Bacteriology kit identified the presence of five *C. difficile*, one *Vibrio* spp., 16 *Aeromonas* spp., 24 EPEC, two EHEC O157, 11 EAEC, and one additional EPEC. These findings were verified by Seegene's Gastrointestinal Panel 1 and 2 kits, except for three samples (n°52, 156, and 168), in which the presence of EAEC was verified through single-plex PCR targeting the *aap* gene (Table 2) and sequencing analysis of the *aap* gene.

Table 2 Results from the retrospective study

Bacterial status	BD MAX or Seegene	Positive culture	AMPLIQUICK	Interpretation
Negative	10	0	7	Concordant
			1 *Aeromonas* spp. (N°5)	Positive
			1 *Salmonella* spp. (N°200)	Positive
			1 *Campylobacter* spp. + EAEC (n°205)	Positive
Campylobacter spp.	113	91	113	Concordant
			2 *Campylobacter* spp. (n°108, 205)	Positive
Salmonella spp.	29	26	29	Concordant
			5 *Salmonella* spp. (n°179, 188, 189, 193, 200)	Positive
			Salmonella spp. (n°180)	Negative
Shigella spp./ EIEC	7	7	6	
			1 negative (n°67)	Positive
EHEC	22	10	18	
			2 EHEC (n°67, 35)	Positive
			2 negative (n°136, 193)	Positive
			2 negative (n°180, 190)	Negative
Yersinia spp.	0	14	14	Positive
P. shigelloides	0	1	1	Positive
C. difficile	0	0	5 (n°162,174,193,196, S133)	Positive
Aeromonas spp.	0	0	16 *Aeromonas* spp. (n°5,15,16,31,32,35,100, 101,113,129,163,166,174, 181,193, 215)	Positive
EPEC	0	0	24 EPEC (n°9,11,15,24,25,29,31,50, 54,67,80,82,89,90,100,101, 120,143,144,161,163,166, 174,190)	Positive
EAEC	0	0	11 EAEC (n°46,51,52,94,123,129,156168,179,204,205)	Positive
ETEC	0	0	1 ETEC (n°168)	Positive
Vibrio spp.	0	0	*Vibrio* spp. (n°181)	Positive

Prospective study

Among the 207 stool samples included in the prospective study, 78 had previously been identified as positive for *Campylobacter* spp., and all were verified to be positive by the AMPLIQUICK Fecal Bacteriology kit (Table 3, Suppl Table 2). Additionally, the kit detected three more samples as *Campylobacter*-positive. Of these, two samples (n°372 and 373) were verified by Seegene Gastrointestinal Panel 1 PCR, while the third (n°243) was positive by a specific single-plex PCR for *C. upsaliensis*, which is not detected by the Seegene Gastrointestinal Panel 1.

Regarding EHEC, eight of the ten samples initially identified as positive were also positive by the AMPLIQUICK Fecal Bacteriology kit, while two samples (n°419 and 428) were classified as negative, which was verified by Seegene Gastrointestinal Panel 2 PCR. Furthermore, the AMPLIQUICK Fecal Bacteriology kit detected four additional samples (n°270, 433, 443, and C31) as positive, all of which were verified by Seegene Gastrointestinal Panel 2 PCR.

The AMPLIQUICK Fecal Bacteriology kit accurately detected the presence of *Salmonella* spp. DNA in 22 samples, *Shigella* spp./EIEC DNA in 33 samples, *Vibrio* spp. DNA in 1 sample, *C. difficile* DNA in 3 samples, and *Yersinia* spp. DNA in 7 samples. Additionally, three samples (n°473, 474, and C39) were identified as positive for *C. difficile* by AMPLIQUICK Fecal Bacteriology and also positive by Seegene Gastrointestinal Panel 1 PCR. One sample (n°C35), which tested positive for *Yersinia* spp. with AMPLIQUICK Fecal Bacteriology, was also positive by Seegene Gastrointestinal Panel 1 PCR. For *Aeromonas* spp., the single sample initially identified as culture-positive was also positive by AMPLIQUICK Fecal Bacteriology. Furthermore, an additional 18 samples positive for *Aeromonas* spp by AMPLIQUICK Fecal Bacteriology kit were subsequently detected as positive by Seegene Gastrointestinal Panel 1 PCR. The AMPLIQUICK Fecal Bacteriology kit also demonstrated a high level of specificity, showing no amplification signal in 47 of the 54 samples classified as negative. The 7 remaining samples were positive for *Campylobacter sp* ($n = 3$), EPEC ($n = 3$) and *Aeromonas* spp ($n = 1$) (Table 3).

For targets not detected during the initial qualification, AMPLIQUICK Fecal Bacteriology identified the presence of 25 EAEC, 22 EPEC, and 7 ETEC. All positive results were verified by Seegene's Gastrointestinal Panel 2 kit, except for eight samples (n°271, 273, 291, 465, 468, C18, C21, and C30), in which the presence of

Table 3 Results from the prospective study

Bacterial status	BD MAX or Seegene	Positive culture	AMPLIQUICK	Interpretation
Negative	54	0	47	Concordant
			3 Campylobacter spp. (n°243, 372, 373)	Positive
			3 EPEC (n°261, 322, 340)	Positive
			1 *Aeromonas* spp. (n°377)	Positive
Campylobacter spp.	78	50	78	Concordant
			3 *Campylobacter* spp. (n°243, 372, 373)	Positive
Salmonella spp.	22	22	22	Concordant
Shigella spp./ EIEC	33	7	33	Concordant
EHEC	10	6	8	Concordant
			2 negatives (n°419, 428)	Positive
			4 EHEC (n°270, 433, 443, C31)	Positive
Yersinia spp.	6	7	7	Concordant
			1 *Yersinia* spp. (n°C35)	Positive
C. difficile	0	3	3	Concordant
			3 *C. difficile* (n°473, 474, C39)	Positive
Aeromonas spp.	0	1	1	Concordant
			18 Aeromonas spp. (n°224, 245, 280, 282, 286, 301, 377, 396, 462, 464, 472, 473, 474, 477, 478, C19, C49, C55*)*	Positive
EPEC	0	0	0	
			22 EPEC (n°233, 261, 265, 266, 271, 273, 274, 276, 295, 303, 322, 340, 419, 428, 433, 443, 455, 466, C24, C38, C54, C56)	Positive
EAEC	0	0	0	
			25 EAEC (n°268, 270, 271, 273, 280, 291, 303, 377, 426, 465, 466, 468, 478, C3, C17, C18, C19, C20, C21, C24, C26, C30, C38, C52, C54*)*	Positive
ETEC	0	0	7 ETEC (n°280, 465, C17, C19, C22, C26, C40)	Positive
			1 ETEC (n°267)	Negative
Vibrio spp.	0	1	1	Concordant

EAEC was verified by single-plex PCR targeting the *aap* gene and sequencing analysis of the *aap* gene. Only one sample (n°267), which was initially classified as negative for ETEC, was detected as positive by AMPLIQUICK Fecal Bacteriology; however, this result was negative by the validation method (Seegene Gastrointestinal Panel 2) (Table 3).

Combined analysis

In total, 401 stool samples were analyzed in this study, including 194 from the retrospective study and 207 from the prospective study. Except for *Yersinia* spp detection, Ct values were higher in culture negative cases (> 4 cycles of difference) (Suppl. Table 11).

Four major discrepancies were observed in three samples (Table 4, Suppl Table 10). Sample n°180 was identified as positive for *Salmonella* spp. by the AMPLIQUICK Fecal Bacteriology kit but this was negative by the validation method. Conversely, it was classified as negative for EHEC by AMPLIQUICK but as positive by the validation method. Multiple attempts to amplify the *invA* gene and the *stx1* and *stx2* genes for sequencing analysis were unsuccessful.

Sample n°190 was classified as negative for EHEC by AMPLIQUICK Fecal Bacteriology but was also positive by the validation method. Similar to sample n°180, multiple attempts to amplify the *stx1* and *stx2* genes for sequencing analysis were unsuccessful.

Sample n°267 was identified as positive for ETEC by AMPLIQUICK Fecal Bacteriology but was negative by the validation method. Multiple attempts to amplify the *sta* gene of ETEC for sequencing analysis failed.

The AMPLIQUICK Fecal Bacteriology kit enabled the additional detection of 5 *Campylobacter* spp., 5 *Salmonella* spp., 1 *Shigella* spp./EIEC, 6 EHEC, 1 *Yersinia* spp., 7 *C. difficile*, 34 *Aeromonas* spp., 46 EPEC, 37 EAEC, and 8 ETEC (Table 4). All these additional detections were found positives by the validation method.

Following statistical analysis of the combined results from both studies, the positive agreement rates of the AMPLIQUICK test ranged from 98.24% *Salmonella* spp to 100% for all other major enteropathogens (Table 5). The negative agreement rates ranged from 99.38% for EHEC to 100% for all other detected targets. Due to the low number or absence of positive cases, the performance of the AMPLIQUICK kit could not be determined for *Vibrio* spp., *Y. pseudotuberculosis*,

Table 4 Results from both studies

Targets	BD MAX or Seegene	Culture positive	AMPLIQUICK	Interpretation
Negative	64	0	54	Concordant
			3 *Campylobacter* spp.	Positive
			3 EPEC	Positive
			2 *Aeromonas* spp.	Positive
			1 *Campylobacter* spp. + EAEC	Positive
			1 *Salmonella* spp.	Positive
Campylobacter spp.	191	141	191	Concordant
			5 *Campylobacter* spp.	Positive
Salmonella spp.	51	48	51	Concordant
			5 *Salmonella* spp.	Positive
			1 *Salmonella* spp. (n°180)	Negative
Shigella spp./ EIEC	40	14	39	Concordant
			1 negative	Positive
EHEC	32	16	26	Concordant
			4 negative	Positive
			2 negative (n°180 and 190)	Negative
			6 EHEC	Positive
Yersinia spp.	6	21	21	Concordant
			1	Positive
C. difficile	0	3	3	Concordant
			7 *C. difficile*	Positive
Aeromonas spp.	0	1	1	Concordant
			34 *Aeromonas* spp.	Positive
EPEC	0	0	-	
			46 EPEC	Positive
EAEC	0	0	-	
			37 EAEC	Positive
ETEC	0	0	-	
			8 ETEC	Positive
			1 ETEC (n°267)	Negative
Vibrio spp.	0	1	1	Concordant
			1 *Vibrio* spp.	Positive
P. shigelloides	0	1	1	Concordant

hypervirulent *C. difficile* strains, Cholera toxin, ETEC, O157 EHEC and *P. shigelloides*.

Discussion

The aim of this study was to evaluate the analytical performance of the AMPLIQUICK Fecal Bacteriology kit. The findings demonstrate that the AMPLIQUICK kit exhibited superior sensitivity compared to traditional culture methods, detecting an additional 89 bacterial pathogens across the 401 samples analyzed. This included 55 *Campylobacter*, 8 *Salmonella*, 25 *Shigella* spp./EIEC, and 1 *Yersinia* spp. The increased sensitivity of the AMPLIQUICK Fecal Bacteriology kit was also evident when compared with the BD MAX automated system currently in use at Bordeaux University Hospital. The AMPLIQUICK kit identified five additional *Campylobacter*, five *Salmonella*, and six EHEC cases, all of which were also positive by Seegene Gastrointestinal Panels 1 and 2, as well as by specific single-plex PCR and sequencing analysis for *C. upsaliensis* (which is not detected by the Seegene Gastrointestinal panel). The inclusion of *C. upsaliensis* as a target broadens the detection spectrum for *Campylobacter* spp., although this species remains rare in France. Overall, the analytical performance of the AMPLIQUICK Fecal Bacteriology kit is comparable to that of other commercially available multiplex diagnostic kits [5–13].

Only a few discordant results were observed in this evaluation. For instance, sample n°267 tested positive for ETEC (Ct = 36.51) with the AMPLIQUICK Fecal Bacteriology kit but this was found negative by the Seegene Gastrointestinal Panel 2. Since both kits target the same genes for ETEC detection (heat-labile toxin and heat-stable toxin), further analyses, particularly sequencing, could help to clarify this discrepancy. Although contamination cannot be ruled out, it is noteworthy that this sample was tested twice using the AMPLIQUICK Fecal Bacteriology kit. Additionally, two EHEC cases detected

Table 5 Performance of the AMPLIQUICK fecal bacteriology kit

	***Campylobacter* spp.**	***Y. enterocolitica***	***Salmonella* spp.**	***C. difficile***	***Aeromonas* spp.**
Positive	196	22	56	11	35
Negative	205	105	344	102	63
Also negative	-	-	1	-	-
Also positive	-	-	-	-	
Positive agreement (IC 95%)	100 (97.60–100)	100 (81.5–100)	98.24 (92–100)	100 (67.86–100)	100 (87.68–100
Negative agreement (IC 95%)	100 (97.71–100)	100 (95.6–100)	100 (98.14–99.98)	100 (95.48–100)	100 (92.84–100)
Overall agreement	100	100	99.75	100	100
	Shigella spp./EIEC	EHEC	EPEC	EAEC	
Positive	39	32	46	36	
Negative	362	319	37	47	
Also negative	-	-	-	-	
Also positive	-	2	-	-	
Positive agreement (IC 95%)	100 (88.83–100)	100 (80.32–99.28)	100 (86.66–100)	100 (87.99–100)	
Negative agreement (IC 95%)	100 (98.69–100)	99.38 (98.85–100)	100 (97.52–99.89)	100 90.59–100)	
Overall agreement	100	99.43	100	100	

IC 95% 95% confidence intervals (calculated using http://www.vassarstats.net/)

by the BD MAX system (cycle threshold [Ct] = 32.6 and 35.2) were not identified by the AMPLIQUICK Fecal Bacteriology kit. The reason for this discrepancy remains uncertain; however, both cases were also positive by Seegene Gastrointestinal Panels 1 and 2 (Ct = 33.23 and 36.34), and the patients' clinical presentations were consistent with EHEC infection. Consequently, these two samples should be considered false negatives.

The risk of contamination is a significant challenge in open multiplex PCR systems, such as the AMPLIQUICK Fecal Bacteriology kit. One possible solution is the introduction of Ct cut-offs to aid in the interpretation of late positive results, as is already applied for *Campylobacter* spp., *Aeromonas* spp., *Vibrio* spp., *Shigella* spp./EIEC, and cholera toxin targets. It could be tempting to compare the Ct values obtained in the present study by comparing them to those reported in similar studies. However, the diversity of pre-analytical conditions, DNA extraction techniques and the different choices of target genes used to detect pathogens, make any comparison hazardous.

The AMPLIQUICK range consists of open kits designed to remain compatible with a wide variety of existing systems and platforms, rather than evolving into closed systems like BD MAX. This flexibility allows AMPLIQUICK Fecal Bacteriology to accommodate laboratories of varying sizes and technical capacities, including both those equipped with automated laboratory instruments and those relying on manual processing by technical staff. A key advantage of open systems is the ability to analyze multiple types of pathogens from a single sample eluate. This includes bacterial targets using AMPLIQUICK Fecal Bacteriology, viral pathogens with AMPLIQUICK Fecal Virology, and parasitic infections with AMPLIQUICK Helminths and Protozoans. Another benefit of open systems is their adaptability to different automated liquid-handling platforms, such as the Microlab NIMBUS from Hamilton and the Autopure 4800 from Allsheng. Biosynex is actively developing a liquid-handling platform to automate the processing of various AMPLIQUICK PCR diagnostic kits. This automation will integrate sample extraction, deposition, and PCR setup, reducing contamination risks and minimizing errors associated with manual processing. Furthermore, it will enable simultaneous testing of 48 patient samples in under 2 h, from sample preparation to PCR results.

Another important aspect of this study is the selection of targets included in the AMPLIQUICK Fecal Bacteriology kit. For *Yersinia* spp., the Biosynex laboratory has chosen to incorporate the *yst* gene, which encodes an enterotoxin [22], and the *aiI* gene, which encodes a membrane protein that facilitates host cell attachment and invasion. The inclusion of these two targets allows detection of both *Y. enterocolitica* and *Y. pseudotuberculosis*, the two primary *Yersinia* species associated with gastrointestinal infections. In contrast, other diagnostic platforms have adopted different strategies for *Yersinia* detection. For example, Novodiag® Bacterial GE+ (Mobidiag, Paris, France) [5] specifically detects *Y. enterocolitica* by targeting the *virF* gene, while BD MAX does not distinguish between pathogenic and non-pathogenic *Y. enterocolitica* [5]. The advantage of the AMPLIQUICK approach lies in its ability to specifically identify only the pathogenic biotypes of *Y. enterocolitica* and *Y.*

pseudotuberculosis, ensuring that only clinically relevant infections are targeted.

Biosynex also opted to include testing for various *E. coli* pathovars. Patients who tested positive exhibited clinical symptoms comparable to those of patients with bacterial infections detected through conventional coproculture, highlighting the clinical relevance of this method for identifying these pathogens. For the detection of EAEC, Biosynex selected the *aggr* (aggregation regulator gene) and *aap* (which encodes the dispersin protein) targets. This choice is particularly important because certain atypical EAEC strains can be *aggr*-negative but *aap*-positive [23]. By targeting both genes, the AMPLIQUICK Fecal Bacteriology kit ensures detection of all EAEC variants, unlike other diagnostic kits such as Novodiag® Bacterial GE +and Seegene Gastrointestinal Panel 2, which rely solely on the *aggr* gene [5, 11].

A limitation of the AMPLIQUICK Fecal Bacteriology kit in detecting *E. coli* pathovars is its inability to differentiate between co-infections and single infections in specific cases. For instance, certain EHEC *O157* strains may carry the *eae* gene, which encodes intimin and is also used in the AMPLIQUICK Fecal Bacteriology panel to detect enteropathogenic *E. coli* (EPEC). Consequently, when a sample tests positive for both *eae* and *stx1/stx2*, it is not possible to distinguish between an EPEC and EHEC co-infection and an EHEC *O157* infection alone, where the strain harbors both *eae* and *stx1/stx2*.

The number of cases of *C. difficile* infection is low in our study. No performance can be calculated. Moreover, GDH test results are more commonly used than bacterial culture (positive culture) as first-step comparator. The performance of the AMPLIQUICK Fecal Bacteriology kit for this major pathogen will therefore have to be assessed by an expert laboratory. Finally, *Plesiomonas spp.* and *Aeromonas spp.* are also incorporated into the panel of enteropathogens detected by the AMPLIQUICK Fecal Bacteriology kit through the inclusion of the *hugA* gene, which encodes a membrane receptor protein involved in heme acquisition [24], and the *gyrB* gene, which encodes the B subunit of DNA gyrase, a key enzyme in type II topoisomerase [25]. Although the pathogenic role of these two bacteria has not been fully elucidated, their inclusion in the detection panel may enhance the documentation of infectious diarrhea in certain patients.

Conclusion

In this study, we used both retrospective and prospective samples to evaluate the analytical performance of the new syndromic PCR kit, AMPLIQUICK Fecal Bacteriology (Biosynex) for diagnosing bacterial digestive infections. Before its launch, this kit demonstrated excellent performance, with sensitivities and specificities approaching 100% for the primary pathogens responsible for community-acquired digestive tract infections.

Furthermore, the kit enhances clinical sensitivity by detecting pathogens that are not identifiable through traditional culture methods, thereby improving identification of bacteria responsible for specific digestive syndromes. Additionally, it selectively detects only pathogenic *Yersinia* strains, increasing clinical specificity through targeted identification.

However, the implementation of this technology requires strict pre-analytical management to minimize the risk of false positives due to cross-contamination or external contamination. Notably, the Biosynex AMPLIQUICK Fecal Bacteriology kit will be available for use on a dedicated automated platform developed for AMPLIQUICK kits, as well as on any open automated platform already present in the user's laboratory.

Abbreviations

PCR Polymerase chain reaction
Ct Threshold cycles
EIEC Enteroinvasive E. coli
EHEC Enterohemorrhagic E. coli
ETEC Enterotoxigenic E. coli

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

PL supervised the study. QB, AC, MJ, and PL analyzed the data and drafted the manuscript. QB, LB, AB, and JA performed the experiments. AC provided some of the samples included in this study. All authors contributed to data interpretation and critically revised the manuscript for important intellectual content.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate

All diagnostic methods were performed as part of routine clinical practice. Patients were investigated in hospital or private settings following good clinical practices. No informed consent was required for the use of human stool samples, as these were sent to the NRCCH for research purposes. Therefore, the need for ethical approval and informed consent has therefore not been waived by an ethics committee, as deemed unnecessary according to the scientific missions of the national reference center mandated by Santé Publique France (www.spf). To ensure patient anonymity, all indirectly identifiable data were removed from this study. Remnants of specimens were preserved at the Centre de Ressources Biologiques Plurithématique of Bordeaux University Hospital under authorization 2017-864-DMT-FI-R with no information regarding patient identity.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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