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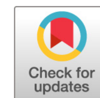

Performance Evaluation of the Novodiag Bacterial GE+ Multiplex PCR Assay

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ABSTRACT The bacteriological diagnosis of intestinal bacterial infections has historically been based on culture on agar plates. However, culture may lack sensitivity, and some enteropathogens, such as pathovars of *Escherichia coli*, may escape routine diagnosis. Our goal was to evaluate the analytical performance of the Novodiag Bacterial GE+ kit for the detection of enteropathogenic bacteria in acute community diarrhea. We included 251 stools in this study (198 retrospective and 53 prospective). The analytical performance was 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 if culture was positive or, in case of a negative culture, if the BD Max extended enteric bacterial panel and/or other real-time PCR (RT-PCR) tests were positive. Of the 251 samples, 200 were positive, and 51 were negative. Overall sensitivities of the Novodiag Bacterial GE+ kit for *Campylobacter* sp., *Salmonella* sp., *Shigella* sp./enteroinvasive *E. coli* (EIEC), *Yersinia enterocolitica*, enterohemorrhagic *E. coli* (EHEC), and enterotoxigenic *E. coli* (ETEC) ranged from 98.98 to 100%, specificities ranged from 98.08 to 100%, positive predictive values (PPVs) ranged from 88.24 to 100%, and negative predictive values (NVPs) ranged from 99.36 to 100%. The analytical performance of the Novodiag Bacterial GE+ kit is excellent. It can be used as a routine tool in the rapid diagnosis of bacterial gastroenteritis. Despite the eNAT tube dilution of the primary sample, the detection of *Salmonella* sp. and EHEC was perfect. The kit has the advantage of only detecting pathogenic *Y. enterocolitica*. Its performance for *Campylobacter* is very satisfactory.

KEYWORDS Novodiag Bacterial GE+, syndromic panel based, analytical performance, digestive infections

Intestinal infections continue to represent a major cause of morbidity and mortality worldwide, with nearly 2 billion cases and 600,000 directly imputable deaths per year, mostly in children under the age of 5 years in developing countries (https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease). Therefore, diagnosis of these infections must be as quick and efficient as possible, especially since the diagnosis speed allows early administration of targeted therapy. The bacterial infection diagnosis is traditionally based on stool culture, which is one of the least efficient techniques, due to a lack of sensitivity and a tedious process requiring 3 days on average to obtain a final result for *Campylobacter* or *Salmonella* species, for example. Moreover, almost 80% of intestinal infection cases remain undetected with this technique, partially due to a lack of detection of certain bacteria, e.g., *Escherichia coli* pathovars (1–3). To overcome these limits, and with the technological progress of the last decade, molecular biology techniques have been developed, including syndromic multiplex panels, which target

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several of the most common pathogens of enteric infections. These multiplex assays also have the advantage to improve laboratory workflow by reducing the analytical process (4).

Despite the theoretical advantages of syndromic panel-based assay use, questions remain concerning its benefits and costs, which may vary among patient populations (4–6). Furthermore, this strategy can become expensive if used on every stool sample without considering the clinical and epidemiological context. Therefore, the implementation of a syndromic panel-based strategy requires clinicians in association with microbiologists to select cases for which a multiplex molecular assay would be useful, based on the Infectious Disease Society of America (IDSA) or American College of Gastroenterology (ACG) guidelines (7–9). Furthermore, the analytical performances and reliability of these panels must be determined. Indeed, the clinical performance of these tests is generally evaluated internally by each of the companies that manufacture and market them. However, it is important to assess their performance independently by defining precisely the reference used for each target and the clinical relevance of the results obtained.

Our aim was to evaluate the analytical performance of the Novodiag Bacterial GE+ kit (Mobidiag, Espoo, Finland), using 251 stool samples (198 retrospective and 53 prospective) for the detection of enteropathogenic bacteria in acute community diarrhea. The analytical performance was calculated using a composite reference standard (CRS). We showed that the analytical performance of the Novodiag Bacterial GE+ kit is excellent. It can be used as a routine tool in the rapid diagnosis of bacterial gastroenteritis.

MATERIALS AND METHODS

Clinical specimens. We included 251 stool samples in this study (198 retrospective and 53 prospective).

The retrospective group was comprised of (i) 80 unpreserved stool specimens, collected between April 2011 and January 2019 at the Pediatric and Adult Emergency Department of the University Hospital Center of Bordeaux (Bordeaux, France), tested and positive by conventional culture, and stored at −80°C within 12 h after reception; and (ii) 118 stool specimens collected between November 2018 and March 2019 from three private laboratories (Bio67, Strasbourg, France; CBM25, Besançon, France; and Exalab, Bordeaux, France) and transported in Cary-Blair medium (Copan Diagnostics, Brescia, Italy). These specimens in Cary-Blair medium were stored at −20°C within 6 h after reception in the respective laboratories and sent frozen to our laboratory. They were previously tested on-site with the BD Max extended enteric bacterial panel (which detects *Campylobacter jejuni* and *Campylobacter coli*, *Salmonella* sp., *Shigella*/enteroinvasive *E. coli* [EIEC], *Yersinia enterocolitica*, *Plesiomonas shigelloides*, *Vibrio* sp., enterohemorrhagic *E. coli* [EHEC], and enterotoxigenic *E. coli* [ETEC]) and by culture; 116 of these 118 samples were positive (according to the BD Max extended enteric bacterial panel result [$n = 118$] and/or culture [$n = 87$]) for at least one enteropathogen. The results obtained for these 198 stool samples are shown in Table 1.

The prospective group was comprised of 53 unpreserved stool samples received from the pediatric and adult emergency departments of Bordeaux University Hospital (Table 1) from February to April 2019 and tested with conventional culture, stored at 4°C, and analyzed with the Novodiag Bacterial GE+ within 2 days. Only 5 of them were positive by conventional culture (Table 1).

None of these 251 stools underwent other freeze-thaw cycles.

Routine methods for bacterial culture. Stool specimens from Bordeaux hospital were tested for bacterial enteropathogens using conventional culture methods. They were plated on Hektoen enteric (bioMérieux, Marcy l'Étoile, France), Campylosel (bioMérieux), and Cefsulodin-Irgasan-Novobiocin (CIN) (Oxoid, Basingstoke, UK) media. Additionally, a selenite-lactose broth (Thermo Fisher Scientific, Waltham, MA) was inoculated, incubated overnight at 35°C, and used to inoculate a second Hektoen enteric plate after enrichment. All plates were incubated 2 days at 35°C in ambient air, except for Campylosel, which was incubated for 3 days in jars using an Anoxomat microprocessor (Mart Microbiology, B.V. Lichtenvoorde, The Netherlands), which creates a microaerobic atmosphere (80 to 90% N_2, 5 to 10% CO_2, and 5 to 10% H_2), and for CIN, which was incubated at 30°C. Bacterial identification was performed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany), as previously described (10).

The 118 retrospective samples were plated on a selective medium based on the bacterium detected by BD Max extended enteric bacterial panel (CIN, Campylosel, or Hektoen enteric); 31 of them were negative for *Campylobacter* culture despite a positive result obtained on the BD Max extended enteric bacterial panel. The *Campylobacter* status of these 31 samples had been confirmed in a previous study with another PCR format, i.e., the PCR Rida Gene bacterial stool panel (R-Biopharm AG, Darmstadt, Germany) (11).

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TABLE 1 Stool samples used in the study

Source (no. of isolates)	Expected result (no. of isolates [species])	BD Max tested	Culture taken
Retrospective samples (198)			
Bordeaux University Hospital (80)	40 (*C. jejuni*)	No	Yes
	18 (*Salmonella* sp.)	No	Yes
	1 (*Salmonella* sp. plus 1 *C. difficile*)	No	Yes (pos for both)[a]
	3 (*C. jejuni* plus *C. coli*)	No	Yes
	2 (*C. coli*)	No	Yes
	3 (*Shigella* sp.)	No	Yes
	2 (*Y. enterocolitica*)	No	Yes
	11 negative	No	Yes
Private laboratories (118)	39 (*C. jejuni*)	Yes	Yes (26 neg)
	3 (*C. coli*)	Yes	Yes (3 neg)
	26 (*Salmonella* sp.)	Yes	Yes
	22 (EHEC)	Yes	No
	13 (ETEC)	Yes	No
	6 (*Y. enterocolitica*)	Yes	Yes
	4 (*Shigella* sp.)	Yes	Yes
	1 (*C. jejuni* plus 1 EHEC)	Yes	Yes (neg for both)
	1 (*C. jejuni* plus 1 ETEC)	Yes	Yes (neg for both)
	1 (*Y. enterocolitica* plus *C. jejuni*)	Yes	Yes
	2 negative	Yes	Yes
Prospective samples (53)	2 (*C. jejuni*)	No	Yes
	1 (*C. coli*)	No	Yes
	1 (*Salmonella* sp.)	No	Yes
	1 (*Y. enterocolitica*)	No	Yes
	48 negative	No	Yes

[a]pos, positive; neg, negative.

Table 1 summarizes the bacterial status of the samples according to the diagnostic tests performed on these 251 samples.

Novodiag Bacterial GE+ testing. The Novodiag Bacterial GE+ assay was performed using a diluted sample in eNAT medium (Copan Diagnostics). The dilution was carried out by introducing feces into a tube using a Floq swab (Copan Diagnostics) or 300 μl of Cary-Blair medium. This tube contains a solution which inactivates infectious agents by chemical lysis within 30 min and stabilizes genomic material by nuclease inactivation. Six hundred microliters of eNAT solution were then transferred to the Novodiag Bacterial GE+ cartridge according to the manufacturer's instructions and put in the Novodiag system.

The Novodiag Bacterial GE+ test is based on automated nucleic acid extraction, amplification, and analysis with two technologies: real-time PCR (fluorescent probes) and microarray (total internal reflection fluorescence [TIRF]-based detection). The test is CE-IVD marked (Conformit Europene marked for *in vitro* diagnostic), as well as the instrument. The total duration of the process (sample and reagents preparation, cassette incubation, and PCR) takes around 105 min (1.75 h). Four modules of 4 cassettes can be connected to the Novodiag system. The Novodiag software automatically performs an analysis with each target reported as positive or negative, with the list of detected targets (Table S1 in the supplemental material). If a control fails, Novodiag reports the run as invalid for every target. According to the manufacturer's instructions, every sample with an invalid result was retested a second time and, if needed, a third time with a diluted sample (300 μl of the first eNAT tube transferred to a second eNAT). These retests were processed within 24 h of the first result.

Confirmation method. Samples showing discordant results between Novodiag Bacterial GE+ and culture and/or expected BD Max extended enteric bacterial panel PCR results were further analyzed by an additional confirmation test (Table 2). Every sample with a discordant positive result concerning *Campylobacter* sp. or *Salmonella* sp. with the Novodiag Bacterial GE+ was confirmed with the PCR Rida Gene bacterial stool panel (R-Biopharm) as previously described (11).

Every discordant result for *E. coli* pathovars detected by Novodiag Bacterial GE+ was confirmed by endpoint PCR for specific virulence-associated genes, which included *ipaH*/*ial* for *Shigella*/EIEC, *lt*/*stp* for ETEC, *aggR* for enteroaggregative *E. coli* (EAEC), *eae*/*bfpA* for enteropathogenic *E. coli* (EPEC) using the primers and PCR conditions described by Oh et al. (12), and stx_1 and stx_2 for Shiga toxin-producing *E. coli* (STEC) using the primers and PCR conditions described by Grad et al. (13). The PCRs contained 2.5 μl of prepared genomic DNA, 5 μl buffer $MgCl_2$ 5× (Promega, Madison, WI, USA), 0.5 μl of deoxynucleoside triphosphate (dNTP) (10 mM, Promega), 0.1 μl of each primer (100 μM, Eurofins Genomics, Ebersberg, Germany), 0.3 μl of GoTaq DNA polymerase (5 UI/μL, Promega), and water to a final volume of 25 μl. PCRs were carried out on a Mastercycler nexus (Eppendorf, Hamburg, Germany) under the following conditions: an initial denaturation step at 94°C for 4 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension of 72°C for 7 min. Amplified DNA products were loaded onto a 1 to 3% agarose gel containing SYBR Safe (Fisher Scientific, Illkirch, France) and read under UV using the Gen Flash system (SynGene, Frederick, MD, USA). Concerning EHEC detection, an overnight enrichment of the samples in brain heart broth (Oxoid) was performed as recommended by Gouali et al.

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TABLE 2 Tests considered for the determination of the initial bacterial status of the samples and confirmation methods used in the study

Pathogen[a]	Test[b]	Confirmation method[c]
Campylobacter coli	Positive culture or PCR BD Max positive	Rida Gene bacterial stool panel
Campylobacter jejuni	Positive culture or PCR BD Max positive	Rida Gene bacterial stool panel
Salmonella sp.	Positive culture or PCR BD Max positive	Rida Gene bacterial stool panel
Shigella sp./EIEC	Positive culture or PCR BD Max positive	*ipaH* and *ial* PCR
Yersinia enterocolitica	Positive culture	Biotyping[d]
EHEC	PCR BD Max positive	stx_1 and stx_2 PCR
ETEC	PCR BD Max positive	*lt* and *stp* PCR
Clostridioides difficile	None	Alethia *C. difficile*
EAEC	None	*aggR* PCR
EPEC	None	*bfpA* and *eae* PCR

[a]Detected by Novodiag Bacterial GE+.
[b]Considered for the determination of the initial bacterial status.
[c]If necessary.
[d]Biotyping performed at the National Reference Center for Yersiniosis.

(14), followed by DNA extraction with MagNA Pure 96 (Roche Diagnostic, Meylan, France) followed by stx_1 and stx_2 PCR detection. Finally, every *Clostridioides difficile* detection was confirmed with Alethia *C. difficile* (Meridian Biosciences, Cincinnati, OH, USA). The Alethia *C. difficile* assay utilizes a loop-mediated isothermal DNA amplification (LAMP) technology to detect the pathogenicity locus (PaLoc) of toxigenic *C. difficile*, which codes for both the toxin A gene (*tcdA*) and the toxin B gene (*tcdB*) (15).

All *Y. enterocolitica* isolates were sent to the National Reference Center for Yersiniosis (Pasteur Institute, Paris, France) for biotyping.

Population and clinical study. The median age of the 251 patients was 30.4 years (±28.2) with a sex ratio of 1.19. Clinical data were collected for the patients hospitalized in Bordeaux University Hospital (Bordeaux CHU) included in the retrospective stool group ($n = 74$) and for all patients who were part of the prospective stool group ($n = 53$). The median age of this population was 22.2 years (±27.1), and the sex ratio was 1.25.

The clinical features collected were presence of diarrhea, fever, abdominal pain, rectal bleeding, nausea, and vomiting. In addition, information on the initiation of a probabilistic antibiotic therapy or oral rehydration, the length of hospitalization, and the presence of a biological inflammatory syndrome (C-reactive protein [CRP] >5 mg/liter) was obtained.

Statistical analysis and composite reference standard. The analytical performances were calculated using a composite reference standard (CRS). 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., EHEC, EPEC, EAEC, ETEC, or *C. difficile*), when the result was concordant with the one previously obtained with the BD Max extended enteric bacterial panel. When a discrepancy was noted, the CRS was defined as positive when at least one independent PCR assay used for confirmation was concordant with the Novodiag Bacterial GE+ (Table 2). The 95% confidence interval (CI) was calculated using the Wilson method.

This CRS was applied for *Campylobacter* sp., *Salmonella* sp., *Shigella* sp./EIEC, *Y. enterocolitica*, EHEC, and ETEC. EAEC and EPEC were excluded because of the absence of a culture result, and they were not included in the BD Max extended enteric bacterial panel. *C. difficile* was also not included in the BD Max extended enteric bacterial panel. For these 3 pathogens, only concordance percentages between Novodiag Bacterial GE+ results and confirmation PCRs were calculated.

To compare clinical features, a Chi-square or F test was performed, with a P value of <0.05 considered significant.

Ethics. All diagnostic methods were performed routinely. All patients were investigated in a hospital or private setting according to good clinical practices. No informed consent for using human stool samples was requested of the patients. Therefore, to ensure subject anonymity, all indirectly identifiable patient data were removed from the present study.

RESULTS

Retrospective study. One hundred ninety-eight stool samples were included in the retrospective study (Table 1). Novodiag Bacterial GE+ detected the presence of *Campylobacter* sp. in 89 of the 90 positive samples. The negative sample corresponded to a *C. jejuni* case for which the culture was negative. The presence of *C. jejuni* was confirmed by a Rida Gene bacterial stool panel both on the primary sample and the eNAT tube. Novodiag Bacterial GE+ correctly detected the samples positive for *Shigella* sp. ($n = 7$) and *Salmonella* sp. ($n = 45$). Furthermore, Novodiag detected 7 *Y. enterocolitica* among 9 stools positive by culture. The 2 *Y. enterocolitica* not detected by Novodiag Bacterial GE+ belonged to the nonpathogenic biotype 1a, as confirmed by the NRC for yersiniosis (data not shown). Novodiag Bacterial GE+ also detected 26

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TABLE 3 Comparison between expected results and those obtained using Novodiag Bacterial GE+ and after confirmation[a]

Expected result (no. of samples)	R (no. detected)[b]	P (no. detected)[c]	Positive culture (no. detected)	Novodiag Bacterial GE+ result (no. and type of bacteria)	Confirmation (no. and type of bacteria)	Not confirmed (no. and type of bacteria)
Negative (61)	13	48	0	45 negative		
				1 (*C. jejuni*)	1 (*C. jejuni*)	
				1 (*C. jejuni* plus EPEC)	*1* (*C. jejuni* plus EPEC)	
				2 (*Shigella* sp./EIEC)	2 (*Shigella* sp./EIEC)	
				3 (*C. difficile*)	3 (*C. difficile*)	
				1 (EAEC)		1 (EAEC)
				5 (EPEC)	2 (EPEC)	3 (EPEC)
				2 (EHEC)	2 (EHEC)	
				1 (EAEC plus EPEC)	1 (EPEC)	1 (EAEC)
C. jejuni (81)	79	2	54	69 (*C. jejuni*)		
				1 (*C. jejuni* plus ***C. difficile***)	1 (*C. difficile*)	
				2 (*C. jejuni* plus **EAEC plus EPEC**)	2 (EPEC plus 1 EAEC)	1 (EAEC)
				2 (*C. jejuni* plus **EHEC**)	2 (EHEC)	
				5 (*C. jejuni* plus **EPEC**)	3 (EPEC)	2 (EPEC)
				1 (*C. jejuni* plus **ETEC**)		1 (ETEC)
				1 negative	1 (*C. jejuni*)	
C. jejuni plus EHEC (1)	1	0	0	1 (*C. jejuni* plus EHEC)		
C. jejuni plus ETEC (1)	1	0	0	*1* (*C. jejuni* plus ETEC **plus EAEC**)	1 (EAEC)	
C. coli (6)	5	1	3	*6* (*C. coli*)		
C. jejuni plus *C. coli* (3)	3	0	3 (for both)	1 (*C. jejuni* plus *C.* coli)		
				1 (*C. jejuni* plus *C. coli* plus **EAEC**)	1 (EAEC)	
				1 (*C. jejuni* plus *C. coli* plus **EAEC plus EPEC**)	1 (EAEC plus 1 EPEC)	
Shigella sp. (7)	7	0	7	7 (*Shigella* sp.)		
Y. enterocolitica (9)	8	1	9	7 (*Y. enterocolitica*)		
				1 (*Y. enterocolitica* plus **EPEC**)	1 (EPEC)	
				1 negative	1 (nonpathogenic *Y. enterocolitica*)	
Y. enterocolitica plus *C. jejuni* (1)	1	0	1 (for both)	**1** (***C. jejuni***)	1 (nonpathogenic *Y. enterocolitica*)	
EHEC (22)	22	0	nd[d]	19 (EHEC)		
				2 (EHEC plus **ETEC**)	1 (ETEC)	1 (ETEC)
				1 negative	1 negative	
ETEC (13)	13	0	nd	9 (ETEC)		
				2 (ETEC plus **EPEC**)	2 (EPEC)	
				1 (ETEC plus **EHEC**)	1 (EHEC)	
				1 (ETEC plus **EAEC**)	1 (EAEC)	
Salmonella sp. (45)	44	1	45	41 (*Salmonella* sp.)		
				1 (*Salmonella* sp. plus ***C. difficile***)	1 (*C. difficile*)	
				2 (*Salmonella* sp. plus **EAEC**)		2 (EAEC)
				1 (*Salmonella* sp. plus **EHEC**)	1 (EHEC)	
Salmonella sp. plus *C. difficile* (1)	1	0	1[e]	1 (*Salmonella* sp. plus *C. difficile*)		
	198	53	135			

[a]Bold represents discordant result obtained by Novodiag Bacterial GE+ compared to expected result.
[b]R, retrospective study.
[c]P, prospective study.
[d]nd, not done.
[e]For *Salmonella* sp. only.

EHEC cases (19 EHEC alone and 7 in combination with another enteropathogen, which included 3 with *C. jejuni*, 3 with ETEC, and 1 with *Salmonella* sp.). Novodiag Bacterial GE+ was negative for one sample that was expected to be positive for EHEC according to the results previously obtained on the BD Max extended enteric bacterial panel. The presence of EHEC was not confirmed by endpoint stx_1 and stx_2 PCR. Novodiag Bacterial GE+ detected 17 ETEC cases (9 ETEC alone and 8 in combination with 1 to 2 other enteropathogens, which included 2 with EPEC, 1 with *C. jejuni*, 1 with *C. jejuni* and EAEC, 3 with EHEC, 1 with EAEC). Finally, 3 *C. difficile* (2 with *Salmonella* sp. and 1 with *C. jejuni*), 14 EPEC cases (3 EPEC alone and 11 with 1 to 3 other enteropathogens), and 9 EAEC cases (1 EAEC alone and 8 in combination with 1 to 3 other enteropathogens) were also detected by Novodiag; the presence of *C. difficile* in the 3 stool samples was confirmed by Alethia *C. difficile*, as well as 11 EPEC (78.6%) and 5 EAEC (55.6%) by endpoint PCR (Table 3).

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TABLE 4 Performance of the Novodiag Bacterial GE+

Pathogen (no. of samples)	Novodiag Bacterial GE+ result	No. of CRS tests with indicated result[a]		
		Positive	Negative	Total
Campylobacter sp. (251)	Positive	94	0	94
	Negative	1	156	157
Salmonella sp. (251)	Positive	46	0	46
	Negative	0	195	195
EHEC (123)	Positive	28	0	28
	Negative	0	95	95
ETEC (119)	Positive	15	2	17
	Negative	0	102	102
Y. enterocolitica (251)	Positive	8	0	8
	Negative	0	243	243
Shigella sp./EIEC (251)	Positive	9	0	9
	Negative	0	242	242

[a]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., EHEC, EPEC, EAEC, ETEC, *C. difficile*]), when the result was concordant with the one previously obtained with the BD Max extended enteric bacterial panel. When a discrepancy was noticed, CRS was defined as positive when at least one independent PCR assay used for confirmation was concordant with Novodiag Bacterial GE+.

Concerning the 13 stools that were expected to be negative, Novodiag Bacterial GE+ detected 1 EAEC and 1 EPEC, which were not confirmed by endpoint PCR.

Prospective study. Among the 53 prospective stool samples, 18 cases (34%) were positive for one or several enteric pathogens using Novodiag Bacterial GE+, whereas only 5 cases (9.4%) (2 *C. jejuni*, 1 *C. coli*, 1 *Salmonella* sp., and 1 *Y. enterocolitica*) were positive by conventional culture. All bacteria species identified by culture were also detected using Novodiag Bacterial GE+. Novodiag Bacterial GE+ detected 4 pathogens that should have been found by conventional culture: 2 *C. jejuni* (confirmed with PCR Rida gene bacterial stool panel) (1 with EPEC as confirmed by endpoint PCR) and 2 *Shigella*/EIEC (confirmed by *ipaH* and *ial* PCR). Novodiag Bacterial GE+ also detected 3 *C. difficile* (confirmed with Alethia *C. difficile*), 2 EHEC (both confirmed by stx_1/stx_2 PCR), 2 EPEC alone (1 confirmed), and 1 EPEC plus EAEC (EPEC confirmed only) (Table 3).

Global study. The analytical process went smoothly for most of the samples. "Invalid" results were obtained on 9 eNAT samples; four contained important traces of blood, and three were too heavily loaded with stools, both situations being conductive to the inhibition of PCR. These problems were solved after retesting (see Materials and Methods). The results for all of the samples included in the present study are summarized in Table 3; 62 of the 251 samples (24.7%) were expected to be negative (based on culture and BD Max extended enteric bacterial panel screening). The 3 main pathogens that comprised this set of samples were first *Campylobacter* sp. (93/251; 37.1%), followed by *Salmonella* sp. (46/251; 18.3%) and pathogenic *E. coli* (37/251; 14.7%). After PCR confirmation of the discordant results obtained using the Novodiag Bacterial GE+ kit, 51 of the 251 remained negative (20.3%); 200 of the 251 were therefore positive (79.7%). Among the 200 positive, 178 were positive for one single pathogen (88.6%) and 22 in combination with 1 to 4 other pathogens (11.4%) (Table 3). Novodiag Bacterial GE+ additionally detected 32 *Campylobacter*, 14 EHEC, and 2 *Shigella* sp./EIEC cases compared to culture only and detected only pathogenic *Y. enterocolitica*. Considering the CRS defined for the present study (see Materials and Methods), Novodiag Bacterial GE+ sensitivity ranged from 98.98% for *Campylobacter* to 100% for the other major enteric pathogens. The specificity fluctuated from 98.08% for ETEC to 100% (Table 4 and 5). Overall agreement (with EPEC, EAEC, and *C. difficile* excluded) was

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TABLE 5 Characteristics of pathogens in this study

Characteristic[a]	Pathogen					
	Campylobacter sp.	EHEC	*Shigella* sp./EIEC	ETEC	*Salmonella* sp.	*Y. enterocolitica*
Sensitivity (% [95% CI])	98.98 (94.45–99.82)	100 (84.98–100)	100 (62.88–100)	100 (74.65–100)	100 (92.29–100)	100 (59.77–100)
Specificity (% [95% CI])	100 (97.60–100)	100 (96.11–100)	100 (98.44–100)	98.08 (93.26–99.47)	100 (98.07–100)	100 (98.44–100)
PPV (% [95% CI])	100 (96.07–100)	100 (84.98–100)	100 (62.88–100)	88.24 (62.26–97.94)	100 (92.29–100)	100 (59.77–100)
NPV (% [95% CI])	99.36 (96.48–99.89)	100 (96.11–100)	100 (98.44–100)	100 (96.37–100)	100 (98.07–100)	100 (98.44–100)

[a]95% confidence intervals were calculated using the Wilson method.

98.80% (95% CI, 96.54 to 99.59%). Concordant percentages between Novodiag Bacterial GE+ were 100% for *C. difficile* (all 6 cases confirmed), 72.2% for EPEC (13 out of 18 cases confirmed), and 50% for EAEC (5 out of 10 cases confirmed).

Demographics and clinical features. The demographic characteristics associated with 128 specimens from patients hospitalized in emergency units of the Bordeaux CHU (74 included in the retrospective stool collection and all patients who were part of the prospective stool collection [$n = 53$]) are presented in Table 6. Those with positive results with Novodiag Bacterial GE+ (confirmed in case of discordance) (92/127; 72.4%) had significantly more fever, diarrhea, abdominal pain, or blood in their feces. Moreover, these patients received probabilistic antibiotic therapy more often (Table 6). In the positive group, the median age was 17 years (±21.6) with a sex ratio of 1.21. In the negative group, the median age was 36.2 years (±34.2) with a sex ratio of 1.33. Among the 93 patients with positive results with Novodiag Bacterial GE+, 11 were positive for a bacterium not detectable by conventional coculture: 4 EPEC, 1 EAEC, 1 EAEC plus EPEC, 2 EHEC, and 3 toxinogenic *C. difficile*. These patients showed characteristics similar to those of other patients in the positive group (data not shown).

DISCUSSION

The diagnosis of acute digestive bacterial infections must be as rapid and precise as possible since a rapid diagnosis conditions the establishment of a targeted therapy at an early stage. Nevertheless, these infections are often underdocumented because conventional diagnostic techniques are long and tedious and require qualified personnel. The use of syndromic PCR panels reduces the mass of analytical work. However, it is necessary to evaluate the analytical performances and the reliability of the resulting diagnoses of the various existing kits. The present study aimed at evaluating the performance of the Novodiag Bacterial GE+ kit.

This study shows that the Novodiag Bacterial GE+ kit has excellent analytical performance to detect bacterial enteropathogens in stools. This new kit showed better sensitivity than culture in accordance with the literature on syndromic molecular diagnosis (15–24). This gain of sensitivity allowed the detection of 2 *Shigella* sp./EIEC and 2 *C. jejuni* from patients with negative culture but suggestive symptoms. Furthermore, Novodiag Bacterial GE+ enabled the detection of the major *E. coli* pathovars, and, in our study, Novodiag Bacterial GE+ performed even better than the BD Max system (25), in particular for ETEC and EHEC detection. Novodiag Bacterial GE+ detects only the pathogenic biotype of *Y. enterocolitica*, which highlights the rationale of the target gene used in this kit. The detection of the major bacterial enteropathogens involved in community-acquired human digestive infections is excellent. The absence of appropriate CRS and the low number of EAEC and EPEC cases did not allow us to properly assess the performance for these pathogens, nor could we evaluate *C. difficile*, which was also beyond the scope of our study, which focused on cases of acute community-acquired digestive infections. If it is deemed necessary, more specialized and targeted studies will allow a future evaluation of this kit regarding these pathogens. Finally, the analysis of clinical features of a set of the patients enrolled in the present study showed the clinical relevance of the Novodiag Bacterial GE+ results.

In the present study, one false-negative *C. jejuni* infection was found. The corresponding sample was positive using the Rida Gene bacterial stool panel with a

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TABLE 6 Clinical features of Bordeaux University Hospital patients

Symptom	Negative group (n = 35) (95% CI)[a]	Positive group (n = 92) (95% CI)[a]	P
Diarrhea	68.6 (52.0–81.5)	98.9 (94.1–99.8)	<0.001[b]
Vomiting	34.3 (20.8–50.9)	41.3 (31.8–51.5)	0.464
Bloody stools	8.6 (3.0–22.4)	44.6 (34,9–54.8)	<0.001[b]
Fever	34.3 (20.8–50.9)	72.8 (62.9–80.8)	<0.001[b]
Biological inflammatory syndrome	60.6 (43.7–75.3)[c]	58.3 (47.6–68.3)[d]	0.835
Rehydration	11.4 (4.5–25.9)	20.7 (13.7–30.1)	0.235
Probabilistic antibiotherapy	37.2 (23.2–53.7)	57.6 (47.4–67.2)	0.038[e]

[a]95% confidence intervals were calculated using the Wilson method.
[b]$P < 0.001$.
[c]Data available for 33 patients.
[d]Data available for 84 patients.
[e]$P < 0.05$.

threshold cycle (C_T) of 31.1 on the primary specimen and 43.6 on the eNAT tube. This deviation corresponds to a 5,000-fold dilution, while, theoretically, it should not exceed 8-fold, thus revealing an overdilution due to a preanalytical error. Accordingly, the dilution in the eNAT tube could, in theory, be associated with a loss of sensitivity and, punctually, the risk of obtaining false-negative results by overdilution. It reveals the importance of mastering preanalytical conditions to obtain interpretable and reliable results. The Novodiag system does not provide amplification curves (or C_T values) or fluorescence intensity values, which could help microbiologists to interpret the results as previously suggested (11), in particular in line with the bacterial inoculum. This also complicates interpretation of the results, particularly in the event of coinfections (11.4% in the present study after confirmation).

The Novodiag Bacterial GE+ can detect EHEC based on the detection of the specific target genes *eae*, stx_1, and stx_2. EHEC infections are sometimes associated with the development of hemolytic uremic syndrome. In France, EHEC strains have a known gene arsenal comprised of *eae* plus stx_2 in 60% of cases (data from the NRC for *E. coli*, Pasteur Institute, France; http://cnr-escherichiacoli-robertdebre.aphp.fr/). However, this profile was found in 2 cases included in the present study. To our knowledge, no EHEC-positive patient developed hemolytic uremic syndrome. Furthermore, in 46.4% of these cases, the Novodiag detected stx_1 only. These different elements illustrate the need for interpretation of these results by the microbiologist in sync with the clinician.

The Novodiag Bacterial GE+ kit allows a reliable result within only 2 h. The use of this type of multiplex PCR implies the exclusive detection of the targets of the proposed panel. *Campylobacter* species other than *C. jejuni* and *C. coli*, *Arcobacter* sp., *Aeromonas* sp., and *P. shigelloides* are undetectable with Novodiag Bacterial GE+. This observation must also be taken into account when developing the strategy for using this kit, in particular with regard to the choice of hospital departments which can prescribe this analysis.

Despite the theoretical advantages of the diagnostic panels compared to standard techniques, their positive consequences on patient care and their economic impact have not been clearly objectified and are extremely variable depending on the populations studied (4–6, 25–28). With a view of the putative implementation of the Novodiag Bacterial GE+ kit at the Bordeaux University Hospital, this study allowed us to target the patient profile for which there would be a benefit. Due to the Novodiag research panel, as discussed above, it is not intended for all hospitalized patients; the aim of the Novodiag Bacterial GE+ kit is to detect the major pathogens responsible for acute gastroenteritis syndromes. In this case, it is of particular interest in community-acquired infections. According to internal data for the year 2018 (data not shown) from the Bordeaux University Hospital, 74% of the enteropathogenic bacteria identifiable by both Novodiag Bacterial GE+ and culture, with the exception of *C. difficile*, came from adult and pediatric emergency and postemergency wards. Therefore, the implementation of the Novodiag technology or any other syndromic PCR in our hospital would

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be more appropriate in terms of benefit for emergency and postemergency wards, especially on all bloody and watery stools. An oriented stool culture strategy, following the results of Novodiag, could be an option for the samples coming from these units.

In conclusion, our study demonstrates the excellent technical performance of the Novodiag kit. This study could serve as a basis for others for the implementation of a syndromic molecular biology technology in terms of workflow adaptation between the prescribing services and the laboratory, all participating, for the abovementioned reasons, in a necessary dialogue between the biologist and the clinician for appropriate use and interpretation, all in the best interest of the patient.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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We declare no conflict of interest. Mobidiag Company (Espoo, Finland), which commercializes the Novodiag Bacterial GE+ test in France, provided the kits but was not involved in the study design or in the data analysis.

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