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Performance of a Multiplex Real-Time PCR Kit for Detection and Differentiation of Enteric Bacterial Pathogens

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Background

Bacterial infections are among the most common causes of infective enteritis worldwide. Stool culture is still commonly performed but it can be cumbersome and may not be sensitive enough to identify the causative pathogen. TaqPath™ Enteric Bacterial Select Panel is a CE-IVD multiplex real-time PCR kit designed for diagnostic testing of most common enteric bacterial pathogens in one reaction: *Campylobacter* (*jejuni, coli, upsaliensis*); *Salmonella spp.* and *Shigella spp./Enteroinvasive E. Coli* (EIEC). This study evaluated the performance of the TaqPath™ Enteric Bacterial Select Panel.

Methods & Results

Preclinical performance evaluation

Contrived samples were created by inoculating cultures of *C. jejuni*, *S. typhimurium* and *S. sonnei* individually into negative stool samples at 2 concentrations (~3-5x limit of detection (LOD) and 10xLOD of the TaqPath™ Enteric Bacterial Select Panel) in 15 replicates. Contrived positive samples and 30 negative samples were tested in parallel using TaqPath™ Enteric Bacterial Select Panel, RIDA®GENE Bacterial Stool Panel I and Allplex™ GI-EB Screening kits. The results of the testing are shown in Table 1.

Table 1. Test performance on contrived samples

Target	TaqPath Enteric Bacterial Select Panel	RIDA GENE Bacterial Stool Panel I	Seegene Allplex GI-EB kit
Campylobacter	100% (30/30)	100% (30/30)	43% (13/30)
Salmonella	97% (29/30)	100% (30/30)	10% (3/30)
Shigella	100% (30/30)	100% (30/30)	100% (30/30)
Negative	100% (30/30)	100% (30/30)	100% (30/30)

Contrived sample analysis demonstrated higher analytical sensitivity of the TaqPath™ Enteric Bacterial Select Panel and RIDA®GENE Bacterial Stool Panel I for *C. jejuni* and *S. typhimurium* compared to Allplex™ GI-EB Screening kit, while all 3 tests showed comparable performance for detection of *S. sonnei*.

Clinical performance evaluation I

In total 407 stool specimens from symptomatic individuals collected in Germany, France and Ivory Coast were tested using TaqPath™ Enteric Bacterial Select Panel and RIDA® GENE Bacterial Stool Panel I and discordant sample resolution was performed using different sets of primers and probes for each target (Figure 1.). Samples with invalid results were excluded (N=12). Study results are summarized in Table 2. Co-infections were detected in 21 samples.

Figure 1. Study design of the clinical performance evaluation I

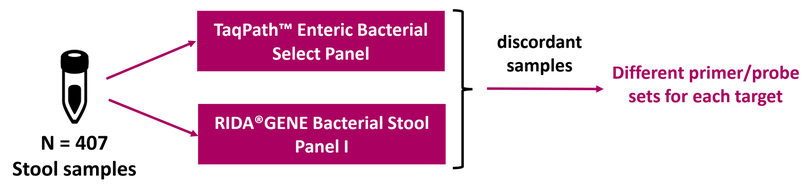


Table 2. Clinical performance of the TaqPath™ Enteric Bacterial Select Panel

		Consensus result								
		Campylobacter spp.			*Salmonella spp.*			*Shigella spp./ EIEC*		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
TaqPath™ Enteric Bacterial Select Panel	Positive	93	0	93	77	7	84	43	0	43
	Negative	2	300	302	0	311	311	0	352	352
	Total	95	300	395	77	318	395	43	352	395
Clinical sensitivity (95% CI)		**98%** (92.6% - 99.7%)			**100%** (95.3% - 100.0%)			**100%** (91.8% - 100.0%)		
Clinical specificity (95% CI)		**100%** (98.8% - 100.0%)			**98%** (95.5% - 99.1%)			**100%** (99.0% - 100.0%)		

Clinical sensitivity of the TaqPath™ Enteric Bacterial Select Panel was 100% for *Salmonella spp.* and *Shigella spp./EIEC*, and 98% for *Campylobacter*; while specificity was 98%, 100% and 100%, respectively.

Clinical performance evaluation II

Testing of 217 stool samples from symptomatic individuals was performed in parallel using the TaqPath™ Enteric Bacterial Select Panel and BD MAX™ Enteric Bacterial Panel (Figure 2.). Two samples were excluded due to invalid results. The result summary is shown in Table 3.

Figure 2. Study design of the clinical performance evaluation II

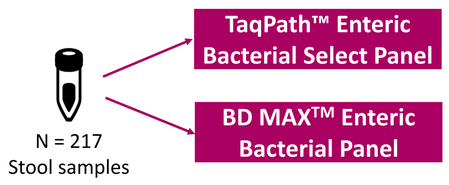


Table 3. Performance comparison of TaqPath™ Enteric Bacterial Select Panel and BD MAX™ Enteric Bacterial Panel

		BD MAX™ Enteric Bacterial Panel								
		Campylobacter spp.			*Salmonella spp.*			*Shigella spp./ EIEC*		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
TaqPath™ Enteric Bacterial Select Panel	Positive	56	5	61	57	1	58	31	1	32
	Negative	0	154	154	1	156	157	0	183	183
	Total	56	159	215	58	157	215	31	184	215
Positive percent agreement (95% CI)		**100%** (93.6% - 100.0%)			**98%** (90.9% - 99.7%)			**100%** (89.0% - 100.0%)		
Negative percent agreement (95% CI)		**97%** (92.9% - 98.6%)			**99%** (96.5% - 99.9%)			**99%** (97.0% - 99.9%)		

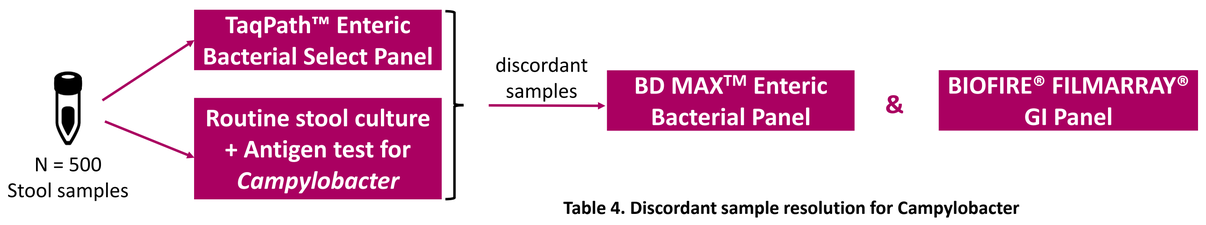
For 3/6 discordant samples routine culture result was available and showed *Campylobacter* positive result in agreement with the TaqPath™ Enteric Bacterial Select Panel. Both molecular tests detected *Campylobacter* in one and *Salmonella* in another sample characterized as negative using routine culture.

TaqPath™ Enteric Bacterial Select Panel shows comparable performance to the BD MAX™ Enteric Bacterial Panel with high positive and negative percent agreement for all 3 targets.

Clinical performance evaluation against routine stool culture

In total 500 stool samples collected from January to March 2023 in Germany from symptomatic patients were tested with TaqPath™ Enteric Bacterial Select Panel and routine stool culture for Salmonella, Shigella and Campylobacter. MALDI-TOF MS (Bruker) was used to identify colonies suspected of Salmonella and Campylobacter. All samples were also tested with the RIDASCREEN® Campylobacter antigen test (R-Biopharm). Shigella spp. was identified by biochemical (api20, api50 CHE, bioMérieux) and serological tests. Discordant samples were tested with 2 resolver methods (Figure 3.).

Figure 3. Study design of the clinical performance evaluation against routine stool culture



Salmonella was detected in 2/500 samples using both routine culture and the TaqPath™ Enteric Panel. *Campylobacter* was detected in 26/500 samples with the TaqPath™ kit, while routine culture detected only 46.1% (12/26) of these infections. The results obtained by the TaqPath™ kit were shown to be true positive for *Campylobacter* using either BD MAX™ Enteric Bacterial Panel or BIOFIRE® FILMARRAY® GI Panel (Table 4.). Four samples showing negative result with BD MAX and culture, but positive with BIOFIRE and TaqPath are likely to represent *C. upsaliensis*.

Table 4. Discordant sample resolution for Campylobacter

Sample ID	TaqPath™ Enteric Bacterial Select Panel		Routine culture result	RIDASCREEN® antigen test	BD MAX™ Enteric Bacterial Panel	BIOFIRE® FILMARRAY® GI Panel
	Camp. Result	Camp. Ct value				
1	Negative	-	Positive	Negative	Negative	N/A ✓
2	Positive	19.36	Negative	Negative	Negative	Positive ✓
3	Positive	26.64	Negative	Negative	Negative	Positive ✓
4	Positive	28.88	Negative	Negative	Negative	Positive ✓
5	Positive	35.81	Negative	Negative	Negative	Positive ✓
6	Positive	25.47	Negative	Negative	Positive	N/A ✓
7	Positive	26.19	Negative	Negative	Positive	N/A ✓
8	Positive	26.91	Negative	Negative	Positive	N/A ✓
9	Positive	28.54	Negative	Negative	Positive	N/A ✓
10	Positive	31.71	Negative	Negative	Positive	N/A ✓
11	Positive	25.88	Negative	Positive	Positive	N/A ✓
12	Positive	22.58	Negative	Positive	Positive	N/A ✓
13	Positive	29.49	Negative	Positive	Positive	N/A ✓
14	Positive	19.05	Negative	Positive	Positive	N/A ✓
15	Positive	23.11	Negative	Positive	Positive	N/A ✓

N/A, results not available as samples were not tested with this method

TaqPath™ Enteric Bacterial Select Panel shows significantly higher clinical sensitivity compared to routine culture for detection of *Campylobacter*, as routine culture testing did not identify >50% of infections.

Conclusion

The TaqPath™ Enteric Bacterial Select Panel is a highly accurate method for quick (<2 hours eluate to result turnaround time) detection and differentiation of most common enteric bacterial pathogens. Compared to culture-based approaches, molecular testing offers higher sensitivity and reduced time-to-result for diagnostic testing of bacterial enteric infections.

CE-IVD In Vitro Diagnostic Use