

Comparison of the BD MAX Enteric Bacterial Panel to Routine Culture Methods for Detection of *Campylobacter*, Enterohemorrhagic *Escherichia coli* (O157), *Salmonella*, and *Shigella* Isolates in Preserved Stool Specimens

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We compared the BD MAX enteric bacterial panel (EBP) to culture for the detection of *Salmonella*, *Shigella*, *Campylobacter*, and Shiga toxin-producing enterohemorrhagic *Escherichia coli* (EHEC) O157 in seeded stool samples. The EBP panel demonstrated superior sensitivity and reliably detected *Salmonella*, EHEC O157, *Shigella*, and *Campylobacter* at concentrations 1- to 2-log₁₀ lower than those needed for culture detection.

The etiologic agents of infectious diarrhea are diverse. The diagnosis of bacterial pathogens is particularly challenging given the large amount of vastly diverse indigenous gastrointestinal flora present in stool (1). There are approximately 10¹¹ bacteria per gram of stool, a population consisting of anaerobes, *Escherichia coli*, *Klebsiella*, *Proteus*, enterococci, and others (2). Individual bacterial populations in stool are very fluctuant, showing changes in response to a variety of environmental cues ranging from antibiotic use to inflammation. Variable amounts of background normal flora can make the isolation of possible pathogens difficult, particularly when these pathogens are shed in small amounts (1, 3). As a result, studies have shown a diagnostic yield of stool culture as low as 1.5%, with a cost per positive culture as high as \$1,200 (4, 5).

Molecular methods can increase sensitivity and specificity compared to stool culture (6). Several real-time PCR assays have been described (7–11). In this study, we evaluated the performance of a real-time PCR assay, the BD MAX enteric bacterial panel (BD Diagnostics, Sparks, MD), which targets *Salmonella* sp., *Campylobacter jejuni* and *Campylobacter coli*, *Shigella* sp., and the *stx*_{1a} and *stx*_{2a} genes in Shiga-toxin-producing *E. coli* and *Shigella dysenteriae*.

Cary Blair-preserved stool samples from clinical patients negative for enteric pathogens by routine stool culture and BD MAX were used to construct pooled stool matrixes for each test organism (four total) and refrigerated at 4°C prior to testing. Test organisms included four unique strains each of *Campylobacter jejuni*, *Salmonella* sp., *Shigella* sp., and enterohemorrhagic *Escherichia coli* (EHEC) O157 (total of 16 strains). These strains were obtained from clinical isolates at a tertiary care medical center between the years of 2009 and 2012. Twelve strain preparations were diluted into negative stool matrixes, achieving concentrations of 1 × 10³ to 1 × 10⁷ CFU/ml (the 4 *Campylobacter* sp. strain dilutions were performed similarly but achieved concentrations of 1 × 10² to 1 × 10⁶ CFU/ml). The chosen organism dilutions best represented the relative abundance of these pathogens in typical clinical samples (typically ranging from 10³ CFU per ml for *Shigella* sp. infections to 10⁶ CFU per ml for *Campylobacter* sp. infections) (12, 13). Prepared concentrations were vortexed to ensure homogeneity. For each test organism, all concentrations were tested in quadru-

plicate using both traditional culture and the BD MAX enteric bacterial panel (EBP).

Stool matrix spiked with *Campylobacter* sp. was cultured to Campy-CVA agar (BD) using a calibrated 10-μl loop, incubated at 42°C using the GasPak EZ Campy system, and screened for gray colonies at 24 to 48 h. Stool matrix spiked with EHEC O157 was cultured to Sorbitol MacConkey (SMAC) agar (Remel) using a calibrated 10-μl loop, incubated in an aerobic atmosphere at 35°C, and screened for colorless colonies at 18 to 24 h. Stool matrixes spiked with *Shigella* sp. and *Salmonella* sp. were cultured to Hektoen enteric (HE) agar (Remel) and xylose lysine deoxycholate (XLD) agar (Remel) using a calibrated 10-μl loop. Plates were incubated in an aerobic atmosphere for 18 to 24 h at 35°C and screened for green colonies with or without black centers on HE agar as well as red colonies with or without black centers on XLD. All colonies identified by screening as presumptive pathogens were confirmed using multiple biochemical and automated tests.

A 10-μl loop was used to transfer the spiked specimen into BD MAX sample buffer tubes, which were vortexed and loaded into the BD MAX instrument along with the BD MAX EBP reagent strip. The automated process (including sample preparation, lysis, DNA extraction, and real-time PCR [RT-PCR]) was initiated and results were available in 3 to 4 h. Results are reported as “negative” or “positive” for each of the following: *Shigella* sp., Shiga-toxin genes, *Campylobacter* sp., or *Salmonella* sp.

Results for all tested organisms are shown in Table 1. At the highest tested concentrations for each organism (1 × 10⁶ CFU/ml for *Campylobacter* sp., 1 × 10⁷ CFU/ml for all others) both the BD MAX and culture had sensitivities of 100%. While sensitivity rates dropped for each method as the concentrations of organisms

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TABLE 1 Comparative sensitivity of culture to the BD MAX EBP assay

Isolate type (from prepared stool samples)	Sensitivity (%) by organism concentration and measurement method									
	10 ⁷ CFU/ml		10 ⁶ CFU/ml		10 ⁵ CFU/ml		10 ⁴ CFU/ml		10 ³ CFU/ml	
	BD MAX	Culture	BD MAX	Culture	BD MAX	Culture	BD MAX	Culture	BD MAX	Culture
<i>Campylobacter</i> ^a	NA ^b	NA ^b	100	100	100	100	100	63.8	100	43.8
EHEC (O157) ^c	100	100	100	75	100	75	87.5	12.5	13.3	0
<i>Salmonella</i> ^d	100	100	100	100	100	81.3	68.8	31.3	43.8	0
<i>Shigella</i> ^d	100	100	100	100	100	75	100	38	81	25

^a At 10² CFU/ml, BD MAX had a 68.8% sensitivity and culture had an 18.8% sensitivity.

^b NA, testing not performed at this concentration.

^c One of 16 samples testing "indeterminate" by BD MAX.

^d Stool culture interpreted as positive when pathogen isolated from either HE or XLD.

dropped, the sensitivity of the BD MAX was always greater than or equal to the sensitivity of culture. At a concentration of 1 × 10³ CFU/ml, the sensitivity of culture ranged from 0% (EHEC and *Salmonella* sp.) to 43.8% (*Campylobacter* sp.). The sensitivity of the BD MAX at this concentration ranged from 13.3% (EHEC) to 100% (*Campylobacter* sp.).

While negative stool matrixes for each test organism were all constructed using the same methodology, they randomly differed in the amounts of indigenous gastrointestinal flora present (data not shown). Cultures from test isolates with large amounts of normal flora background were more difficult to interpret; rare colorless non-lactose-fermenting colonies may have been hidden by heavy growth of fermenting organisms. Cultures with a lesser amount of background stool flora allowed pathogen detection even at the lowest tested concentration (1 × 10³ CFU/ml). BD MAX sensitivities were independent of normal flora background. There was, however, a wide range of sensitivity at the 1 × 10³ CFU/ml concentration for each organism (13.3% for EHEC to 100% for *Campylobacter* sp.). This variability may reflect individual sensitivities of each probe or variable amounts of interfering substances in the stool matrixes.

The detection of DNA-based targets allows the BD MAX to function as both a screening and confirmatory test simultaneously. In contrast, culture-based screens using selective and differential medium require biochemical testing for pathogen confirmation. Time-consuming biochemical work-ups may be performed only to reveal that an organism is normal flora. In this study, approximately 20 to 30% of the samples contained colonies of *Citrobacter* sp., *Proteus* sp., and *Providencia* sp. that were unnecessarily characterized since they produced H₂S, making them difficult to distinguish from *Salmonella* sp. This phenomenon also contributes to the poor sensitivity of culture since the identification of one H₂S-producing colony as normal flora may be used to discount other H₂S-producing colonies as normal flora when actual *Salmonella* sp. colonies are present. Another benefit of nucleic acid amplification tests is the ability to detect low levels of fastidious organisms despite poor growth. This may explain why the BD MAX detected 100% of *Campylobacter* sp. at 1 × 10³ CFU despite a culture detection rate of 43.8%.

While efforts were made to construct similar negative stool samples to spike organisms into, variable differences in quantity and consistency of normal flora background were observed. While these differences allowed us to observe several interesting phenomena (discussed above) efforts could be made to control for these variables in further studies. This

could have been done by performing all testing from aliquots of a single large constructed negative stool sample. It should be kept in mind, though, that patient samples typically vary in normal flora, and a single homogenous specimen may not reflect real-world performance. Another weakness of this study was that all specimens were artificially constructed; therefore it is difficult to make conclusions about clinical utility. Prospective clinical studies are needed to further characterize the BD MAX enteric bacterial panel's clinical sensitivity/specificity in comparison to culture and other nucleic acid amplification tests (NAATs) and to assess the impact on patient care.

Several limitations of molecular assays such as the BD MAX should be kept in mind. Since these tests are based on the detection of specific genetic targets, they are incapable of detecting pathogens for which a target is lacking. Another criticism of molecular assays is that they leave a laboratory without an isolate for susceptibility and epidemiologic testing. Subsequent culture of all positive stools could remedy this, though occasional samples may yield a positive molecular test and negative culture. Despite these limitations, as demonstrated, the BD MAX enteric bacterial panel has a higher sensitivity at low levels of concentration for enteric pathogens compared to culture. These qualities make the BD MAX a useful diagnostic tool and perhaps a possible replacement for culture-based methods.

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The BD MAX enteric bacterial panel is not available in the United States.

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