

# Rapid Detection of *Campylobacter* Antigen by Enzyme Immunoassay Leads to Increased Positivity Rates

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***Campylobacter* antigen detection by enzyme immunoassay (EIA) provides rapid results compared to traditional culture. However, concern exists regarding specificity. Verification studies of an EIA compared to culture revealed a positive predictive value (PPV) of 91%, whereas PPV fell to 42% during routine diagnostic testing. We suggest all positive EIA results be confirmed via culture.**

*Campylobacter* enteritis is a food- and waterborne zoonotic illness and one of the most common causes of infectious diarrhea in the United States (1, 2). While identification of the etiological agent does not typically affect treatment outcomes, as the majority of these infections are self-limited, laboratory diagnosis is essential for epidemiological studies and outbreak tracking through strain identification and typing. Conventional laboratory diagnosis of campylobacteriosis is based on the recovery of the organism from stool specimens by microaerophilic culture. Current recommendations for the recovery of *Campylobacter* stipulate that cultures be held for a minimum of 72 h prior to signing out a negative result (3); however, a recent laboratory surveillance by the Centers for Disease Control and Prevention found that, of laboratories surveyed, 66% reported negative results at 48 h, while only 33% reported negative results after 72 h (4). In contrast to traditional culture methods, more rapid methods for the detection of *Campylobacter* antigens in stool, including enzyme immunoassay (EIA) and lateral flow systems, require only 1 to 2 h until results (5). Herein, we describe the verification and subsequent implementation of a *Campylobacter* EIA at a large university clinical laboratory that serves both acute and tertiary care hospitals and a network of outpatient clinics.

*Campylobacter* cultures were performed on stool specimens transported in C&S medium (Medical Chemical Corporation, Torrance, CA), streaked for isolation on Campy CVA agar plates (BBL, Sparks, MD), and incubated for 48 h in a microaerophilic environment at 42°C. All morphotypes growing on Campy CVA agar plates were Gram stained for microscopic morphology and tested for oxidase reactivity to evaluate for the presence or absence of *Campylobacter* spp. The rate of recovery of *Campylobacter* by culture is tracked through laboratory electronic records. *Campylobacter* EIA was performed using Meridian Biosciences Premier CAMPY (Meridian Biosciences, Cincinnati, OH), per the manufacturer's instructions. Results of EIA testing were read both visually and spectrophotometrically using a dual wavelength of 450/630 nm (ELX800; Biotek Instruments Inc., Winooski, VT). All protocols for this study were reviewed and approved by the UCLA institutional review board.

Prior to implementation of the Premier CAMPY, laboratory verification of the assay's performance characteristics included testing 60 remnant, deidentified stool specimens submitted to the clinical laboratory for *Campylobacter* culture between August and November 2011. *Campylobacter* culture results were recorded prior to freezing (10 positive for *Campylobacter*, 50 negative for

*Campylobacter*) (Table 1), and the specimens were deidentified and stored in C&S at -20°C for further testing by EIA. The EIA demonstrated 98% concordance, 98% specificity, and 98% sensitivity compared to the culture results, similar to what was previously reported (5). One discordant sample was found to be positive by EIA but negative by culture. Chart review revealed gastroenteritis-like symptoms, including diarrhea with abdominal pain, fever, and nausea, which is consistent with *Campylobacter* infection. Furthermore, culture results for both *Salmonella* and *Shigella* were negative, suggesting the causative agent may have been *Campylobacter*. Based on these results, the laboratory replaced traditional culture for *Campylobacter* with the EIA for routine testing.

In the first week of clinical testing with the EIA, a positivity rate of 11.3% ( $n = 9/75$  specimens; 6/9 were outpatients) was observed, in stark contrast to the 1.7% positivity observed by culture in the previous 4 weeks. Although an increased number of positive stools were expected (due to the increased sensitivity of the EIA compared to that of culture) (5), this increase was higher than what others have reported when converting to the EIA (5, 6). Further, three clinicians caring for different patients voiced concern of false-positive *Campylobacter* EIA results to the laboratory director. All three patients were on the pediatric transplant service and had no risk factors for *Campylobacter* exposure; two of these patients were on total parenteral nutrition. At this point, the laboratory returned to *Campylobacter* cultures for clinical testing in order to further investigate the specificity of the EIA.

Stool samples submitted for *Campylobacter* culture ( $n = 119$ ) in the subsequent 2 weeks were stored at -20°C and batch tested by the EIA. To ensure that the EIA performed consistently between users, batch testing was divided equally among the five clinical laboratory scientists who typically perform *Campylobacter* testing. In order to resolve discrepant results, 16S rRNA gene sequence analysis (7) was performed on isolates recovered from

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TABLE 1 Accuracy of study results for Premier CAMPY EIA compared to those for the reference method

Study	EIA result	No. of samples		% (range)			
		Culture positive	Culture negative	Sensitivity	Specificity	PPV	NPV
Initial verification study	Positive	10	1	100 (66–100)	98 (88–100)	91 (57–100)	100 (91–100)
	Negative	0	49				
Study following implementation <sup>a</sup>	Positive	3	4	75 (22–98)	96.5 (92–99)	42.9 (12–80)	99 (94–100)
	Negative	1	111				

<sup>a</sup> Discrepant results resolved by PCR analysis.

specimens that were EIA negative, and culture-negative, EIA-positive samples and a random sample of culture/EIA concordant samples ( $n = 12$ ) were sent to the Wadsworth Center (New York State Department of Health) for PCR analysis.

Over the 2 weeks, 5.98% of the EIA results were positive ( $n = 7$ ), whereas 2.6% ( $n = 3$ ) of culture results were positive. Four samples negative by culture but positive by EIA tested negative by PCR, indicating 3.4% false-positive (FP) results by the EIA. Patients with FP results ranged in age from 55 to 89 years, and 75% were from an outpatient setting (3 of 4). No enteric pathogens were identified via aerobic bacterial stool culture for any of these samples; however, other uncommon *Campylobacter* species causing gastroenteritis cannot be ruled out. The single EIA-negative, culture-positive isolate was determined to be a *Campylobacter jejuni*/*Campylobacter coli* strain with 98.6% identity by MicroSeq sequencing of the 16S rRNA subunit, suggesting that this was a false negative (FN) by EIA; however, since only 500 bp are sequenced, the possibility of the isolate being *Campylobacter lari* could not be ruled out (8). Importantly, the positive predictive value (PPV) of the EIA went from 91% (95% confidence interval [CI], 57 to 100%) during initial evaluation to 43% (95% CI, 42 to 80%) in the subsequent studies (Table 1). Negative predictive values (NPV) remained high, at 99 to 100% (Table 1).

Analysis of the positivity rates pre- and post-EIA implementation show *Campylobacter* prevalence to be 1.74% in the 4 weeks preceding EIA testing and 1.71% in the 4 weeks following EIA testing, suggesting that the increased positivity rate was not due to an increased number of positives but rather an increase in FP reads by the EIA. Likewise, in our small sample set of 117 samples where culture results were reported and EIA was batch tested, 3 positives

were reported via culture and 5 positives were reported via EIA, for a 2.6 and 4.3% positivity rate, respectively. While these numbers are small, the *in vivo* testing of the Premier EIA in our laboratory suggests that EIA should not be used to replace traditional culture methods but may function as a screening tool in high-volume laboratories.

The discrepant results of the EIA between the initial verification and the subsequent clinical testing are disconcerting. The original small sampling size ( $n = 60$ ) of the verification assay may have contributed to the variance between the testing. Determining the number of specimens to include in a verification study is a challenge faced by all clinical laboratories, and this report highlights the necessity for a thorough verification, which includes review of all discrepant results and careful evaluation of patient demographics included in the assay's clinical trials for FDA approval. For the EIA, FDA performance claims were based entirely on outpatient specimens, whereas specimens received in our laboratory encompass both outpatients and many inpatients with complicated medical histories. As such, a more thorough verification of the assay's performance for patients not included in the FDA trials, such as inpatients on TPN, could have been performed. Many laboratories perform verifications based on convenience sampling rather than on an informed cross-section of patients, and results such as observed herein are the risk of such limited verification studies. In addition, inappropriate inpatient testing for gastroenteritis seems to have a role in the false positivity rate, as three of our initial cases that gave a positive EIA result were from patients hospitalized for greater than 72 h. Reeducation of hospital house staff on the importance of pretest predictive value was performed following completion of this study.

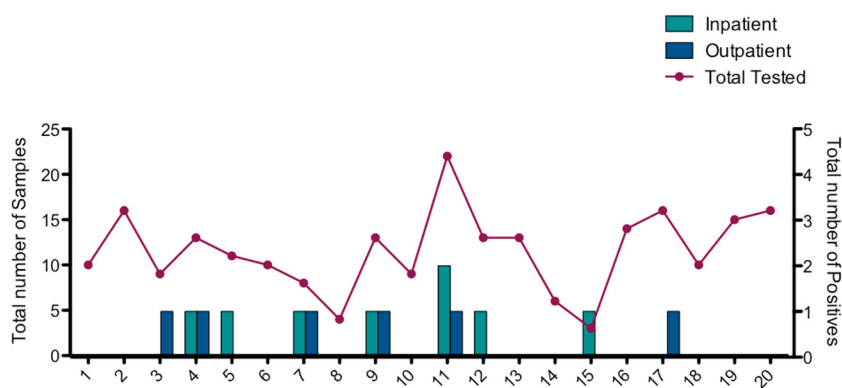
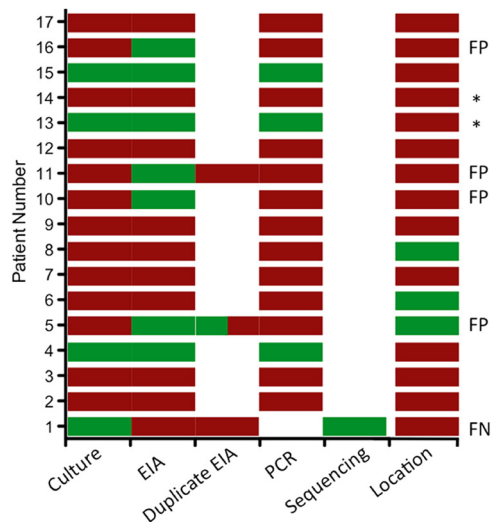


FIG 1 *Campylobacter*-positive samples during *Campylobacter* EIA testing. The total number of specimens tested is compared to the total number of positive *Campylobacter* EIA specimens. Positive specimens were plotted by patient location; light blue bars represent number of positive *Campylobacter* samples from inpatients, while dark blue bars represent positive samples from outpatients.



**FIG 2** Comparison of culture and EIA results with PCR as an arbitrator test. On 20 April 2012, EIA testing was replaced by culture to report patient results. All stool samples received between 16 and 27 April were collected and batch tested via EIA. All positive culture or EIA specimens and a random sampling of samples negative for both culture and EIA were sent to the Wadsworth Center for verification via PCR. Four FP and 1 FN were observed. Colored bars represent testing performed: red bars represent a negative test result (or outpatient for location), green represents a positive result (or inpatient for location), while a white box represents test not performed. \*, pediatric patients.

Ultimately, our initial verification results were similar to those previously published on the *Campylobacter* EIAs and lateral flow systems (5). Our results show that while the EIA performed well in our initial verification study, *in vivo* clinical testing demonstrated a high number of FP (Fig. 1 and 2). The *Campylobacter* EIAs provide a rapid alternative to laboratory culture for identification of

*C. jejuni* and *C. coli*, however, laboratories are urged to verify positive results with culture.

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