Revisiting the roles of culture and culture-independent detection tests for *Campylobacter*.

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Abstract:

Culture-independent detection testing (CIDT) for *Campylobacter* has become an area of intense controversy and confusion among laboratorians in the field of clinical microbiology. To date, the true analytical and clinical performance of stool antigen CIDTs versus truly optimized culture conditions is unknown. In this issue of *Journal of Clinical Microbiology* (C. Fitzgerald, colleagues, and the campylobacter diagnostics study working group, J. Clin Microbiol XX: YYY-ZZZ, 2016, URL) report comprehensive performance data for four *Campylobacter* stool antigen CIDTs versus culture and molecular diagnostics.

Gastrointestinal infections attributable to *Campylobacter* spp. represent a significant challenge to human health as well as the food safety industry. *Campylobacter jejuni* and *Campylobacter coli* (to a lesser extent) collectively account for the majority of cases of campylobacteriosis in the United States with estimates upward of 1.3 million cases per year (1). *Salmonella* has historically been considered the most prevalent enteric bacterial pathogen, however in recent years *Campylobacter* infections have approached *Salmonella* in disease prevalence based on public health reporting data (2). Unlike *Salmonella*, culture for *Campylobacter* has been shown by multiple studies to be insensitive and poorly standardized across laboratories in the United States (3, 4). This suboptimal sensitivity may also suggest that the true incidence of campylobacteriosis is higher than is currently reported based on existing culture practices and the use of stool antigen culture-independent diagnostic tests (CIDTs).
CIDTs that can directly detect *Campylobacter* antigen from preserved stool samples have been commercially available in the United States for over a decade. The first tests were cleared by the US Food and Drug Administration (FDA) in enzyme-linked immunosorbent assay (ELISA) format, with a rapid immunochromatographic assay (ICA) format gaining FDA clearance in 2009. These assays allow laboratories to significantly shorten the turn-around-time (TAT) for *Campylobacter* detection from greater than 72 hours to less than 24 hours; or in the case of ICA tests, less than an hour. The rapidity of which test results are available has potential significance for clinical microbiology laboratories that are under growing pressure to provide more rapid test results, with less available workforce; these tests however must generate clinically meaningful results. A significant challenge with *Campylobacter* stool antigen CIDTs has been the lack of consistent results in published studies. While some studies have described excellent sensitivity and specificity versus conventional *Campylobacter* culture, sometimes with enhanced sensitivity compared to culture (5, 6), others have reported poor specificity and variable sensitivity (7-10). Still others have shown that these assays potentially have broader detection within the *Campylobacter* genus than what the manufacturers have indicated in their instructions for use (further complicating the true analytical performance) (9-12). This collective body of work has culminated into a quagmire of conflicting/inconsistent data and even contentious opinions as to the best use of these assays (if any) for clinical care in the diagnosis and reporting of campylobacteriosis. Currently the public health reporting criteria for a confirmed case of campylobacteriosis is contingent upon recovery of an isolate from stool culture; with CIDTs only serving as supportive evidence for a probable case (http://wwwn.cdc.gov/nndss/conditions/campylobacteriosis/case-definition/2015/).

With increasing adoption of CIDTs for *Campylobacter* there are lingering concerns as to how these results will be used in the clinical microbiology laboratory, whether isolates will cease to be provided for public health investigations due to cessation of culture, whether the lack of inclusion of CIDTs in the case definition will result in decreased case reporting, and most importantly whether the stool antigen CIDTs
in particular are truly providing a better alternative to conventional culture (both analytically and clinically).

In this issue of *Journal of Clinical Microbiology*, we highlight an ambitious, prospective eight-center study of stool antigen CIDTs for *Campylobacter* lead by Fitzgerald and colleagues in the *Campylobacter* diagnostics study working group. This study not only provides a comprehensive multi-center evaluation of four commercial *Campylobacter* stool antigen CIDTs, but it also evaluated multiple conventional *Campylobacter* culture media, including Campy CVA, which has been adopted by many labs in the United States but for which comprehensive comparison data to other *Campylobacter* culture media is absent from the body of published literature. The study was conducted at eight independent centers located across various regions of the United States and included academic and non-academic medical centers, state and county public health laboratories, and the Centers for Disease Control and Prevention. A total of 2767 stool samples from patients with gastrointestinal illness were tested with four different commercial *Campylobacter* selective culture media, four commercial stool antigen CIDTs (three FDA cleared), and one PCR specific for *Campylobacter jejuni/coli* (not FDA cleared).

There are two major findings of this study that have broad implications for *Campylobacter* detection. The first is demonstrating enhanced culture recovery of *Campylobacter jejuni/coli* by using two specific culture media [Campy cefoperazone, vancomycin, amphotericin B (CVA) and modified cefoperazone charcoal deoxycholate agar (mCCDA)]. Of the various *Campylobacter* selective media used, more isolates were recovered with the combination of mCCDA and CVA media than any single medium or other combinations of media. CVA is the second most commonly used *Campylobacter* selective media in the United States (3), but it recovered 5% fewer *C. jejuni/C. coli* isolates than using mCCDA and CVA together. This is an important and easily addressable diagnostic gap to highlight with current stool culture practices for *Campylobacter* in the United States.
The second major finding and the primary focus of this study was demonstrating that stool antigen CIDTs have highly variable sensitivity, specificity, and positive predictive value (PPV) in a low prevalence setting when mCCDA and Campy CVA combination culture is used as the reference culture method. True cases in this study were defined based on specific laboratory test combinations including any culture positive or positive by a single stool antigen CIDT and PCR. Non-cases were defined as illnesses in which specimens were positive by only stool antigen tests or PCR but not both. Using these definitions, of 202 non-cases that were positive by at least one CIDT, only one sample was positive by all four stool antigen CIDTs and PCR, but negative by culture. Furthermore, 75% of these non-cases were positive by only one CIDT and negative by all other methods (antigen tests, cultures, and PCR), suggesting very poor analytical specificity for the stool antigen CIDTs. These cases were further qualified by the fact that non-cases were less likely to have clinically compatible symptoms to support a diagnosis of campylobacteriosis, also calling into question the clinical specificity of these assays.

Taken together, despite reasonably high specificity (greater than 95%), the positive predictive value of three of the stool antigen CIDTs ranged only from 36-51% (slightly better than a flip of a coin, at best), while the negative predictive values (NPV) all exceeded 99% as would be expected in such low prevalence settings. The assay with the lowest PPV was an ICA assay, which is ironically the assay with the broadest superficial appeal to clinical microbiology laboratories (rapid TAT, no sample batching, simple to perform) particularly when compared to an ELISA.

Though cross-reactivity with other Campylobacter species has been reported for stool antigen CIDTs previously, the authors did not investigate this aspect of the analytical specificity of the assays (9-12). Nonetheless it is important to consider that “non-cases” were typically not clinically compatible with the positive stool antigen CIDT results, which argues against the idea that false positive results are largely attributable to pathogenic Campylobacter spp. such as C. upsaliensis. While reactivity with C. upsaliensis occurs (one such instance was detected in this study), this likely does not explain the
multitude of perceived false positives in the study. The authors appropriately conclude that stool antigen CIDTs do not perform adequately to serve as a stand-alone diagnostic tool for campylobacteriosis, a valid conclusion which echoes other investigators in Europe (7).

This is an important conclusion that deserves discussion since these data would not support the laboratory practice of replacing Campylobacter culture with stool antigen CIDTs entirely. Laboratories that have adopted stool antigen CIDTs as their sole detection tool for Campylobacter spp. should revisit this practice in their institution in light of these data. One approach for laboratories that are intent on reducing time-consuming, low-yield Campylobacter cultures may be instead to consider screening with stool antigen CIDTs, and reflexing only the positives to culture as has been advocated previously (7). This approach is not without significant limitation; despite the high NPV, the sensitivity of the stool antigen CIDTs versus both culture and case definition were below 90% in this study, meaning even in a low prevalence setting, some Campylobacter-positive specimens will not be detected. These overall troubling test characteristics paired with growing institutional pressures to reduce TAT and perform more testing with fewer employees leads to significant confusion and uncertainty for clinical laboratories in determining the most appropriate choice for testing for Campylobacter.

A final point discussed by the authors of significance for the future of this discipline is the availability of molecular tests for detecting enteric bacterial pathogens. At the time that this study was conducted (2010), there were no commercial PCR assays for Campylobacter cleared by the FDA. As of the time this commentary was written (2016), five molecular methods for the detection of Campylobacter had been cleared by the FDA for in vitro diagnostic use. Early studies have shown increased detection of C. jejuni/C. coli versus culture, however even these methods have been plagued with perceived imperfect specificity (e.g. false positives) possibly due to; the imperfection of culture as a reference method, cross-reactivity with other nucleic acid targets, or a function of the lack of a standardized molecular method for resolution testing. These assays may or may not detect (but do not
differentiate) other closely related pathogenic Campylobacter species including C. upsaliensis or C. lari (manufacturer design dependent). Once again, this is an issue that will require additional investigation to clarify the significance of this broader detection capability versus more restrictive conventional cultures. The incidence of false negatives in early studies conversely was extremely low, which likely represents the most significant improvement over stool antigen CIDTs.

As this fledgling field evolves and increasingly more laboratories adopt molecular detection for Campylobacter, we stand to face similar concerns as those perceived for stool antigen CIDTs; namely a lack of culture isolates for public health utilization/outbreak investigation and increased “positive” specimens that do not correlate with conventional culture methods. Several early studies with molecular CIDTs have described excellent sensitivity and specificity versus traditional culture; however as we have already discussed, the same was initially reported for stool antigen CIDTs. Studies such as this work should be replicated using molecular methods to investigate the utility of this next generation of Campylobacter detection in real-time using the CVA-mCCDA two-media culture standard described in this study.

Specific, data-driven, best practice guidelines for Campylobacter detection would be invaluable to clinical microbiology laboratories in order to make informed decisions as to the most appropriate use of Campylobacter diagnostics. Such a document is currently in preparation by the CDC’s Campylobacter Best Practices Working Group. Questions that could be addressed include: Should CVA and mCCDA both be routinely used for Campylobacter cultures? How should stool antigen testing for Campylobacter be implemented if at all? What role do molecular CIDTs have in the diagnosis of Campylobacter? How do CIDTs practically fit into the case definitions for campylobacteriosis?

Despite some outstanding questions, this study readily serves as an important foundation for understanding the limitations of stool antigen CIDTs for Campylobacter, forces us to rethink the
inadequacy of our culture-based procedures, and opens the figurative door for the next step of investigation into molecular testing methods for Campylobacter.


