Changing Diagnostic Testing Practices for Foodborne Pathogens, Foodborne Diseases

Active Surveillance Network, 2012–2019

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Diagnostic Testing for Foodborne Illness

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Abstract

**Background.** Pathogen detection has changed with increased use of culture-independent diagnostic tests (CIDTs). CIDTs do not yield isolates, which are necessary to detect outbreaks using whole-genome sequencing. The Foodborne Diseases Active Surveillance Network (FoodNet) monitors clinical laboratory testing practices to improve interpretation of surveillance data and assess availability of isolates. We describe changes in practices over eight years.

**Methods.** During 2012–2019, 10 FoodNet sites collected standardized data about practices in clinical laboratories (range, 664–723 laboratories) for select enteric pathogens. We assessed changes in practices.

**Results.** During 2012–2019, the percentage of laboratories that used only culture methods decreased, with largest declines for *Vibrio* (99% to 57%) and *Yersinia* (99% to 60%). During 2019, the percentage of laboratories using only CIDTs was highest for Shiga toxin-producing *Escherichia coli* (43%), *Campylobacter* (34%), and *Vibrio* (34%). From 2015 to 2019, the percentage of laboratories that performed reflex culture after a positive CIDT decreased, with largest declines for *Shigella* (75% to 42%) and *Salmonella* (70% to 38%). The percentage of laboratories that routinely submitted isolates to a public health laboratory decreased for all bacterial pathogens examined from 2015 to 2019.

**Conclusions.** By increasing use of CIDTs and decreasing reflex culture, clinical laboratories have transferred the burden of isolate recovery to public health laboratories. Until technologies allow for molecular subtyping directly from a patient specimen, state public health laboratories should consider updating enteric disease reporting requirements to include submission of isolates or specimens. Public health laboratories need resources for isolate recovery.
Background

Public health enteric infectious disease surveillance relies on clinical laboratories to submit isolates to state public health laboratories (SPHLs), which conduct whole-genome sequencing (WGS) to determine serotype, subtypes, and antimicrobial resistance, and use this information to detect and investigate outbreaks and sporadic cases. To detect these infections, laboratories are increasingly using culture-independent diagnostic tests (CIDTs), which do not yield isolates [1]. CIDTs include both nucleic acid amplification tests and antigen-based tests; some may offer increased sensitivity and provide results more rapidly than culture-based methods [2-4]. Syndromic panels are CIDTs that test for several pathogens; these offer the advantage of simultaneous testing for multiple bacterial, viral, and parasitic pathogens from a single specimen [5].

Complicating the interpretation of CIDT results is that sensitivity and specificity may differ among these assays, and that they might detect genetic material being shed following resolution of an infection rather than the live pathogen. Monitoring the adoption of changing laboratory testing practices can improve the understanding of enteric pathogen disease trends and burden, and can help in assessing the effectiveness of public health interventions [6].

The Foodborne Diseases Active Surveillance Network (FoodNet) is a collaboration among CDC, 10 state health departments, the U.S. Department of Agriculture’s Food Safety and Inspection Service, and the Food and Drug Administration. FoodNet personnel conduct active, population-based surveillance for laboratory-diagnosed infections caused by Campylobacter, Cryptosporidium (until 2018), Cyclospora, Listeria, Salmonella, Shiga toxin-producing...
Escherichia coli (STEC), Shigella, Vibrio, and Yersinia in 10 sites that account for approximately 15% of the U.S. population. In 2012, FoodNet staff began routinely asking clinical laboratories about diagnostic testing practices to provide context for disease surveillance. We describe changes in laboratory testing practices for pathogens captured by FoodNet surveillance from 2012 to 2019.

Methods
We collected standardized data elements for diagnostic laboratory practices specific to individual pathogens from hospital and reference laboratories by phone, email, and in person for the previous six months. Collection methods differed by site. For laboratories unresponsive to requests, we monitored for changes, and we assumed no change in laboratory practices from the previous year if we did not detect meaningful changes in reporting or in submission of specimens to the public health laboratory. Yearly metrics were not kept about laboratories unresponsive to requests; site personnel estimated that, on average, 5% (range 0–20%) were unresponsive to requests from year to year; most unresponsive laboratories did not test for enteric pathogens. State health officials submitted data to CDC based on pre-determined cycles. During 2012–2019, we conducted 13 noncontiguous cycles and analyzed data from the cycle that represented the most complete data for a specific year. We collected data for 2012, 2014–2017, and 2019 during April–August. For 2013 we collected data from December 2013 to February 2014, and data for 2018 from October 2018 to January 2019.

We collected data for Campylobacter, Listeria, Salmonella, STEC, Shigella, Vibrio, and Yersinia during all years, for Cryptosporidium data during 2012–2017, and for Cyclospora during 2018–
2019. We collected information on reflex culture and specimen submission practices for bacterial pathogens during 2015–2019. Data collected for *Listeria* were specific to its detection from blood and cerebrospinal fluid (CSF) specimens, and specific to stool specimens for all other pathogens. Practices for STEC were specific to culture methods to detect *E. coli* O157 on differential and selective media, and CIDTs that detect Shiga toxin, O157 antigen, or their gene targets from stool or broth samples.

We defined syndromic panels as FDA-approved polymerase chain reaction-based panels with more than five targets designed to diagnose enteric infections. Most syndromic panels are designed to detect enteric pathogens from stool samples, with the exception of *Listeria*. *Listeria* is included in syndromic panels designed to identify pathogens typically isolated from CSF specimens and blood culture. Laboratory-developed tests (LDTs) are validated and controlled by a single laboratory, and often use commercially available reagents. Syndromic panel tests and LDTs are types of CIDT tests. We defined reflex culture as a culture done in response to a positive CIDT result.

We calculated rate ratios (RRs), 95% confidence intervals (CI), and p-values using SAS software version 9.4 (SAS Institute, Cary, North Carolina), and determined statistical significance (p <0.05) using the 2-tailed Cochran-Mantel Haenszel $\chi^2$ test. Percentages were calculated among non-missing values for pathogen-specific questions. Data are summarized by pathogen based on the number of laboratories that reported testing for the pathogen each year.
**Results**

During 2012–2019, sites received data annually from between 664 and 723 clinical laboratories serving the FoodNet catchment area. Some were in outpatient practice settings and hospitals; others were commercial reference laboratories. Most tested for *Campylobacter*, *Salmonella*, *Shigella*, and STEC (Table 1). Although testing practices varied by pathogen and year, most laboratories began adopting CIDTs in 2015, most often PCR-based syndromic panels, and increased this use in subsequent years, expanding to all enteric pathogens (Figures 1 and 2). Increased use of CIDTs corresponded with fewer laboratories attempting reflex culture, and fewer submissions of isolates to SPHLs; now many laboratories send broth or stool specimens to SPHLs (Figures 3 and 4).

**Bacteria**

*Campylobacter*

The number of laboratories using any method (culture-based, CIDT, or both) to test stool specimens for *Campylobacter* decreased significantly during 2019 compared with 2012 (RR = 0.82 [95% CI 0.71–0.94]) (Table 1). Among laboratories reporting test type, the percentage (and number) identifying *Campylobacter* by only culture methods decreased from 89% (402) in 2012 to 53% (202) in 2019, whereas those using CIDT alone or in combination with culture increased from 11% (49) in 2012 to 47% (181) in 2019 (Figure 1). During 2012, antigen tests (96%) were the most common type of CIDT reported (Figure 2). By 2019, 72% used syndromic panels. Few laboratories (22% [13]) performed reflex culture for *Campylobacter* during 2015 and that proportion had further decreased by 2019 (13% [24]) (Figure 3), despite more laboratories using CIDTs. During 2015 to 2019, the percentage of laboratories routinely submitting *Campylobacter*
isolates to SPHLs decreased from 55% to 48%, and the percentage routinely submitting stool specimens increased from 9% to 33% (Figure 4).

**Listeria**

The number of laboratories able to test blood and CSF specimens for *Listeria* increased significantly during 2019 compared with 2012 (RR = 1.34 [95% CI 1.13–1.60]) (Table 1). During 2012, culture was the only method (Figure 1). During 2019, 29% (87) of laboratories reported using a CIDT with gene targets for *Listeria*. Most using CIDT (78% [63]) performed the test with culture, 10% (8) performed reflex culture following a positive CIDT, and 12% (10) used CIDT alone. Among laboratories that used CIDTs, 60% (49) used a syndromic panel designed to identify *Listeria* from blood culture specimens, 27% (22) used a syndromic panel designed to identify *Listeria* from CSF specimens, 10% (8) used both panel types, and 4% (3) used a laboratory-developed PCR test. During 2019, most laboratories (92% [274]) routinely submitted *Listeria* isolates to SPHLs, 3% (9) submitted only specimens, and 3% (10) reported not routinely submitting specimens or isolates.

**Salmonella**

The number of laboratories testing for *Salmonella* decreased significantly during 2019 compared with 2012 (RR = 0.81 [95% CI 0.71–0.93]) (Table 1). Among laboratories reporting test type, the percentage (and number) that identified *Salmonella* by only culture methods decreased from 98% (452) in 2012 to 64% (252) in 2019 whereas those using CIDT alone or in combination with culture methods increased from 2% (8) in 2012 to 36% (141) in 2019 (Figure 1). Among laboratories using CIDTs, the percentage using syndromic panels increased from 50% (3) in
2013 to 98% (137) in 2019. Most laboratories (70%) reported performing reflex culture during
2015; by 2019 this percentage had decreased to 38% (Figure 3). During 2015 to 2019, the
percentage of laboratories routinely submitting Salmonella isolates to SPHLs decreased from
93% (330) to 81% (312), and the number routinely submitting stool specimens increased from
3% (10) to 34% (130) (Figure 4).

**Shigella**

The number of laboratories testing for Shigella decreased significantly during 2019 compared
with 2012 (RR = 0.82 [95% CI 0.71–0.93]) (Table 1). Among laboratories reporting test type,
the percentage (and number) that identified Shigella only by culture methods decreased from
98% (451) laboratories in 2012 to 64% (253) in 2019 whereas those using CIDT alone or in
combination with culture methods increased from 2% (9) in 2012 to 36% (140) in 2019 (Figure
1). Among laboratories using CIDTs, the percentage using syndromic panels increased from 60%
(3) in 2013 to 98% (136) in 2019. Most laboratories (75%) reported performing reflex culture
during 2015; during 2019, this decreased to 42% (Figure 3). During 2015 to 2019, the percentage
of laboratories routinely submitting Shigella isolates to SPHLs decreased from 89% (314) to
79% (297), and the percentage routinely submitting stool specimens increased from 3% (9) to
34% (128) (Figure 4).

**Shiga toxin-producing Escherichia coli (STEC)**

The number of laboratories testing for STEC decreased significantly during 2019 compared with
2012 (RR = 0.83 [95% CI 0.72–0.96]) (Table 1). Among laboratories reporting test type, the
percentage (and number) using culture methods alone to identify Escherichia coli O157
decreased from 45% (183) in 2012 to 14% (50) in 2019 whereas the percentage using CIDTs in combination with culture methods to detect O157 or Shiga toxin increased from 37% (149) in 2012 to 43% (153) in 2019 (Figure 1). Laboratories using CIDTs alone to detect O157 or Shiga toxin increased from 18% (74) in 2012 to 43% (152) in 2019. During 2012, most laboratories (99%) using CIDTs reported using an antigen-based test, whereas during 2019 just 54% used antigen-based tests, 36% used syndromic panels, and 10% used both methods (Figure 2). During 2015 to 2019, the percentage of laboratories routinely submitting STEC isolates to SPHLs decreased from 64% (199) to 59% (205), and the percentage routinely submitting stool specimens increased from 16% (50) to 47% (162) (Figure 4).

**Vibrio**

From 2012 to 2019, the number of laboratories testing for *Vibrio* declined, but the change was not statistically significant (RR = 0.90 [95% CI 0.77–1.05]) (Table 1). Among laboratories reporting test type, the percentage (and number) that identified *Vibrio* by only culture methods decreased from 99% (319) in 2012 to 57% (164) in 2019, whereas the percentage using CIDTs alone or in combination with culture methods increased from 1% (3) in 2012 to 43% (126) in 2019 (Figure 1). LDTs were the only type of CIDT reported during 2012–2014. By 2019, among laboratories using CIDTs, 98% (124) used syndromic panels. Most laboratories (69%) did not perform reflex culture during 2015, and even fewer did during 2019 (26% [32]) (Figure 3). During 2015 to 2019, the percentage of laboratories routinely submitting *Vibrio* isolates to SPHLs decreased from 84% (215) to 71% (208), and the percentage routinely submitting stool specimens increased from 5% (12) to 41% (118) (Figure 4).
Yersinia

The number of laboratories testing for Yersinia decreased significantly during 2019 compared with 2012 (RR = 0.82 [95% CI 0.70–0.95]) (Table 1). Among laboratories reporting test type, the percentage (and number) that identified Yersinia by only culture methods decreased from 99% (368) in 2012 to 60% (185) in 2019, whereas the percentage using CIDTs alone or in combination with culture methods increased from 1% (2) in 2012 to 40% (122) in 2019 (Figure 1). LDTs were the only type of CIDT reported during 2012–2014. By 2019, among the 122 laboratories using CIDTs, all used syndromic panels. Most (67%) did not perform reflex culture during 2015 and fewer (25%) did in 2019 (Figure 3). During 2015 to 2019, the percentage of laboratories routinely submitting Yersinia isolates to SPHLs decreased from 74% (208) to 72% (221), and the percentage routinely submitting stool specimens increased from 4% (11) to 37% (115) (Figure 4).

Parasites

Cryptosporidium

From 2012 to 2017, the last year FoodNet conducted surveillance for Cryptosporidium, the number of laboratories testing for it was similar. Most laboratories used a single test type during 2012 (84% [211]) to 2017 (83% [221]). During 2012, the most common test used was an antigen test (70% [176]), followed by microscopy (45% [112]), and LDT (1% [2]). During 2017, antigen tests (62% [166]) and microscopy (31% [82]) were still the most common methods; however, 26% (68) of laboratories reported using a PCR test. Use of syndromic panels increased from 1% (2) in 2013 to 24% (65) in 2017.
During 2018 and 2019, the years *Cyclospora* data were collected, the number of laboratories testing for it was similar. On average, 62% of laboratories included tests for *Cyclospora* as part of routine enteric screening; the most common was a syndromic panel (87%). Among those not including *Cyclospora* in routine enteric screening, 10% of laboratories tested for it as part of ova and parasite testing, and 29% performed testing on clinician request; microscopy (63%) was the most common method used.

**Discussion**

The increased adoption of CIDTs continues to alter the landscape of enteric pathogen detection and affect the interpretation of surveillance data. Beginning in 2013, following the introduction of FDA-approved syndromic panels, clinical laboratories began shifting from culture-based methods to CIDTs for enteric bacterial and parasitic pathogens [7]. This shift largely correlates with an increase in infections diagnosed in FoodNet sites from 2013 through 2019 [1, 4, 8-11].

During 2012–2019, the number of clinical laboratories testing for enteric bacterial pathogens decreased, which might be due in part to laboratory mergers, acquisitions, and outsourcing [12]. Changes differed by pathogen. Decreases in clinical laboratory testing were greatest for *Campylobacter, Salmonella, and Shigella*, which had previously been the pathogens most often included in the routine culture of stool specimens [13]. Conversely, more laboratories indicated the ability to identify *Listeria* in blood and CSF samples in 2019 than in previous years.
Since 1995, clinical laboratories have increasingly adopted CIDTs that detect Shiga toxins produced by STEC [14]. Although E. coli O157 is readily identified by culture using differential and selective media, there is not a selective agar for detecting non-O157 STEC strains. In 2011, the rate of non-O157 infections surpassed that of O157 infections, and this was strongly correlated with increases in CIDT use [15]. During 2012–2019, we observed that many laboratories were discontinuing culture methods for STEC and increasingly adopting syndromic panels. The use of CIDTs to detect STEC may improve the timeliness of STEC diagnoses. Prompt and accurate diagnosis of STEC infections, especially those caused by E. coli O157, can decrease the risk for serious complications and outcomes, such as renal damage and death. However, for accurate diagnosis of STEC infection, CDC recommends that all stools submitted for routine testing from patients with acute community-acquired diarrhea be simultaneously cultured for E. coli O157 and tested with a CIDT that detects Shiga toxins [14]. Some syndromic panels can differentially detect the O157 antigen and also Shiga toxins, which might influence a clinical laboratory’s decision to drop culture methods. Specimens that only detect Shiga toxin are reported as STEC-positive; however, reflex culture should be attempted to confirm that the organism producing the toxin is E. coli; some species of Shigella occasionally produce Shiga toxin [16].

Adoption of syndromic panels also changed the practices among clinical laboratories testing for parasitic pathogens. By 2017, nearly a quarter of laboratories used a syndromic panel to detect Cryptosporidium. By 2019, most laboratories used a syndromic panel routinely to test for
Conventional diagnostic testing using culture and microscopy might rely on the clinician to choose the correct test, might be sequential, and typically tests for one pathogen at a time [17]. Many bacteria can be difficult to culture, and syndromic panels, which are more sensitive and test for multiple pathogens from the same specimen, can decrease turnaround time for patient diagnosis. Adoption of CIDTs might also benefit clinical laboratories by decreasing the time and skill needed for pathogen detection. However, culture is still needed for diagnosis of antimicrobial resistant bacterial infections. As the number of clinical laboratories using CIDTs increased, the percentage performing reflex culture decreased, and submission of isolates to SPHLs was replaced by submission of stool specimens. As a result, the burden of recovering a bacterial isolate has progressively fallen upon SPHLs, which lack resources to fully absorb this additional testing [6].

The recovery of isolates by culture, whether by clinical or public health laboratories, remains an essential component of public health efforts to monitor trends and to detect and respond to outbreaks [18]. Public health laboratories have transitioned to using WGS for subtyping enteric pathogens; this method provides better discriminatory power and precision than PFGE for identifying related isolates, and has improved outbreak investigations [19]. However, current WGS technology relies on the ability to recover isolates [11]. We found that the percentage of laboratories in FoodNet sites submitting either specimens or isolates to the SPHL increased from 2015 to 2019 for pathogens except STEC, which decreased slightly. This might be partly due to recognition of legal requirements. In 2015, a review of state legal requirements found that 47 states had disease reporting laws for at least one enteric pathogen, requiring clinical laboratories
to submit clinical materials to public health laboratories [18]. State regulations varied widely by pathogen; submission of specimen versus isolate was not typically specified. In 2015, all 10 FoodNet sites had pathogen specimen submission requirements for at least *Listeria*, *Salmonella*, and STEC.

Our findings are subject to limitations. First, the clinical laboratories included in the study are those that serve FoodNet’s surveillance catchment area and may not represent laboratories nationally. Second, data collection methods differed by site and relied on individual laboratory participation. Third, the number of laboratories using blood culture tests that can detect *Listeria* may be underrepresented because some may incubate blood on site and send likely positives to another facility for isolation. Fourth, the data only describe testing practices and do not consider the number of tests performed by each laboratory. Responses from a small office laboratory have equal weight in our analysis as responses from a major commercial laboratory.

**Conclusions**

Clinical laboratories have an important role in public health surveillance. They are increasingly using CIDTs without reflex culture, which transfers the burden of isolate recovery to under-resourced public health laboratories [20]. Although CIDTs offer many advantages, such as simultaneously testing for multiple pathogens from a single specimen, isolates are necessary to conduct WGS, identify pathogen subtypes, determine antimicrobial resistance, detect outbreaks, and help link illnesses to likely sources. These isolate-based analyses are essential to implementing and evaluating public health prevention activities. In the future, advanced laboratory methods, such as metagenomics, may allow for molecular subtyping directly from a
patient specimen. Until then, states should consider updating their enteric disease reporting
requirements to include submission of isolates or specimens to SPHLs and identify adequate
resources and partnerships for isolate recovery.

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Disclaimer
The findings and conclusions in this report are those of the authors and do not necessarily
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Patient consent
This study does not include factors necessitating patient consent.

Potential conflicts of interest
All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of
Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the
manuscript have been disclosed.
References


Table 1. Number and percentage of clinical laboratories testing for surveyed pathogens on site by year and pathogen — Foodborne Diseases

Active Surveillance Network, 2012–2019

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*Data are unavailable for years in which the pathogen was not included in the survey: *Listeria* (2013–2017), *Cryptosporidium* (2018–2019), *Cyclospora* (2012–2017)

†Shiga toxin-producing *Escherichia coli*

§Diagnostic laboratories for which data was provided
Figure Legends

Figure 1. Percentage of laboratories using culture-based tests only, culture-based tests and culture-independent diagnostic tests (CIDTs), or CIDT only, by pathogen, year, and test type — Foodborne Diseases Active Surveillance Network, 2012–2019

Culture only  Culture and CIDT  CIDT only

Figure 2. Number of laboratories using antigen-based tests, syndromic panels, antigen-based tests and syndromic panels, and laboratory-developed tests to detect *Campylobacter* and Shiga toxin-producing *Escherichia coli* (STEC), by pathogen, year, and test type — Foodborne Diseases Active Surveillance Network, 2012–2019

Antigen-based test  Syndromic panel  Antigen-based test and syndromic panel  Laboratory-developed test
Figure 1. Percentage of laboratories using culture-based tests only, culture-based tests and culture-independent diagnostic tests (CIDTs), or CIDT only, by pathogen, year, and test type — Foodborne Diseases Active Surveillance Network, 2012–2019

Abbreviations: N = number of laboratories testing for pathogen

*Listeria was not included in the survey during 2013–2017.

Figure 1
197x234 mm (x DPI)
Figure 2. Number of laboratories using antigen-based tests, syndromic panels, antigen-based tests and syndromic panels, and laboratory-developed tests to detect *Campylobacter* and Shiga toxin-producing *Escherichia coli* (STEC), by pathogen, year, and test type — Foodborne Diseases Active Surveillance Network, 2012–2019
Figure 3. Percentage of laboratories* that performed reflex culture on site after pathogen detection by culture-independent diagnostic test, by pathogen—Foodborne Diseases Active Surveillance Network, 2015 and 2019

**Abbreviations: N = number of laboratories using a CIDT test**

*Among laboratories with information available. In 2015, data on reflex culture was unknown for 16 laboratories for Campylobacter, 5 for Salmonella, 4 for Shigella, 1 for Vibrio, and 0 for Yersinia. In 2019, unknown for 1 laboratory for Campylobacter, 3 for Salmonella, 3 for Shigella, 1 for Vibrio, and 0 for Yersinia.

Figure 3
176x140 mm (x DPI)
Figure 4. Percentage of laboratories that routinely sent broth, stool, isolates, or nothing to a state public health laboratory in 2015 compared with 2019, by pathogen — Foodborne Diseases Active Surveillance Network

Abbreviations: N = number of laboratories testing for pathogen

*Percentage among laboratories when known. Some laboratories routinely submit multiple specimens. In 2015, data on specimen submission was unknown for 69 laboratories for Campylobacter, 74 for STEC, 74 for Salmonella, 73 for Shigella, 47 for Vibrio, and 55 for Yersinia. In 2019, unknown for 8 laboratories for Campylobacter, 12 for STEC, 9 for Salmonella, 21 for Shigella, 14 for Vibrio, and 11 for Yersinia.

Shiga toxin-producing Escherichia coli.