

# 2018 FORUM ON CULTURE- INDEPENDENT DIAGNOSTICS: CHARTING A PATH FOR PUBLIC HEALTH

## Meeting Summary

March 2022



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## EXECUTIVE SUMMARY

Foodborne illnesses affect an estimated 48 million Americans each year, resulting in 280,000 hospitalizations and 3,000 deaths. These illnesses are largely preventable, especially if we can understand the agents, vehicles, and settings that lead to illness. Public health agencies are tasked with conducting disease surveillance to monitor the burden (health impact on the population) and trends in foodborne and diarrheal illnesses. Surveillance is also used to detect outbreaks caused by problems in the food supply chain. Surveillance information is used by public health departments, the food industry, regulatory agencies, and the public to make informed decisions about risks and mitigation approaches, to monitor the effectiveness of control programs, and to limit the health and economic impacts of outbreaks. Although not widely recognized outside of the public health community, activities informed by disease surveillance are key parts of our national food safety system.

Clinical microbiology, which is a primary source of information for surveillance, is undergoing a significant technological revolution that holds immense potential benefits for both public health and clinical medicine, but these changes pose important challenges for disease surveillance. Additionally, there are non-laboratory sources of data for surveillance, such as reported outbreaks (with or without laboratory data) and syndromes which are reported with or without laboratory data, such as botulism, HUS. The use of microbiological culture (i.e., growing bacteria on a petri dish) has been used to diagnose illness since the time of Pasteur. Culture-independent diagnostic tests (CIDTs; tests that do not rely on culture) have been slowly introduced over the last 30 years, but the use of CIDTs has dramatically accelerated with the recent introduction of molecular-based “syndromic panels” that can simultaneously test for many pathogens, including bacteria, viruses, and parasites. These panels have several advantages for clinicians and their patients. Their rapid turnaround time, high sensitivity, and ability to identify multiple types of microorganisms at once will benefit both patient care and public health surveillance in several ways. However, public health professionals need to make significant adjustments to the way they use such data in adapting to this new data stream.

CIDT results cannot be interpreted in exactly the same manner as culture, leading to both clinical and public health management conundrums. Furthermore, unlike culture-based tests, CIDTs do not produce pure samples of bacteria (i.e., isolates) as part of the test process. For most bacterial syndromes tracked by public health, isolates are needed for DNA sequencing and other advanced characterization methods that provide detailed information on the microorganism’s disease-causing potential (i.e., virulence) and susceptibility to antibiotic treatment as well as how closely the microorganism is related to similar microorganisms in other patients, animals, food, or the environment. This information has become a cornerstone of current foodborne disease surveillance systems. A lack of isolates and their associated DNA sequences will make it increasingly difficult to detect dispersed clusters of illness, monitor trends in antibiotic resistance, attribute illnesses to possible sources, and interpret trends in data collected through surveillance. Public health surveillance systems that currently require isolates include PulseNet, the nation’s molecular subtyping network for foodborne disease surveillance, and the National Antibiotic Resistance Monitoring System (NARMS), which informs the development of public health interventions and policies designed to protect people from the threat of resistant enteric infections. These at-risk systems are key components of our national food safety system, are directly responsible for preventing hundreds of thousands of cases per year and provide key information for informing food safety and antibiotic use policy.

The problems created by the new CIDT technologies are complex. Solving these problems requires comprehensive, collaborative, and multi-disciplinary approaches. Fleshing out strategies for addressing these challenges was the impetus behind development of the 2018 Forum on Culture Independent Diagnostic Testing, sponsored by the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), the Council of State and Territorial Epidemiologists (CSTE), the Pew Charitable Trusts, and The Ohio State University (OSU). The Forum brought together leaders from government, academia, and the private sector to raise awareness of the issues and develop new ideas for addressing the wide range of problems and potential to adapt public health to changing microbiology practice. The Forum focused on foodborne and other enteric diseases, but the scope also included surveillance for other infectious disease syndromes impacted by CIDTs. Attendees explored three specific issues: (a) adapting the process of public health decision-making to the new types of data; (b) preserving isolate-based surveillance until CIDT-compatible

surveillance mechanisms are in place; and (c) developing technological approaches to characterizing pathogens for public health activities without the need for culture. Key discussion points for these three issues are summarized below.

## Public Health Decision Making

The adoption of CIDs is necessitating re-evaluation of several practices related to the collection of health data and its use in public health decision making. CIDs have created new opportunities for monitoring diseases for which little data were formerly available, such as cyclosporiasis and enterotoxigenic *Escherichia coli* disease. For pathogens already under laboratory-based surveillance, the difference in methodology requires adjustments in the way cases are defined and counted, and how that information is used to estimate disease burden and track trends. CIDs have increased the frequency of reported co-detections (i.e., signals for more than one pathogen in single specimens), but current surveillance systems are not designed to effectively track, use, or interpret this type of data. Other challenges have emerged such as how to interpret positive CIDT results for certain microorganisms (described further in Section III), and for how best to use CIDs to evaluate cases for possible exclusion from sensitive settings such as daycare centers or food service operations.

Major action items proposed for the public health community during the forum included (1) explore new surveillance opportunities to take advantage of data streams made available by CIDs, such as enterotoxigenic *E. coli* (EPEC) infections and cyclosporiasis; (2) modify data collection systems to obtain additional test-type and testing denominator data; (3) conduct studies needed to understand CIDT performance and use characteristics, with the goal of adjusting burden and trend estimates; (4) resolve CIDT interpretation issues by conducting studies to better understand the medical and public health significance of selected pathogens such as EPEC, EPEC, and *Vibrio* spp., and co-detections, and (5) convene a workgroup to identify knowledge gaps and develop guidelines for use of CIDs in sensitive settings, such as daycare and food service.

## Maintaining Isolate-based Surveillance

The current approach for maintaining the flow of isolates to public health agencies in the near-term involves multiple strategies to encourage and facilitate clinical or Public Health Laboratories (PHLs) to conduct culture on CIDT positive specimens (i.e., “reflex” culture). To conduct reflex culture, CIDT specimen collection methods must be compatible with culture-based methods. The medical device industry has indicated that research and development efforts in the microbiology testing realm is moving toward the inclusion of specimen inactivation steps, wherein pathogenic microorganisms are killed prior to testing. This facilitates the safety of point-of-care (POC) testing, but such steps would make reflex culture of the inactivated specimens impossible. Determining which laboratories will perform reflex culture, whether it is the public health laboratory, the clinical or reference laboratory, or a combination of the two, poses another challenge. There are significant and complex issues that impact this decision, including pathogen viability, laboratory funding, and legal requirements.

A variety of potential actions were discussed to minimize impacts including: (1) increase active communication between public health, clinical laboratories, non-governmental organizations (NGOs), and other stakeholders to increase awareness and promote interim solutions; (2) work with the Center for Medicare & Medicaid Services (CMS), and other accreditation agencies to increase compliance with local reporting rules and explore potential for workload credits or other means of minimizing fiscal impacts of reflex culture; (3) work with the medical device industry to promote compatibility of test systems with reflex culture, and (4) improving the efficiency of isolate recovery.

## Moving Towards Culture-Independent Molecular Characterization

Isolate recovery from CIDT positive specimens is the only viable approach for maintaining isolates in the short run, but it is slow, expensive, and unlikely to be sustainable in the long term. A better approach would be to develop methods for high-level characterization of pathogens (e.g., high-resolution strain identification, detection of potential antibiotic resistance, and virulence determination) directly from clinical specimens. Such methods have the potential to significantly increase the speed and effectiveness of the surveillance process and synchronize public health technology with that of clinical microbiology. Several challenges need to be overcome first.

The main approaches discussed at the Forum involve the tools of metagenomics, a new sequence-based technology commonly used in the study of microbial communities. The various types of metagenomic tools have potential benefits and limitations and, given the importance of subtype-based surveillance to the health and well-being of the nation, it seems prudent to explore multiple approaches simultaneously. Two approaches being explored are highly multiplexed amplicon sequencing (HMAS) and “shotgun” metagenomics. HMAS appears most likely to be deployable in the near-term, and most participants felt that this work should be actively explored. The use of shotgun metagenomics to characterize pathogens directly in specimens is likely to become practical at some point the not-so-distant future with continuing advancements in bioinformatics and information technology. Towards that end, participants encouraged research into targeted enrichment methods such as CRISPR-Cas9-based methods to improve the signal-to-noise ratio, and approaches such as Hi-C formalin cross-linking and long-read sequencing to reduce phasing issues. For both HMAS and shotgun methods, further research is needed into methods to stabilize specimens and IT and bioinformatics infrastructure to handle and process data.

## **Conclusion**

The emergence of CIDTs will require a concerted and coordinated response by public health professionals and many partners to take advantage of new opportunities that the technology provides while, at the same time, averting the substantial negative effects on our ability to monitor and control infectious diseases. To potentially solve a wide array of issues, participants proposed that knowledge gaps be identified, studies be designed to fill gaps, modification to data systems and disease reporting rules/regulations be implemented, and funding to facilitate the changes be allocated.

## INTRODUCTION

Clinical microbiology is undergoing a major technological revolution that holds great potential benefits and challenges for public health surveillance of infectious diseases. Diagnostic technology using nucleic acid-based methods, referred to as culture-independent diagnostic testing (CIDT), is making it possible for physicians to quickly obtain information about which pathogen(s) are making their patients sick without waiting for slower culture-based testing. A few one-or-two-target CIDTs have been in use for over 30 years, which has selectively impacted the diagnosis and surveillance of the specific diseases those tests detect. In recent years, the introduction of “syndromic panels” that simultaneously test for many pathogens, including bacteria, viruses, and parasites in a single workflow, is dramatically changing the diagnostic landscape.

CIDTs are changing how infections are diagnosed in ways that benefit the patient and the clinician. Traditional bacterial culture and identification methods usually take several days to complete, require high technical expertise and may lack sensitivity in some circumstances. The standard enteric culture panel includes a limited number of pathogens such as *Salmonella* and Shiga toxin-producing *E. coli* (STEC). Physicians may specifically order tests for additional bacterial pathogens such as *Vibrio cholerae* and *Yersinia enterocolitica* or parasites such as *Giardia lamblia*, *Cryptosporidium parvum*, or *Cyclospora cayetanensis*, but these tests are available only in selected laboratories and physicians may be less likely to be familiar with or specifically suspect these agents. Previously, culture-based tests for other enteric pathogens, such as enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), or norovirus had been available only in research centers or selected public health laboratories and were not practical for routine diagnostics. Additionally, testing for a wide range of bacterial, viral, and parasitic pathogens required a complex series of workflows in the clinical laboratory. In the traditional culture workflow, which typically includes antibiotic susceptibility testing, the results are generally reported after the passage of 2-3 days for bacterial agents. Until such results are available, treatment is empiric. This may result in antibiotic treatment when it is not generally indicated (e.g., STEC infection, uncomplicated salmonellosis), or lack of treatment where it may be beneficial for selected patients and agents (e.g., *Campylobacter jejuni*, enterotoxigenic *E. coli*), or treatment failure. In contrast, the new syndromic panels have a rapid turn-around time (e.g., hours) allowing for targeted therapy, may be more sensitive than culture, and a single test can simultaneously detect multiple pathogens including those not formerly part of the routine testing panel. As a result, use of CIDTs will likely result in more patients being diagnosed and receiving faster and better initial treatment. However, case-specific antibiotic resistance and virulence information are not available for CIDTs unless reflex culture (i.e., culture test triggered by a positive CIDT result) is conducted. This could result in treatment failure in infections caused by organisms with undetected resistance to standard antibiotics (e.g., fluoroquinolone resistant *Shigella* spp. and cephalosporin resistant *Salmonella* in patients with invasive disease, such as sepsis). Finally, the new types of data produced by CIDTs have created interpretation challenges for the clinical community. Physicians are now routinely receiving reports with unclear clinical implications, such as reports of agents for which they have limited experience (e.g., enterotoxigenic ETEC adenovirus F 40/41), of uncertain pathogenicity in the general population (e.g., enteropathogenic *E. coli* (EPEC), *Pleisiomonas shigelloides*), variable pathogenicity (e.g., *Vibrio* spp., Shiga toxin-producing *E. coli*, astrovirus) or reports of multiple agents detected in single specimens.

The introduction of CIDTs into clinical diagnostic practice also has advantages and disadvantages for public health surveillance. Use of CIDTs will likely result in more diagnosed and reported cases of infections, particularly for pathogens such as *Cyclospora*, *Y. enterocolitica* and non-STEC *E. coli* which were more difficult to detect using traditional methods. Culture-based surveillance is relatively slow, which results in a considerable time lag between illness onset and outbreak detection. Faster diagnosis and more reporting means that some local outbreaks are likely to be detected and investigated sooner. Increased testing and diagnosis may lead to better information about illness in our populations. However, the introduction of CIDTs requires that a multitude of public health monitoring systems adapt to the new type of data.

Accurate counting of cases is essential for estimating disease burden, monitoring trends, and investigating outbreaks. Since the performance characteristics of CIDTs are different from culture and from each other, use of CIDTs has the potential for introducing considerable uncertainty into surveillance and control activities. These approaches need to be adjusted to account for the characteristics of CIDTs, but the adjustments are not straightforward. Many issues need to be addressed, such as: How should trends be adjusted to account

for differences in test performance characteristics and use patterns? Which conditions should be counted for co-detections? Can CIDT results be used to predict infectivity in circumstances where sensitive populations need to be protected? In addition, the CIDT testing process does not yield the pure strain of pathogenic bacteria (“isolate”) that is produced during traditional bacterial culture. These isolates are used for advanced characterization of bacteria including antibiotic susceptibility, virulence potential, and strain identification. Reflex culture is generally not part of the CIDT process, which creates economic, procedural, and legal issues that must be addressed. Lack of isolates makes it difficult to detect dispersed clusters of illness, to monitor trends in resistance, attribute illnesses to possible sources, or in some circumstances to gauge risk to the public. For the near future, if the flow of isolates to the public health system is not maintained, fewer outbreaks will be identified and controlled, fewer gaps in the food safety system will be identified, and less new information critical to controlling foodborne disease will be obtained.

Solving the challenges created by CIDTs requires comprehensive, collaborative, and multi-disciplinary approaches. Recognizing this need, in 2012 the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), and the Council of State and Territorial Epidemiologists (CSTE) organized a “Forum on Culture-Independent Diagnostics” to preemptively chart a path for public health and address the anticipated issues. The 2012 Forum raised awareness about the impact of CIDTs on public health surveillance systems and helped guide ensuing activities by the groups. By 2018, the trend towards CIDTs had accelerated and the impacts on public health surveillance programs had become more evident. The CDC, APHL, CSTE, The Pew Charitable Trusts, and The Ohio State University (OSU) organized the 2nd Forum on Culture-Independent Diagnostic Testing to build upon the 2012 meeting and the intervening 6 years of experience with CIDT trends. This forum, held in Washington, D.C. on May 8 and 9, 2018, brought together leaders from government, academia and the private sector with expertise in clinical and public health microbiology, epidemiology, infectious disease medicine, molecular biology, economics, public health and public policy. The goals of the forum were to:

1. Increase awareness among participants about the scope of the problem, current impacts, and likely future impacts
2. Evaluate current efforts to address CIDT issues in public health and identify knowledge gaps
3. Generate new ideas for moving forward.

To achieve these goals, the Forum included plenary sessions to familiarize all participants with the major topics, and separate tracks to explore three specific issues: (a) public health practice; (b) strategies for maintaining the availability of isolates and specimens for isolate-based surveillance if needed; and (c) advanced technologies that in the future will allow pathogens to be characterized for public health purposes without the need for culture. This paper provides background information on the impact of CIDTs on foodborne disease surveillance and summarizes the forum discussions and potential pathways for adapting our foodborne disease surveillance system to changing clinical diagnostic practices.



## BACKGROUND

In the United States, each year an estimated 48 million Americans become ill, 280,000 are hospitalized and 3,000 die as a result of food contaminated with microorganisms or their toxins (Scallan, Griffin, et al., 2011; Scallan, Hoekstra, et al., 2011). Globally, foodborne disease causes an estimated 600 million illnesses and 420,000 deaths annually, particularly affecting children (Havelaar et al., 2015). Foodborne disease has also been associated with chronic sequelae, including irritable bowel syndrome, reactive arthritis, kidney disease and neurological dysfunction (Batz et al., 2013; Buzby, 2001; Hoffmann & Scallan Walter, 2020; Keithlin et al., 2014). The economic impact of these illnesses is significant (Buzby & Roberts, 2009; Flynn, 2014).

### Enteric Disease Surveillance Overview

Enteric infections are thought to be largely preventable, but progress towards the goal of reducing the number of enteric illnesses requires an understanding of which pathogens, foods, and other exposures are making people sick and how transmission can be stopped or reduced. Surveillance networks, such as FoodNet in the United States, play a central role in deepening our understanding of enteric illnesses. The data such networks collect help estimate the burden of illness, identify trends, evaluate the effectiveness of control measures, and attribute illness to specific foods and other exposures. Federally supported programs such as Outbreak Net Enhanced help detect and investigate outbreaks. In the United States, information is largely gathered through case-based surveillance, outbreak surveillance, and laboratory-based surveillance. Each of these data collection methods is impacted by CIDs.

“Case-based surveillance” relies on the collection of reports of cases of illness. These case reports include information such as the symptoms of illness, when those symptoms started, demographic information about the ill person (age, sex, state of residence), and key risk factor information (e.g., travel, activities, foods consumed) for the specific infection. CDC conducts case-based surveillance for botulism, cholera and other *Vibrio* illnesses (including *V. parahaemolyticus* and *V. vulnificus* infections), listeriosis, and typhoid and paratyphoid fever infections by collecting case report forms for each person who is diagnosed with a case of one of these illnesses (CDC, 2018b). These data are reported to CDC by state and territorial public health departments. Case-based surveillance may or may not also include information gathered in laboratory-based surveillance, described below. Each US state and territory maintains a list of diseases for which reporting by clinical laboratories and clinicians is required (“notifiable” diseases). The diseases and associated information included in the report varies by jurisdiction. The CDC also has a list of notifiable diseases but reporting by states is voluntary.

“Outbreak surveillance” is conducted by local, state, and federal agencies, working in close collaboration and using tools and approaches that have been tested and modified over time. Many of these approaches are explained in the Council to Improve Foodborne Outbreak Response (CIFOR) Guidelines for Foodborne Disease Outbreak Response (CIFOR (En-US), 2020), which covers aspects of outbreak surveillance from legal preparedness, team roles and communication through laboratory and epidemiology practice to environmental considerations and control measures. This type of surveillance is used for outbreaks reported by citizens or healthcare providers, and for outbreaks detected by other mechanisms such as PulseNet, described below.

“Laboratory-based” surveillance generally refers to the collection of information about bacteria, viruses, and parasites that have been identified by laboratory testing of ill persons. Certain types of microorganisms are isolated and identified from patient specimens by clinical laboratories, and the isolates are then submitted to state, territorial or local public health laboratories (PHLs) for further characterization. Isolates are tested by PHLs to determine their serotype, virulence characteristics, and genomic profile (also called a subtype). PHLs use this data to detect and investigate outbreaks within their jurisdictions, and to discover potential linkages between local cases and those in other jurisdictions. Using reports from PHLs, CDC analyzes laboratory-based surveillance data at the national level. PulseNet and the National Antibiotic Resistance Monitoring System (NARMS) are examples of laboratory-based surveillance systems and are described further below.

PulseNet is the nation’s molecular subtyping network for enteric disease surveillance, with a focus on *Salmonella*, *Shigella*, Shiga toxin-producing *E. coli* (STEC), *Listeria monocytogenes*, *Campylobacter*, *Vibrio* spp., and *Y. enterocolitica*. Potential CIDT impacts to this network were a driving force behind development

of the Forum. PulseNet is the primary US detection mechanism for dispersed enteric disease outbreaks, such as outbreaks due to contaminated distributed food products. PulseNet consists of 82 local, state, and federal health and regulatory laboratories in all 50 states and Puerto Rico. Each participant uses a highly standardized “DNA fingerprinting” method — pulsed-field gel electrophoresis (PFGE) in the past, and now whole genome sequencing (WGS) — to characterize pathogen isolates from ill patients that were submitted by clinical laboratories, or from food testing laboratories in their jurisdictions. As with PFGE, WGS depends on the availability of isolates. Realizing the potential of WGS, therefore, requires addressing CIDT issues. PulseNet works by comparing the genetic profiles of isolates submitted to the national

PulseNet database to group together closely related cases that are most likely to share a common exposure, such as a food. Epidemiologists at the local, state, and federal levels triage and investigate clusters to identify the source, if possible. Molecular “matches” in the PulseNet databases between clinical isolates and food or environmental isolates obtained as part of routine regulatory activities also provide a hypothesis for investigators. When regulated foods are suspected, investigations are conducted jointly with FDA or USDA, who may conduct “trace-back” investigations to triangulate on the responsible source, conduct microbiology testing of suspect foods or food environments, investigate suppliers in the food production chain, or order recalls. Local, state, and federal agencies work together to take action, such as warning the public or taking other types of actions. A representative sample of clinical isolates are also forwarded from PHLs to CDC and tested for antimicrobial resistance as part of the National Antimicrobial Resistance Monitoring System (CDC, 2020b). These data are a primary source of information for treatment guidelines. Summaries of investigated enteric outbreaks are reported to the National Outbreak Reporting System (NORS). Annual analysis of those reports provides a source of data for attribution estimates for specific categories of foods (CDC, 2018a).

Investments in PulseNet have had a substantial return on investment in terms of averted illnesses and associated cost savings. A 2016 economic analysis focusing on benefits at the state level showed that PulseNet prevents at least 270,000 cases of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* infection annually, saving society an estimated \$507 million per year in costs and productivity losses (Scharff et al., 2016). PulseNet increased the number and tempo of recalls, leading to an additional in \$37 million in health-related costs avoided. The cost of running PulseNet was estimated to be \$7.3 million, resulting in about \$70 saved for every \$1 invested. During 2018-2019, the transition to whole genome sequencing (WGS) began, which provides more detailed and precise information, and is likely to greatly increase the cost effectiveness of PulseNet and associated programs.

## CIDT Impact on Enteric Disease Surveillance

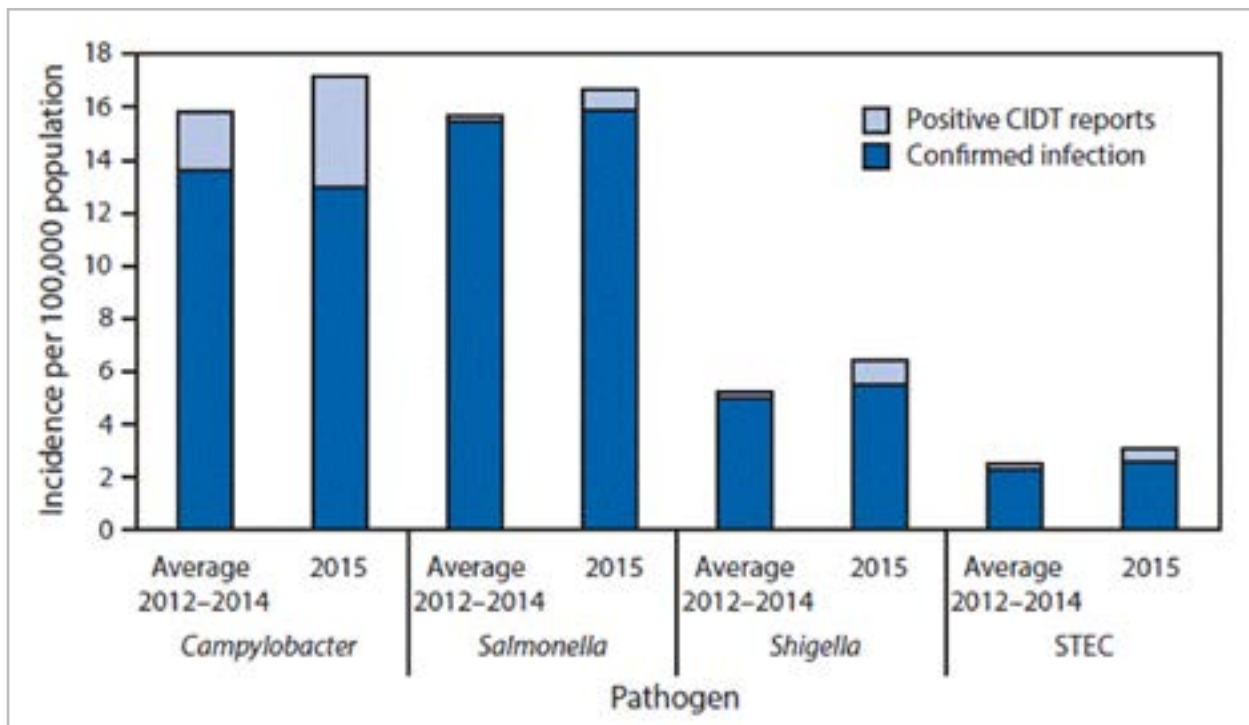
Clinical laboratories have been increasing the use of CIDTs in recent years to diagnose enteric infection. For bacterial enteric pathogens, the earliest such tests focused on individual pathogens, such as antigen-based assays for *Campylobacter*, or for *E. coli* O157-specific proteins. More recently, broad PCR diagnostic panels have enabled testing for a variety of pathogens at once.

Since enteric disease surveillance activities were built around the use of culture-based methods to diagnose illnesses, several surveillance activities have been affected by adoption of CIDTs. Case counts based on culture alone are decreasing, while those based on CIDTs are increasing. Since the performance characteristics of CIDTs are different from culture and different from each other, interpretation of disease burden and trends over time has become problematic (Langley et al., 2015). Furthermore, the use characteristics of CIDTs (e.g., how often and for what reasons the tests are ordered) may be different from culture. It seems likely that in emergency rooms and pediatric practices, the speed of getting a diagnosis would be particularly appealing. However, this trend may be countered to some extent by health care reimbursement policies. The impact of CIDTs on enteric test use practices remains to be evaluated.

The use of CIDTs has already affected the way CDC reports progress towards public health goals for foodborne illness. FoodNet data from 2015 showed that the number of *Campylobacter*, *Shigella*, *Salmonella* and STEC cases diagnosed by CIDT increased 122% in 2015 when compared to 2012 – 2014 data (**Figure 1**) (Huang et al., 2016). Difficulties in knowing whether to attribute these case increases to increased and broader testing (with CIDTs) or to actual increases in the underlying diseases led CDC to, in 2016, stop issuing its annual report card on food safety using simple comparisons of how recent results compare to previous years. The new

approach to presenting the data will continue until the reported numbers can be adjusted for the effects of CIDT use.<sup>a</sup>

**Figure 1.** Cases diagnosed by CIDT on average for 2012 – 2014 compared to 2015 (Huang et al., 2016)



One important challenge is that CIDTs do not by themselves yield the bacterial isolates needed by public health to characterize pathogens to the strain level, measure antibiotic resistance, and determine virulence potential. As a result, isolates may: (1) not be recovered, eliminating the possibility of molecular tracking or resistance testing; (2) be recovered by reflex culture by clinical laboratories at their own expense; or (3) be recovered by PHLs at public expense. The potential impacts include reduced sensitivity of cluster detection and exposure assessment, and financial implications for clinical laboratories and/or PHLs. Case-specimens not subject to reflex culture are lost to the system, lowering the sensitivity of outbreak detection and trend analysis. For those specimens subjected to reflex culture, the turnaround time of patient result to cluster detection may be extended from several hours to several days or more, depending on the organism and culturing process. Extended turnaround time lowers the effectiveness of epidemiological investigations which typically rely on patient recall. Isolate recovery by healthcare providers is not generally reimbursed by insurers, including Medicare, as isolate recovery is, with some exceptions, considered a public health function and not essential for patient care. The non-reimbursed costs associated with reflex culture can be substantial, contributing to financial hardship for clinical laboratories already experiencing financial cutbacks. Reflex culture may alternatively be conducted by PHLs, which are also not funded for this relatively new activity. These laboratories must therefore choose between competing public health priorities. These issues will be covered in more detail below.

<sup>a</sup> Editor’s Note: In 2019 (after completion of the 2018 CIDT Forum), the following update was published in the MMWR (Tack et al., 2020): “The number of bacterial infections diagnosed by CIDT (with or without reflex cultures) increased 65% in 2018 compared with the average annual number diagnosed during 2015–2017; the increase ranged from 29% for STEC to 311% for *Vibrio*. In 2018, the percentage of infections diagnosed by DNA-based syndrome panels was highest for *Yersinia* (68%) and *Cyclospora* (67%), followed by STEC (55%), *Vibrio* (53%), *Shigella* (48%), *Campylobacter* (43%), and *Salmonella* (33%), and was lowest for *Listeria* (2%). In 2018, a reflex culture was attempted on 75% of specimens with positive CIDT results, ranging from 64% for *Campylobacter* to 100% for *Listeria*.”

## CIDT IMPACT ON PUBLIC HEALTH DECISION MAKING

As previously discussed, increasing use of CIDTs is affecting the ways in which public health agencies monitor foodborne and other enteric illnesses, investigate outbreaks, and implement measures that prevent spread of infection and contamination of food. In short, increasing use of CIDTs is affecting the data that are the basis for public health practice.

### CIDTs Offer Potential for Diagnosing a Broader Array of Pathogens and Pose New Challenges in Results Interpretation

One major benefit of syndromic panel CIDTs is their ability to test for an array of pathogens that are difficult or impossible to culture. These new data provide surveillance opportunities to public health but also present challenges in interpretation.

Traditional diagnostic methods typically involve testing a specimen for several suspected pathogens using specific assays such as culture, enzyme immunoassays (EIA), and ova and parasite exam. The most frequently cultured enteric pathogens per routine testing are *Salmonella*, *Campylobacter*, *Shigella* and STEC (Humphries & Linscott, 2015), while other tests must be ordered specifically to detect less common pathogens. Syndromic panel CIDTs, however, can test for a range of pathogens without a specific order, including some pathogens not readily diagnosed by culture. For example, norovirus was rarely diagnosed in clinical laboratories until the advent of CIDTs and now can be diagnosed by several panels. The parasite *C. cayetanensis* cannot currently be cultured, its detection by stool microscopy requires specialized skills, and testing has not been widely available. After the adoption of syndromic panel CIDTs that include a target for *Cyclospora*, cases increased from 300-600 per year between 2013 and 2016, to 1065 cases in 2017 and 2,299 in 2018 (CDC, 2019). This increase may partly reflect increased transmission in the United States, as 2018 was the first year in which produce grown in the US was confirmed through laboratory findings to be contaminated with *Cyclospora*. However, a greater factor is likely the rapid increase in syndromic panel diagnosis (CDC, 2020a). Similarly, identifying pathotypes of *E. coli* other than STEC, such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli*, enteroaggregative *E. coli*, and enteroinvasive *E. coli*, has not been routine in clinical laboratories. Even identifying STEC other than O157:H7 is not straightforward. Increasing use of multi-pathogen CIDT panels has increased diagnosis of non-O157 STECs as well as other diarrheagenic *E. coli* pathotypes. The Minnesota Department of Health reported that 39% of ETEC-positive patients did not have a history of foreign travel (Medus et al., 2016). ETEC had previously been recognized as a frequent cause of traveler's diarrhea, and therefore was not tested for unless the patient had recently traveled abroad. The Minnesota finding suggests that much more illness may be acquired in this country. These examples demonstrate how the new CIDT data are expanding our understanding of enteric disease and opening new opportunities for tracking and controlling disease. Better understanding of diseases detected by CIDTs is urgently needed to guide both the medical and public health communities in appropriate treatment and response.

### Fit for Intended Use—CIDT Applications in Other Settings

The CIDT technology is based on detecting the presence of DNA or RNA, not on detection of an intact cell (e.g., *C. cayetanensis*) or viable living pathogen (e.g., *Salmonella* or STEC). Commercial kits must satisfy clinical and analytical performance characteristics required by FDA for its approval process (i.e., 510(k) approval). Performance requirements are typically only established for primary diagnosis by clinical laboratories of patients presenting with enteric illness. CIDTs are attractive in other circumstances, such as testing for carriage/shedding in asymptomatic individuals in the outbreak setting or for test-of-cure for patients who have recovered from their illnesses, as there is often considerable need for rapid turnaround of results. For example, workers in a restaurant-associated outbreak or children in a daycare outbreak setting may need to be screened to identify shedders and thereby prevent continuing transmission. Once determined to be positive, these individuals may need sequential screening tests to demonstrate that they are no longer infectious before returning to work or school. Traditional culture/identification methods can take 2-3 days, resulting in considerable economic impact on cases and businesses.

However, it is important to note that use of CIDTs for carriage, shedding, and test-of-cure is considered “off-label.” CIDTs were not designed to be used in these types of patient populations, and many questions

are unanswered about how well the tests perform in these settings. It remains to be established how CIDT results correlate with infectivity, and how results are affected by transient colonization, consumption of dead organisms, or persistence of nucleic acid after successful treatment or cure. Another consideration is that extraneous sources of DNA can create false positive results. In 2018, one *in vitro* diagnostic manufacturer notified laboratories that their agar transport medium was contaminated with non-viable *Vibrio* DNA, leading to false positive diagnoses of *Vibrio* infections in PCR syndromic panel testing (Nebraska Department of Health and Human Services, 2018). Additionally, research to understand the prevalence of infections with enteric pathogens in asymptomatic individuals using both CIDTs and traditional methods would help assess the clinical and public health relevance of CIDT detections. Finally, since CIDT assays are based on the presence of specific microbial genetic markers, mutation of those markers could result in failure of the testing panels to detect the pathogen when it is present. This problem occurred in Sweden in 2006 when *Chlamydia trachomatis* developed a mutation in the detection target. When the problem was identified, it was estimated that between 20% – 65% of *Chlamydia* cases nationwide carried the variant, and up to 78% of all cases were found to have the mutation in local areas (Soderblom et al., 2006). While the ecology of *C. trachomatis* is very different from enteric pathogens, it appears that only limited mechanisms, such as complaints to device manufacturers which are then reported to FDA, are in place to monitor changing performance characteristics of CIDT panels.

Using traditional methods, co-detections (i.e. finding more than one pathogen in a single specimen, which may or may not indicate a polymicrobial infection in the patient) are occasionally recorded, and multiple agents are sometimes found in single outbreaks, such as those involving direct contact with farm animals (Conrad et al., 2017). With the increase in syndromic CIDT panel use, the frequency of co-detections has changed from a relatively rare curiosity to a common occurrence. For example, studies at the Minnesota Department of Health found that a high proportion of ETEC positive specimens were also positive for one or more of 10 different pathogens (personal communication, David Boxrud, Minnesota Department of Health Public Health Laboratory). Co-detections pose interpretation challenges for the physician (e.g., deciding which result to use in treatment decisions) and the public health practitioner (e.g., how to count, and how to manage interventions in an outbreak setting).

It is not clear how often co-detections represent true polymicrobial infections, or whether one pathogen is primarily responsible for disease and the detection of other signal(s) is incidental. Interpretation of microbiology results in the public health setting traditionally relies on a combination of epidemiologic and clinical information to determine whether one organism is more plausible as the cause of the symptoms than the others. However, many enteric pathogens cause similar symptoms, and causality is likely to be difficult to tease apart. Mechanisms for reporting cases and strategies for using the data to estimate burden and trends have yet to be established.

## **Adapting Case Definitions for Public Health Surveillance to Include CIDTs**

Surveillance case definitions provide a consensus set of criteria for classifying and reporting cases in a uniform manner and are integral to disease reporting. Standardized surveillance definitions are critical to produce comparable data for monitoring trends in illnesses over time and to guide effective public health actions. The growing use of CIDTs is changing case definitions and complicating their use.

Case definitions typically include clinical and laboratory characteristics such as compatible illness symptoms, laboratory confirmation for a specific pathogen, and/or an epidemiologic link to another case (See text Box 1 at the bottom of the document for an example). In outbreak settings, additional specific criteria may be used to refine the definition, such as molecular subtype, and time, place and personal characteristics of the patient.

Standardized case definitions for infectious disease surveillance are revised and approved by the Council of State and Territorial Epidemiologists (CSTE). These definitions usually categorize cases as confirmed, probable, or suspect. Confirmed cases are often defined as patients from whom the pathogen was isolated, and probable cases may be defined as those with symptoms consistent with the illness and an epidemiologic link to a confirmed case. Suspect cases are often defined as those with symptoms consistent with the illness without associated laboratory test confirmation.

CIDT results are being incorporated into case definitions, most often by defining the case with compatible illness and a CIDT detection alone as a probable case. However, important questions remain unresolved. It is not clear how conflicting results should be interpreted if both culture and CIDT are conducted. Is a CIDT positive, reflex culture-negative case different from a CIDT positive case that was not cultured at all? How should variation in CIDT sensitivity and specificity be handled? It is impractical to tailor definitions on the performance of specific types of tests, and to revise case definitions whenever a new test is introduced, or an old test is modified. Some laboratories develop their own CIDTs instead of using commercially available FDA-cleared/approved tests, which raises the question as to whether results from such laboratory-developed tests (LDTs) should be treated the same as those from FDA-cleared/approved tests. As with the commercial tests, this could be addressed by classifying positive results from FDA-cleared/approved CIDTs as probable cases and those from laboratory-developed CIDTs as suspect cases. However, current routine surveillance does not collect information on the exact types of tests used for each reported case.

Some CIDTs may not clearly distinguish frank pathogens (microorganisms that always cause disease, even in a healthy individual) from opportunistic pathogens (microorganisms that can take advantage of a weakened host to cause disease but may not cause illness in a healthy individual). For example, although differing strains of *Y. enterocolitica* can cause a range of symptoms, CIDTs do not distinguish between pathogenic and less-pathogenic strains. Similarly, the significance of finding an *E. coli* with a genetic marker for the virulence-conferring *eae* gene (and calling it an EPEC) in an older child or adult is unclear, which is a problem both for clinical interpretation and public health decision-making.

## **Adapting the Public Health Approach to Burden and Trends Analysis**

Adjusting ongoing surveillance to account for test differences is critical to continuing to compare trends year to year and to looking back at historical data. Following trends over time is important to know whether disease control strategies are working. Making the needed adjustments is complex and depends on having information about the performance characteristics of CIDT tests, the frequency with which they are used (i.e., counting both negative and positive results), and the changes in healthcare provider behavior concerning whom to test and when. An early attempt to adjust *Campylobacter* reporting for an array of CIDTs found that with such adjustments, the incidence did not decline between 2012 and 2015, though the incidence based on cultures alone declined substantially in that time period (Gu et al., 2018). This also highlighted the value of knowing the total volume of tests performed, by type of test, to make such adjustments.

Addressing these challenges will require new information on CIDTs and their use. To interpret surveillance data appropriately, public health agencies may need to know the number of different types of tests performed in the lab and, if both CIDTs and culture are ordered, the results of both tests. For example, in New York City clinical laboratories are required to report CIDT positive and negative results to public health agencies. The substantial burden that this reporting may impose would be lessened if labs simply reported the total number of tests conducted each year. Other states, however, do not want clinical laboratories to report negative test results, except for negative culture results on the same specimen that produced CIDT-positive test results. Most surveillance reporting is structured to report positive test results and not negative test results.

The importance of incorporating CIDTs led CSTE to pass a policy position statement at the 2013 annual conference that outlines their concerns about the rapid development of new CIDTs and the need for a fluid, collaborative process between CSTE, CDC, FDA and APHL that addresses the application of new test methods to surveillance case definitions (CSTE, 2013). CSTE has also begun to incorporate CIDT results for enteric disease case definitions, such as the 2017-approved case definition for STEC which incorporated CIDT results in both probable and suspect case classifications (CSTE, 2017). This process is not simple and continued work on standardizing definitions for all enteric pathogens should remain a key priority. Opportunities for modifying case definitions occur annually.

## Excluding Infected Persons From Child Care, Patient Care and Food Service Settings

Public health authorities set criteria for whether or not to exclude someone infected with a contagious pathogen from a high-risk setting, such as food service establishments, child care facilities, and high-risk patient care settings (Heymann, 2015). Local and state jurisdictions use these criteria to develop regulations for excluding people from these settings and for clearing them for return. Exclusion and clearance criteria vary by organism and setting. For example, persons with simple diarrheal illness without a specific diagnosis are typically excluded from daycare until diarrhea has ceased for 24 hours, while children with *E. coli* O157 infection may be excluded until they have two consecutive negative stool cultures. The FDA model Food Code suggests criteria for excluding ill or infected workers from food service establishments (FDA, 2017).

These exclusion and clearance criteria have traditionally used bacterial culture-based methods or other standard diagnostic tests. The use of CIDTs raise several issues. Culture identifies a living pathogen which is indicative of the potential for transmission, but the process of culture takes too much time to be practical for businesses, schools, and other settings. CIDTs offer rapid results but use of these assays for infectivity or test-of-cure is “off-label.” A convalescent person may continue to shed detectable pathogen DNA for longer than they shed viable organisms detectable by culture. Hypothetically speaking, a patient who is no longer infectious may still produce a CIDT-positive test result and continue to be excluded from high-risk settings. Additionally, differences in sensitivity and specificity among CIDT tests might affect the consistency and reliability of testing for exclusion and clearance. A balance will need to be struck between protecting uninfected individuals in high-risk settings and avoiding unnecessarily lengthy exclusions for individuals who are no longer infectious to others. One could screen a group rapidly by CIDT in high-risk transmission settings for diseases such as STEC infection and salmonellosis. The best approach to clearance remains to be established. A CSTE work group was formed after the 2018 Forum to begin development of interim guidelines.

The cost of exclusion screening and clearance testing is another consideration yet to be resolved. The cost of repeated testing needed for clearance may not be covered by health insurance providers. Culture-based tests are often provided at no cost by PHLs. If the use of CIDTs, which are considerably more expensive than culture, becomes routine for screening and clearance testing, a fair and rational funding mechanism needs to be identified.

Wide-ranging suggestions were made by Forum participants to solve problems associated with decision making. These are summarized in [Table 1 \(Appendix 2\)](#).

## MAINTAINING ISOLATE-BASED SURVEILLANCE

Maintaining isolate-based surveillance systems will remain a public health priority until new technologies that can characterize pathogens directly from clinical specimens are ready for implementation. However, ensuring the availability of isolates and specimens from clinical and reference laboratories to public health agencies in support of national public health surveillance is challenging and requires multi-stakeholder coordination and collaborations. Solving the complex issues requires identifying nodes of opportunity in the total testing and reporting processes. See [Table 2 \(Appendix 2\)](#).

### General Challenges to Maintaining Isolate-based Surveillance

As previously described, molecular characterization of foodborne pathogens (e.g., PFGE or WGS) is a powerful tool for detecting outbreaks and monitoring trends in pathogen virulence and antimicrobial resistance. Yet both PFGE and WGS require isolates, which are not directly provided through the CIDT testing process. For most CIDT test systems, specimens are analyzed directly without a culture step. The additional step of reflex culturing a positive patient specimen to recover the bacterial pathogen identified with the CIDTs is necessary until culture-independent molecular characterization methods can be validated and deployed (see section “Moving Towards Culture-Independent Molecular Characterization” below). It is not clear if the responsibility for reflex culture should reside with clinical laboratories, reference laboratories, public health laboratories, or some combination of laboratories. Significant issues that impact this decision includes funding, workforce, and

geographic distance. For example, the cost of reflex culture could be as high as 18 million dollars per year for PulseNet and NARMS pathogens based on a CDC estimate (2019 unpublished data, CDC), and it is not clear who is responsible for bearing these costs or where funds would come from.

Clinical laboratories are under increasing financial pressures as reimbursements shrink, and many laboratories now lack capacity needed to meet varying public health isolate submission requirements. As more clinical laboratories implement CIDTs, there are economic incentives to eliminate culture. In some states, clinical laboratories (including clinical reference laboratories) are legally mandated to provide an isolate to PHLs, but those laboratories may be performing this task without being recognized for their contributions to public health, both in terms of effort and expense. Clinical laboratories could potentially be reimbursed for reflex culture as they are for other tests, but reimbursement options are unclear because (a) reimbursement is for work performed on behalf of a patient, and for most pathogens reflex culture is not required for patient care and therefore may be considered duplicative; (b) the conditions under which reimbursement would be appropriate have not been defined for the various healthcare payers in the US; and (c) test-based reimbursement requires a test order and test code but a specific reimbursement test code for reflex culture from positive CIDT specimens does not currently exist. Finally, clinical laboratories charge or receive funding for activities that cannot be charged to individual patients and considered overhead, such as quality monitoring, training, administration, etc. Resources for these activities are determined through workload monitoring, but there are no current mechanisms in place to capture isolate recovery for public health.

PHLs typically perform tests for patient care such as newborn screening and diagnosis of sexually transmitted diseases, tuberculosis, and exotic diseases. These types of tests are done under regulatory oversight and are eligible for reimbursement. However, a significant portion of the PHL testing portfolio includes tests that are conducted to benefit the general population but not specifically the patient whose specimen is being tested, and therefore this work may be ineligible for patient-based reimbursement. PulseNet and NARMS activities are in this category. All states receive some funding for public health activities from the federal government, and some states receive additional funding from state or local governments. However, funding for public health was stretched thin before this issue arose, and most PHLs have not identified a mechanism for conducting large numbers of reflex cultures that could potentially come from clinical laboratories that are unable or unwilling to perform reflex cultures. Furthermore, isolate recovery at the PHL level increases total turnaround time, and the additional stress to any pathogen present will be increased during transport and may lower recovery rates. Both of these limitations lower the sensitivity of surveillance activities. For foodborne disease this means that fewer outbreaks are detected and solved, less cases are prevented, and problems in the food supply are not identified and corrected.

Once the responsibility for reflex culture has been determined, technical challenges remain in determining the most effective and efficient methods for isolate recovery. Primary culture procedures for diagnostic testing are well established, but these can be complex and resource, time, and labor intensive, and perhaps not even appropriate for recovery of an isolate where the agent to be isolated is already known (as in reflex culture of CIDT positives). Sensitivity is a key performance measure for diagnostic culture, but requirements may be different for isolate recovery where patient management is not at stake. Guidance documents for streamlined isolate recovery are currently lacking.

CIDT specimen collection methods must be compatible with culture-based methods if isolates are to be obtained for public health efforts. Importantly, culture-based methods require that the organism be viable (i.e., alive). CIDTs, on the other hand, can detect living or non-living genetic targets. The medical device industry appears to be moving towards point-of-care CIDT testing devices that may include specimen inactivation immediately after collection to assure safety in the non-laboratory setting. Such a move, should it occur, would have detrimental if not catastrophic consequences for isolate recovery.

Finally, attempts at the state or local level to create legislative solutions to the challenges posed by CIDT implementation can be problematic themselves. Each state has different rules for reporting of infectious disease and submission of isolates or other materials, as described below. How these rules are worded may impact enforceability or ability of clinical laboratories to seek reimbursement or workload credit. The diversity of reporting rules also creates logistical problems for reference laboratories that are serving multiple states.



## Current Approaches to Addressing Challenges in Maintaining Isolate-based Surveillance

Who conducts reflex culture, and who pays? This continues to be one of the more difficult issues in our public response to CIDTs and was one of the more active areas of discussion in the Forum. Most clinical and reference laboratories that perform reflex culture are doing so voluntarily. Other clinical laboratories send CIDT positive patient specimens (e.g., stool) to a PHL for isolate recovery. Some states provide courier services for efficient and timely transport of specimens to PHLs, while others rely on the US mail or private transport companies. A few jurisdictions have pursued mandating reflex culture by the reporting clinical laboratory. In all situations, laboratories conduct this work without specific compensation or workload credit. In at least one state, the legislature has funded the state PHL to perform this function. Although the laboratory community has engaged in considerable discussion about reimbursement and funding, there is no national consensus on the best approach or path forward.

### State Reporting Rules and Submission Requirements

All states require reporting of selected illness to local or state health departments. Most states additionally require submission of isolates or other clinical materials to local or state public health laboratories for selected reportable infectious diseases, such as salmonellosis or STEC disease. How these laws are written directly impacts a number of activities related to a CIDT response, such as (i) robustness of isolate submission, (ii) flexibility of the surveillance system to changing testing paradigms, (iii) potential for clinical laboratories to advocate for additional support, (iv) ability of states to obtain isolates or other clinical materials from out-of-state reference laboratories, and (v) potential for laboratory regulatory authorities to monitor compliance with public health mandates. To better understand state variation in requirements for submission of isolates or other clinical materials by clinical laboratories, APHL analyzed state regulations for enteric isolate submission in Dec 2015 (APHL, 2016b), and developed a position statement for establishing legal requirements for the submission of enteric disease isolates and/or clinical material to PHLs in 2015 (APHL, 2015). Subsequent work by the CDC Law Project has examined state disease reporting statutes and regulations requiring clinical laboratory submissions to health departments to identify characteristics of the legal language that could facilitate isolate submission (Hulkower, 2019). The legal framework used by jurisdictions to govern disease reporting requirements and direct reporting and submitting procedures varies greatly. Most jurisdictions' laws only require mandatory submission of isolates without instructions for submission if CIDT methods were used, and without a list of alternatives if no isolate is available. It is not clear if regulatory efforts to mandate reflex culture have been successful, nor is there consensus on the wisdom of that approach, especially without solving reimbursement issues discussed elsewhere.

### Expedited Isolate Recovery

Establishing best practices for isolate recovery remains a challenge, and the cost, turnaround time, and labor involved in isolate recovery are limiting factors. Laboratory tests for diagnostic purposes are designed to maximize their performance characteristics (e.g., sensitivity, specificity, predictive value). While these qualities are also important for isolate recovery for surveillance purposes, the issues are slightly different because the results are already known, and a falsely negative reflex culture would negatively impact surveillance but not patient care. Therefore, the emphasis of on-going isolate recovery studies is obtaining maximum recovery using the least number of resources.

It is currently common practice for many PHLs to use five or more plating media, multiple enrichments, and extensive screening of individual culture "picks" for *Salmonella*-positive stool specimens received, which is very labor intensive, costly and time prohibitive. Although specimen collection and diagnostic culture procedures have been used routinely for more than a half century, there are surprisingly little scientific data available to justify current recommendations. CDC, APHL and state PHLs have partnered to develop guidance on optimized isolation workflows for the two pathogens most reported to PulseNet, STEC, and *Salmonella* (Williams-Newkirk, 2019). Preliminary research at CDC examined optimal transport and storage conditions, culture media, and optimal workflow. Testing performed at CDC examining specimen transport conditions (media and temperature) and the most common plating and enrichment media found that holding specimens at room temperature instead of the traditionally recommended refrigerator temperature allowed for recovery from stools spiked with

as little as 100 *Salmonella* bacteria per milliliter of specimen without enrichment. With enrichment, recovery was possible with as few as 10 *Salmonella* bacteria per milliliter of stool. This is a significant improvement over the frequently cited sensitivity threshold of  $\geq 1000$  bacteria per milliliter of stool needed for practical isolate recovery (Kuijpers et al., 2018). Early results suggest that not all of the time-honored assumptions may be justified. In 2019 a new optimized CDC workflow for *Salmonella* recovery was piloted at three PHLs in parallel with their standard protocols to evaluate efficacy. If broadly adopted, this workflow is anticipated to reduce material costs and labor by as much as 80%, while having a minimal impact on the number of isolates available for surveillance. It may be possible to further reduce the cost of reflex culture using specimen screening protocols to limit recovery protocols to those specimens most likely to yield a viable isolate. This type of research needs to be extended to other enteric pathogens.

## Communication and Cooperation Between Key Partners

Engagement and communication between clinical and PHLs and other stakeholders will be paramount in solving the issues discussed at the Forum. A number of different approaches have been taken to address the challenges associated with maintaining isolate-based surveillance until alternatives can be implemented. Beginning in 2012, consultations began among clinical, public health and reference laboratories, APHL, accreditation organizations, professional lab organizations, AdvamedDx (a trade association for the medical device industry), CDC, CMS and FDA. These consultations resulted in increased awareness among device manufacturers involved in designing test systems including (a) the importance of isolate recovery and, (b) preparing for the future of culture-independent public health testing. Public health language was collaboratively developed for inclusion in package insets for newly cleared or approved molecular multi or single analyte detection panels that detect reportable pathogens. Development of resources such as the joint 2016 “APHL/ASM Interim guidelines for submission of enteric pathogens from positive culture-independent diagnostic test specimens to public health” has aided the collaboration between clinical and PHLs, which is critically important to maintaining the availability of isolates and specimens (APHL, 2016). While progress has been made, exploring opportunities to continue to improve engagement and communications remains a critical need.

## Identified Gaps and Potential New Solutions

Despite the ongoing approaches described above, a large number of gaps and potential solutions were articulated by Forum participants.

### 1. Who Conducts and Pays for Reflex Culture?

#### Current Gaps

As described earlier the nationwide cost of reflex culture is considerable, and there is no national consensus as to which laboratory should perform the culture and who should bear the associated costs.

#### *Sources other than Medicare or private payers:*

- Some states reported having a mechanism for payment to clinical laboratories for reflex culture — how widely used is this mechanism?
- Funds for isolate recovery are generally insufficient in PHLs. This applies to both states that pay clinical laboratories for reflex culture, and those that conduct isolate recovery in the PHL. What role can or should the Federal government play?
- Some states have successfully obtained local funding for isolate recovery — how widely has this occurred? How was this accomplished, and could it be applied elsewhere?
- Are there other ways in which clinical and reference laboratories can be encouraged/incentivized to participate in isolate recovery/ retaining specimens for public health surveillance activities?

***Reimbursement through Medicare, private payers, or clinical laboratory overhead:***

- Payers are not currently part of the solution. Could they be?
- For purposes of patient management, a reflex culture on CIDT positive specimens is considered a duplicative test by CMS because there is already a result from the CIDT informing clinical management. A duplicative test, even if a provider orders it may not be reimbursed. Could reflex culture activities be bundled with standard test operating procedures, and reflected in the reimbursement?

**Potential Solutions**

***General***

- Establish a workgroup to consider reimbursement and other isolate recovery incentives, including (but not limited to) those described in the paragraphs below.
- To ensure understanding by both providers and insurers, definitions would need to be developed for “Diagnostic stool” (specimen used with CIDT for primary patient diagnosis) and “Isolate recovery stool” (specimen used to comply with public health mandates).

***Sources other than Medicare or private payers:***

Establish a workgroup to consider options, such as:

- Payment (or workload credit) via coding and coverage.
- Develop relevant codes so that reflex culture, isolate recovery and shipment of isolates can be documented and properly reimbursed and/or work credit assigned.
- Develop funding and billing systems to reimburse clinical and reference laboratories for reflex culture
- Identify jurisdictions that have successfully secured public funding for isolate recovery (e.g., Colorado) and study strategies used to see if they could be applied more widely. Identify and fund courier service to transport CIDT positive specimens from clinical laboratories to public health laboratories. This would reduce costs for clinical laboratories and would reduce turnaround time issues.

***Reimbursement through Medicare, private payers, or clinical laboratory overhead:***

- Explore with the medical device manufacturers and CMS the idea of incorporating reflex culture into the protocol for the primary test (e.g., CIDT) to allow the cost of reflex culture to be built into reimbursement.
- Fee for service: Improve documentation with CPT (medical billing code) or modifiers.
  - Streamlined process for new CIDT codes.
  - National Council on Compensation Insurance (NCCI) edits should allow for “co-coding” of CIDTs and CPTs where indicated.
  - Possible CPT/HCPCS (healthcare common procedure coding system) modifier to indicate when a procedure is for public health purposes.
  - Encourage payers to reimburse testing “performed for public health purposes.”
  - Develop codes for shipping and handling for clinical laboratories.
- Prospective payment: CPT or modifiers and practice guidelines to recognize the need for isolate recovery for public health. Payment for services in a defined clinical episode of care are bundled into a single payment amount, e.g., DRGs, APCs, care bundles. Services must still be documented through correct coding.
  - Correct and complete coding are critically important to document work input for laboratories used in “productivity” and staffing assessment.

- Ensure that algorithms and protocols for testing include compliance with infection prevention and public health requirements.
- Value based payment: Establish lab metrics to show that labs are in compliance with public health mandates.
- Payment incentives for compliance with infection prevention and public health requirements with specific monitors to assess compliance. For example:
  - Did the laboratory report XXX disease?
  - Did the laboratory attempt culture recovery for XXX disease?
  - Did the laboratory submit XXX isolate to public health?

## 2. Role of Laboratory Regulations and Accreditation

### Current Gaps

It is unclear whether laboratory regulations can be used to facilitate compliance with public health requirements. For example, if activities associated with state reporting rules are included in regulatory standards, clinical laboratories may be able to effectively claim reflex culture activities as part of overhead costs. The Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations include federal standards applicable to all US facilities or sites that test human specimens for health assessment or to diagnose, prevent, or treat disease ([www.cdc.gov/clia](http://www.cdc.gov/clia)). CMS, in partnership with CDC and FDA, manages the CLIA program. There are seven CMS approved organizations with accrediting authority under CLIA, including the College of American Pathologists (CAP Clinical laboratories in 2 states, New York [except for physician office laboratories] and Washington. These organizations are exempt from meeting CLIA requirements as they are subject to state licensure requirements approved by CMS as meeting or exceeding CLIA regulations). The CLIA regulatory standards and other standards established through the approved accreditation organizations focus on the quality and reliability of clinical laboratory testing. What opportunities exist to increase awareness of CIDs and public health needs among CMS state and regional surveyors and accreditation organizations inspectors? What role can CMS and accreditation organizations play in encouraging compliance with: a) state and local reporting and submission rules; b) following manufacturer's instructions; and c) public health recommendations/mandates?

- For laboratories with College of American Pathologist's (CAP) accreditation, the laboratory General Checklist (GEN.20374) – Federal/state/local regulations, asks if your laboratory has a policy to ensure compliance with applicable federal/state/local laws and regulations.
- Discussion held around feasibility of proposing new checklist question related specifically to CIDs and whether it would pass the CAP checklist committee review to propose adding this to the Microbiology Checklist or whether this would be considered a duplication of the general checklist requirement (GEN.20374).

### Potential Solutions

- Discuss opportunities for informing CMS state and regional surveyors and accreditation organizations inspectors about CIDT challenges.
- Engage with CAP and propose new CAP microbiology checklist question: Are you complying with public health mandates from your state/local requirements for isolate/specimen submission with respect to CIDs?
- Partner with professional laboratory organizations to develop practice guidelines for syndromes of public health concern that delineate appropriate testing algorithms for public health testing.

### 3. Role of State Reporting Rules

#### Current Gaps

Reporting of infectious disease and submission of isolates or specimens is under the jurisdiction of State and/or local governments. The wording and legal underpinning for these rules varies greatly, creating problems previously described. Since reporting rules are periodically reviewed and revised, national leadership would not only help state and local governments but would also help ameliorate several the issues.

#### Potential Solutions

Continue with efforts to strengthen reporting rule language nationwide with regard to isolate/specimen submission through the CDC Law Project. Although reporting rules exist under different legal structures in different states and a “one-size-fits-all” approach to nationwide standardization is unlikely to work, it may be possible to create model for a variety of legal frameworks.

### 4. Isolate Recovery Protocols

#### Current Gaps

As described earlier, there is a general lack of guidance on standardized media, transport, and storage conditions to optimize isolate recovery which has different goals than primary diagnosis. Creation of a guidance document is limited by a lack of data to support recommendations. For example, even though recommendations for storage and transport of specimens for pathogenic *E. coli* have been available for decades, there seems to be little if any data in the literature to support such recommendations. Furthermore, Cary Blair media (the most common transport medium for enteric culture) from different vendors are not the same and have different transport temperatures stabilities. While some progress has been made in increasing our basic understanding of optimal transport conditions and culture media for recovery of *Salmonella*, as described earlier, more work needs to be done to identify optimal workflows, and basic research needs to be extended to other enteric pathogens such as STEC, ETEC, *Shigella* spp., *Campylobacter* spp., *Vibrio* spp., and *Yersinia* spp. Finally, there is no consensus on if or how the results of reflex culture should be reported.<sup>b</sup>

#### Potential Solutions

- Continue to develop “best practices” for performing efficient isolate recovery.
  - National adoption of optimized isolate recovery workflows for *Salmonella* and Shiga toxin-producing *E. coli* (STEC).
  - Continue to develop other streamlined isolation protocols for specific pathogens for use in PHLs.
- Develop evidence-based guidance for specimen prioritization and appropriate decision points to reduce unsuccessful recovery efforts.
- Continued engagement between clinical and PHLs to ensure specimen correctly preserved to obtain isolates as appropriate, if a test is initially CIDT positive.
- Include suggestions for reporting in final isolate recovery protocol.
- Support routine courier services in each state to bring CIDT positive specimens to state PHLs.

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<sup>b</sup> Editor’s Note: If the isolate recovery protocol is optimized for speed and cost rather than performance characteristics appropriate for diagnostic testing, the results should only be reported to epidemiology partners.

## 5. General Communication Issues

### Current Gaps

Potential funders of reflex culture (e.g., CMS or other federal agencies, state or local governments, or private payers) may not be aware of the cost effectiveness of surveillance and outbreak investigations. Coordinated education and outreach to multiple stakeholders to increase awareness of CIDTs and impact on public health is needed. Key stakeholders include clinicians (to increase awareness and understand the tests they are ordering), public health officials (to encourage advocacy), PHLs (to educate local public health departments on emerging technologies), payers (to understand issues of reimbursement), CMS state and regional surveyors, and accreditation organization inspectors (to assist with enforcement of reporting rules), and diagnostic device manufacturers (to reinforce awareness during product development that viable specimens are needed for reflex culture). CDC and NGOs such as APHL and CSTE are also key stakeholders and are essential for nationwide coordination.

### Potential Solutions

- Conduct and disseminate studies to demonstrate the societal benefit and economic costs of surveillance.
  - Update CDC cost analysis to identify what are the cost estimates to public health and clinical laboratories to perform reflex culture.
  - Develop economic modeling to demonstrate population benefit (for advocacy with state legislators, federal government, etc.)
  - Increase scientific and analytic publications on the burden and costs associated with foodborne illness outbreaks and the positive impacts of foodborne disease surveillance systems.
  - Collaboratively develop a communication plan and toolkit that includes stakeholder specific resources with consistent messaging about public health needs, and that can be used by multiple stakeholders.
  - Increase engagement of payers to help in the recognition of value and to encourage payment.
  - Educate laboratories about state reporting laws to ensure they are aware of the possible need for a second specimen depending on the device they are using, as well as potential need to isolate and submit specimens.
- Consider development of national level model language for requiring or requesting referral of isolates or CIDT positive specimens from clinical laboratories to PHLs. (also see “reference laboratory issues” section)

## 6. Communication and Coordination Between Clinical Laboratories and PHLs

### Current Gaps

Communication and coordination between clinical laboratories and PHLs is specifically needed when clinical laboratories implement CIDT panels because of the following issues: (a) Understanding of shared contribution may be lacking; (b) two-way information flow is absent or inconsistent, and (c) there is limited guidance or framework for engagement – for example, specific PHL needs such as turnaround time are not always clearly defined and communicated to clinical laboratories. There is a need in general to recognize the value of laboratory services in the context of population health.

### Potential Solutions

- Educating clinical laboratories on (1) the value of CIDTs, and (2) the value of laboratory testing including reflex culture for population health.
- Increasing active coordination between clinical laboratories and PHLs by state-wide conference calls or jointly developed journal articles or seminars (to improve understanding of shared contribution) and jointly developing guidance documents to clarify PHL requirements.

- Promoting active engagement between PHLs and clinical laboratories as new CIDTs come on the market before implementation in clinical laboratories. This engagement would include a mutually acceptable plan to provide isolates and specimens and define a two-way information flow between clinical and PHLs.
- Continuing discussions with stakeholders at conferences and meetings and extend the scope to other infectious diseases where CIDTs are likely to have an impact.

## 7. Communication Among Clinical Users, Public Health, and the Medical Device Industry

### Current Gaps

Continued engagement is needed between diagnostic device manufacturers and users (clinical and PHLs) about how current and future novel diagnostic collection devices will support industry development as well as isolate recovery for public health needs.

- Different CIDT vendors have different specimen requirements, which poses a problem for follow-up reflex testing.
- There are no recommendations as to actions for clinical laboratories to take when isolate recovery is not possible.
- End users/public health will need to be aware of changes to collection devices or protocols which could impact isolate recovery.
- Manufacturers working on new collection devices that inactivate specimens should consider protocol modifications to make their devices compatible with public health activities.

### Potential Solutions

- Recommendations should be developed for clinical laboratories on how to proceed with required reporting/ submission when CIDTs are exclusively used, and specimens are not suitable for isolate recovery. This may include (future) submission of inactivated (non-viable) material or DNA to laboratories for advanced culture-independent pathogen characterization and changes to wording in reporting rules.
- Continue dialog between diagnostic device manufacturers and users (clinical laboratories and PHLs) with follow-up meeting about novel collection devices to support industry development as well as isolate recovery for public health needs.
- Manufacturers should consider modification of specimen collection language to support public health activities, such as collection of a second specimen (e.g., rectal swab) or splitting a specimen (e.g., stool) which can be preserved for isolate recovery.
- Encourage collaboration amongst clinical and PHLs, regulatory agencies, and device manufacturers to ensure that laboratories can maintain compliance with public health mandates when using current and future novel diagnostic devices.

Specifically, to the last bullet, public health partners should work with FDA and manufacturers to amend package inserts to include language alerting users that testing needs to be compatible with public health submission and reporting mandates. Device restrictions may require additional specimen collection from the patient to maintain compliance. If a test system and/or collection device cannot preserve the specimen for subsequent isolate recovery, then the manufacturer and end user need to be aware of this, and language should be provided in the package insert that clearly states the device's inability to support isolate recovery.

## 8. Reference Laboratory Challenges

### Current Gaps

Reference laboratories (i.e., laboratories that serve multiple healthcare institutions) receive specimens from multiple different laboratories and often from long distances, but there are no standards for media, transport, and storage that need to be met. For instance, freezing may inhibit subsequent growth of some organisms. The specimen submitted may also not be appropriate for culture. Reference laboratories also work with multiple state PHLs and reporting requirements differ. Standardization of what is needed or mandated would be very beneficial and allow greater efficiency. Strategies for handling and interpreting discrepant results between the CIDT and reflex culture need to be better defined.

Unresolved issues:

- It is not clear who is responsible for initiating/ordering an isolate recovery culture. Reference laboratories generally require a test order to provide additional testing on a specimen.
- Varying state PHL reporting, and submission requirements are challenging to keep up with.
- Do reference laboratories that perform CIDT need to create a specific stool CIDT test code with a reflex to an isolate recovery culture?
- Do reference laboratories need to create specific isolate recovery test codes for their laboratory clients that perform CIDT in-house?

### Potential Solutions

- Develop model reporting and isolate submission language for state reporting rules and encourage their use.
- Consider flexibility in state requirements regarding materials to be submitted (e.g., specimen or swab in Cary Blair transport medium vs isolates).

## 9. Lack of Interoperability/IT Challenges

### Current Gaps

- Clinical and public laboratory information and data systems are not interoperable; they use multiple different systems and multiple vendors.
- Reporting needs to be easier and in real-time.

### Potential Solutions

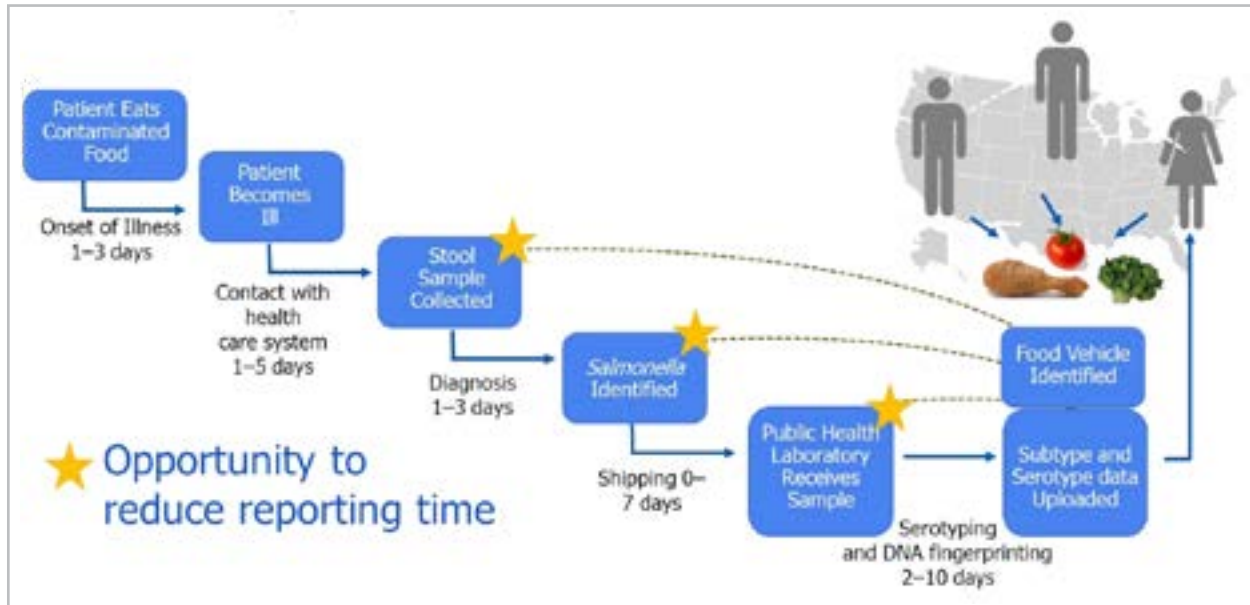
- Modernization of laboratory data architecture.
- Automated ways to push results to public health within system that generates alert triggers. Get the data timely to the people who can do something actionable with the data, even if isolates are not yet available.
- User-friendly bioinformatics platforms for data uploading, analysis and reporting.
- Future diagnostic tests need to ensure compatibility and efficient data flow between clinical laboratories and PHLs.



## MOVING TOWARDS CULTURE-INDEPENDENT MOLECULAR CHARACTERIZATION

Maintaining the flow of culture isolates to public health is necessary in the short term, but the process is expensive, slow, and unlikely to be sustainable in the long-term. The development of assays for characterizing pathogens directly from clinical specimens, without the need for a culture step (i.e., culture-independent pathogen characterization) is needed to speed up the surveillance process and make public health activities compatible with those of clinical diagnostics.

Figure 2. Direct-from-specimen Tests: Reduce Time to Actionable Results. Source: \_\_\_\_\_



### Direct-from-specimen Pathogen Characterization: General Challenges

Stool specimens are complex, containing hundreds or thousands of bacterial, viral, fungal, and protist species as well as human, plant, animal, and other eukaryotic DNA. Additionally, stool contains a wide variety of other digested or undigested materials. There may be multiple bacterial strains in the stools that are genetically similar to pathogens and therefore are hard to distinguish, or disambiguate, from the pathogens of interest. In general, the cost of molecular-based methods is high, the analysis software is not designed for public health needs, and IT demands are high.

The requirements of practical direct-from-specimen pathogen characterization assays for public health include (but are not limited to):

- Sensitivity and specificity; sufficient ability to separate pathogen signal from background commensal flora while fulfilling resolution requirement
- WGS-level strain resolution, compatible with isolate-based surveillance
- Antibiotic resistance and virulence determinant identification capabilities
- Turnaround time equal or less than isolate-based surveillance (e.g., < 5 working days)
- Cost similar to isolate-based surveillance (e.g., < \$150/specimen)
- Compatibility of specimen collection protocols with specimens collected for clinical diagnostics
- Practical workflow (e.g., works with a standard workday, equipment fits in standard laboratory, etc.)

- Compatibility with existing or feasible IT infrastructure and bioinformatics software
- Compliant with privacy concerns (e.g., HIPAA or other state/federal data privacy regulations).

Two general approaches have been proposed, each with its own benefits and challenges including: (a) targeted (amplicon) sequencing; and (b) shotgun (unbiased) sequencing. A third approach, single-cell sorting and sequencing, was discussed. See [Table 3 \(Appendix 2\)](#).

## Targeted (Amplicon) Sequencing: Discussion Overview

Targeted sequencing of amplification products, such as 16S profiling, is commonly used in studies of microbial communities. It is now being explored as an approach to characterize pathogens directly from stool specimens using large numbers of targets, generating an output compatible with an isolate-based WGS bioinformatics workflow. This approach is attractive because it largely depends on existing technology and overcomes issues of sensitivity inherent in shotgun approaches by utilizing amplification products. However, because targeted sequencing uses hundreds or thousands of pathogen-specific targets, it: (1) requires considerable up-front development for each agent; (2) may not provide suitable specificity for pathogens which share a majority of genomic loci with commensal flora (e.g., STEC); and (3) requires a cost-effective platform capable of large-scale multiplexing. Note: amplicon sequencing approaches are sometimes referred to as “metagenomics” methods due to their historical use of for characterization of microbial populations. However, species-specific applications cannot technically be so classified.

Both mechanical and chemistry-based highly multiplexed amplification sequencing (HMAS) platforms have been described. Mechanical platforms involve the use of microfluidics to simultaneously amplify large numbers of targets. Chemistry-based methods involve the use of pooled PCR reactions facilitated by proprietary chemistries. CDC has down-prioritized the use of chemistry-based HMAS due to the large amount of optimization required for each assay, hands-on-prep time, and poorly-scaling workflow. Instead, it has focused on a commercial microfluidic HMAS platform for development of a direct-from-specimen assay to characterize *Salmonella* spp. to the strain level. This involves identification of short, informative regions of single copy genes using thousands of pathogen-specific primers. Additionally, other markers for antibiotic resistance genes, virulence, serotype, and potential markers for consumed food can be easily added to the HMAS primer panel.

Unknown or unresolved issues include:

- Mitigation of off-target amplification
- Crosstalk between microfluidic chambers
- Reagent contamination
- Limit-of-detection (LOD)/sensitivity/specificity
- Interpretation of data, including calling genes with partial data and appropriately weighting sequence abundance when establishing call confidence
- Difficulty in bioinformatically resolving results from closely related mixed infections.

## Amplicon Sequencing: General Conclusions, Issues, Potential Solutions

- Microfluidic Highly Multiplexed Amplicon Sequencing (HMAS) panels seemed to the group to have a reasonable chance of achieving surveillance goals give sufficient investment in research and development.
- The utility of HMAS panels would be increased if antibiotic resistance determinants, virulence genes, and serotyping genes were included in panels with targets designed for strain typing (e.g., cgMLST). Although initial results with a panel targeting AMR genes seem promising, several challenges remain including sensitivity and specificity of primers, and off-target amplification.
- The HMAS approach may be applicable for other enteric (e.g., STEC, *Vibrio* spp., etc.) and non-enteric pathogens, improving its utility as a cross-cutting technology.

- Commercially available platforms for the efficient execution of HMAS panels in PHLs already exist, and both the per-specimen cost and specimen-to-subtype turnaround times are potentially compatible with public health limitations.
- The types and volume of data produced by HMAS panels are compatible with existing IT infrastructure in PHLs.
- New approaches are needed for separating core-genome pathogen sequence information from closely related commensal flora, such as *E. coli* O157:H7 and commensal *E. coli*. Approaches such as differential read quantitation are currently being explored.
- Emerging/novel highly multiplexed amplification technology that could potentially meet the program specifications should be evaluated when/if it becomes available.

## Shotgun (Unbiased) Metagenomics Overview

Shotgun metagenomic approaches involve sequencing of all genetic material in a sample. As will be described, current approaches may seek to enrich the sample for the pathogen(s) of interest, but once the sample is prepared, sequencing is largely an unbiased process. This approach may have wide application in infectious disease and should be compatible with its emerging use in clinical diagnostics. The method has potential as both a primary diagnostic method and an advanced pathogen characterization method, and thus may be important for increasing our understanding of disease causation and carriage and may further decrease turnaround time for both diagnostics and public health.

Metagenomics in clinical practice: Metagenomics is rapidly transitioning from a diagnostic test of last resort for critical illnesses such as meningitis, encephalitis, and sepsis to a primary diagnostic method. It seems likely that this trend will expand into other syndromes such as respiratory and gastrointestinal illnesses as tests improve and costs decline. Medical providers will use this data to diagnose their patients, and public health will need to tap into this data stream to gather information about trends that more broadly affect the population.

Metagenomics in public health: In the outbreak setting, shotgun metagenomics will be a powerful tool for pathogen discovery. However, for the method to be practical for surveillance several bottlenecks need to be resolved including: (a) sensitivity, (b) “phasing” or separating pathogen reads (at needed taxonomic levels) from similar reads originating with commensal flora, (c) cost, (d) software limitations, and (e) IT demands. Increasing the sensitivity and specificity of pathogen signals may be achieved by wet lab methods, bioinformatics methods, or combinations of the two. Increasing pathogen signals in the wet lab involves enrichment of pathogen nucleic acids (i.e., “target enrichment”) by depletion of non-target DNA or by capture of pathogen reads. Wet lab enrichment has the potential of reducing overall cost by reducing the amount of sequencing required to reach the desired level of coverage, which also lowers the amount of data to be processed, uploaded, and stored. Bioinformatics binning methods can be used to isolate pathogen signals and distinguish them from commensal sequences. The ability to separate pathogen and commensal sequences is also referred to as phasing.

## Shotgun (Unbiased) Metagenomics Approaches and Issues: Targeted Enrichment, Phasing, Data Gaps, Software Gaps, Infrastructure Limitations

### Targeted Enrichment

Each of the following have potential for addressing signal-to-noise issues but require additional research and development to become practical for surveillance.

- Biotinylated bait-capture approaches, including commercially available kits, work by preferentially binding and pulling down sequencing library DNA that match the target sequence, generally the pathogen of interest. Remaining challenges include cost, hands on time of workflow, and disambiguation of common sequence targets that are found in both commensals and pathogen.

- CAS9 approaches such as Finding Low Abundance Sequences by Hybridization (FLASH) and Depletion of Abundant Sequences by Hybridization (DASH) (pioneered by the Crawford and DeRisi laboratories at the Chan Zuckerberg BioHub and by the University of California, San Francisco) have been successfully applied for use with antibiotic resistance markers and depleting human genomic material where sequences are well defined. It is not clear how the specificity of the method would impact its ability to enrich for loci with undefined variation, which is an inherent requirement for markers used for bacterial pathogen strain typing. In other words, how much miss-matching does the method allow? The method has not been commercialized and it is not clear if its cost would be problematic in a surveillance setting. Further research is needed to answer these questions. Potential solutions needed to fill the gaps: Optimization for tackling high number of targets, enrichment for very low abundant organisms, and human DNA removal.

## Phasing Approaches

CDC is addressing phasing by evaluating Hi-C, a formaldehyde-based DNA cross-linking method with bioinformatic deconvolution. CDC is also considering long-read sequencing as the cost and error-profiles improve. High-fidelity, low-cost and long-read low-cost sequencing methods would aid in solving phasing issues, but such methods are not currently available. Finally, phasing by read quantification may allow separation of pathogen signals from closely related commensal strains, such as *E. coli* O157:H7 and commensal *E. coli*.

## Data Gaps

There are a number of data gaps that impact outcomes of metagenomics-based analyses such as incomplete databases, mis-annotation, bias in databases, misclassification of organisms that lead to incorrect pathogen identification, and insufficient numbers of well-curated reference genomes that represent the diversity of organisms found in specimens and samples.

## Software Gaps

Additional software development is needed for practical and deployable metagenomic analysis in the public health workflow.

## Infrastructure Limitations

Compute power, storage capacity, and network bandwidth may limit practical implementation of shotgun metagenomics.

## Shotgun Metagenomics: Potential Solutions

### Specimen Collection and Storage

Nucleic acid preservation methods are needed for practical implementation of any metagenomics-based surveillance method. The complexity of the stool microbiome presents challenges to standardizing specimen collection and developing truly uniform specimen transport media – all of which have the potential to impact the ability to directly sequence pathogens in clinical materials. The ideal point of the testing process for stabilizing nucleic acids is during specimen collection at the point of care. Several commercial kits have been successful in stabilizing DNA and community composition and could be ideal for use both in clinical and public health settings. The results of recent evaluations have been encouraging, but further research is needed.

### Databases and Data Quality

- Increase in the number of well-curated genomes in public databases to better represent diversity.
- High quality data in repository databases are needed for robust and accurate data interpretations.
- For detection and accurate annotation of AMR and virulence factors (by any of the described methods), a comprehensive, well-curated database is essential.

## General Knowledge and Data Gaps

- Development of performance metrics (e.g., limit of detection, sensitivity, specificity) to evaluate outcomes, such as outbreak detection and investigation and trend analysis.
- Additional metagenomic data from healthy individuals is needed.

## Research and Development

Broaden research base: better engagement of people from academia, PHLs, clinical laboratories, and commercial vendors is needed to figure out the best approach that fit public health requirements.

## Implementation Issues

- Visualization modules with minimal computational resources should be considered to meet diverse end user needs.
- Metagenomics analyses in public health should be unified computing, extensibility, security, auditability, reproducibility, multi-audience flexibility and data sharing.
- A common language is needed between bioinformaticians and epidemiologists to facilitate data interpretation.
- Unifying reporting across the states will be useful when considering adoption of metagenomics-based approaches in public health.

## Single-cell Sorting and Sequencing

Microfluidic high-speed cell sorting is currently being used for human cells and has the potential to isolate pathogens on a cellular level thereby eliminating the need for culture. This approach would solve a number of intrinsic problems with direct-from-specimen pathogen characterization. PCR-activated cell sorting has been used to separate pathogenic and non-pathogenic species in simple mixtures but has not been demonstrated in complex and high-density mixtures such as stool. If feasible, such a technology would make possible characterization such as whole genome multilocus sequence typing (wgMLST), a high-resolution strain characterization method, for a wide range of pathogens. Challenges include the amount and variety of background cells versus pathogens and the lack of low-cost / high-throughput approaches methods for stool specimens still need to be developed to be able to evaluate this technology. The technology is in its infancy, and much more research to determine its efficacy and practicality for surveillance.

## Direct-from-specimen Pathogen Characterization: Conclusions

- Shotgun metagenomics has the potential for being the method-of-choice for clinical diagnosis of selected invasive illnesses (see “Metagenomics in Clinical Practice” above) and should prove equally important for understanding illness on a population level. Sequencing costs are continuously falling, and new technologies are emerging which could result in inexpensive high fidelity long reads. As a consequence of these trends, it is highly likely that current bottlenecks such as cost, lack of adequate software, phasing issues, and data bandwidth will be overcome in the near future.
- Metagenomics provides a more complete picture of the disease state than HMAS, and protocols are likely to be more universal and less agent-specific. When used as a surveillance tool metagenomics has the potential for not only allowing practical direct-from-specimen pathogen characterization, but also opening up new opportunities for understanding diseases on a population basis.
- As the eventual use of metagenomics in routine clinical practice appears likely. It therefore seems incumbent upon public health to consider this future when planning development of IT and bioinformatics systems, so that this rich data stream can be tapped to discover patterns of diseases in the population that suggest modifiable risks.

- Although the likelihood of HMAS success is high, its utility has not yet been proven. Given the high imperative of replacing isolate-based sequencing it seems prudent for public health to continue pursuit of this widely applicable line of research. This research avenue will allow public health to expand its surveillance repertoire and position itself for the next wave of clinical microbiology.
- Single-cell sorting and sequencing is an intriguing idea that address all of the intrinsic difficulties of characterizing bacterial pathogens directly in specimens. However, this method is still at the concept stage, has the least amount of supporting research of the approaches described, and is the furthest from practical application.

## **CONCLUSION**

The adoption of CIDTs into microbiology laboratory practice represents a major technological shift, impacting both clinical medicine and public health programs that depend on the data. These new tests have considerable potential for improving clinical diagnostics and public health. Reaping those benefits and avoiding harm to systems built upon culture-based data will require concerted and coordinated efforts of multiple public and private entities.

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## GLOSSARY

<b>AMR:</b> Antimicrobial resistance	<b>FDA:</b> Food and Drug Administration
<b>APCs:</b> Ambulatory payment classification	<b>FLASH:</b> Finding low abundance sequences by hybridization
<b>APHL:</b> Association of Public Health Laboratories	<b>HCPCS:</b> Healthcare common procedure coding system
<b>CAP:</b> College of American Pathologists	<b>HMAS:</b> Highly multiplexed amplicon sequencing
<b>CDC:</b> Centers of Disease Control and Prevention	<b>IT:</b> Information technology
<b>CgMLST:</b> Core genome multi-locus sequence type	<b>LDTs:</b> Laboratory-developed tests
<b>CIDTs:</b> Culture-independent diagnostic tests	<b>LOD:</b> Limit-of-detection
<b>CIFOR:</b> Council to Improve Foodborne Outbreak Response	<b>NARMS:</b> National Antibiotic Resistance Monitoring System
<b>CLIA:</b> The Clinical Laboratory Improvement Amendments	<b>NCCI:</b> National Council on Compensation Insurance
<b>CMS:</b> Center for Medicare and Medicaid Services	<b>NGOs:</b> Non-governmental organizations
<b>CPT:</b> Medical billing code	<b>NORS:</b> National Outbreak Reporting System
<b>CRISPR:</b> Clustered regularly interspaced short palindromic repeats	<b>OSU:</b> The Ohio State University
<b>CSTE:</b> Council of State and Territorial Epidemiologists	<b>PFGE:</b> Pulsed-field gel electrophoresis
<b>DASH:</b> Depletion of abundant sequences by hybridization	<b>PHLs:</b> Public health laboratories
<b>DRGs:</b> Diagnosis-related group	<b>POC:</b> Point-of-care
<b>EAEC:</b> Enteroaggregative <i>E. coli</i>	<b>STEC:</b> Shiga toxin-producing <i>E. coli</i>
<b>EIA:</b> Enzyme immunoassays	<b>USDA:</b> United States Department of Agriculture
<b>EPEC:</b> Enteropathogenic <i>E. coli</i>	<b>wgMLST:</b> Whole genome multilocus sequence typing
<b>ETEC:</b> Enterotoxigenic <i>E. coli</i>	<b>WGS:</b> Whole-genome sequencing

# APPENDIX 1: 2018 SURVEILLANCE CASE DEFINITIONS FOR SHIGA TOXIN-PRODUCING *E. COLI* (STEC)

From: <https://wwwn.cdc.gov/nndss/conditions/shiga-toxin-producing-escherichia-coli/case-definition/2018/> (accessed April 29, 2019)

## Clinical Criteria

An infection of variable severity characterized by diarrhea (often bloody) and/or abdominal cramps. Illness may be complicated by HUS (note that some clinicians still use the term thrombotic thrombocytopenic purpura [TTP] for adults with post-diarrheal HUS).

## Laboratory Criteria for Diagnosis

### Confirmatory Laboratory Evidence

- Isolation of *E. coli* O157:H7 from a clinical specimen OR
- Isolation of *E. coli* from a clinical specimen with detection of Shiga toxin or Shiga toxin genes.

### Supportive Laboratory Evidence

- Isolation of *E. coli* O157 from a clinical specimen without confirmation of H antigen, detection of Shiga toxin, or detection of Shiga toxin genes, OR
- Identification of an elevated antibody titer against a known Shiga toxin-producing serogroup of *E. coli*, OR
- Detection of Shiga toxin or Shiga toxin genes in a clinical specimen using a culture-independent diagnostic test (CIDT) and no known isolation of *Shigella* from a clinical specimen. OR
- Detection of *E. coli* O157 or STEC in a clinical specimen using a CIDT.

## Epidemiologic Linkage

- A clinically compatible illness in a person that is epidemiologically linked to a confirmed or probable case with laboratory evidence OR
- A clinically compatible illness in a person that is a member of a risk group as defined by public health authorities during an outbreak.

## Criteria to Distinguish a New Case from an Existing Case

- A new case should be created when a positive laboratory result is received more than 180 days after the most recent positive laboratory result associated with a previously reported case in the same individual. (See formula referenced in Appendix B of the 2017 CSTE Position Statement [17-ID-10] for details on time period calculation, hierarchy of dates and interpretation). OR
- When two or more different serogroups/serotypes are identified in one or more specimens from the same individual, each serogroup/serotype should be reported as a separate case.

## Case Classification

### Suspected

- Identification of an elevated antibody titer against a known Shiga toxin-producing serogroup of *E. coli* in a person with no known clinical compatibility, OR
- Detection of Shiga toxin or Shiga toxin genes in a clinical specimen using a CIDT and no known isolation of *Shigella* from a clinical specimen in a person with no known clinical compatibility, OR
- Detection of *E. coli* O157 or STEC in a clinical specimen using a CIDT in a person with no known clinical compatibility, OR
- A person with a diagnosis of post-diarrheal HUS/TTP (see HUS case definition).

## Probable

- A person with isolation of *E. coli* O157 from a clinical specimen without confirmation of H antigen, detection of Shiga toxin or detection of Shiga toxin genes, OR
- A clinically compatible illness in a person with identification of an elevated antibody titer against a known Shiga toxin-producing serogroup of *E. coli*, OR
- A clinically compatible illness in a person with detection of Shiga toxin or Shiga toxin genes in a clinical specimen using a CIDT and no known isolation of *Shigella* from a clinical specimen, OR
- A clinically compatible illness in a person with detection of *E. coli* O157 or STEC/EHEC from a clinical specimen using a CIDT, OR
- A clinically compatible illness in a person that is epidemiologically linked to a confirmed or probable case with laboratory evidence, OR
- A clinically compatible illness in a person that is a member of a risk group as defined by public health authorities during an outbreak.

## Confirmed

A person that meets the confirmatory laboratory criteria for diagnosis.

## APPENDIX 2: ISSUE, SOLUTION, AND ACTION TABLES

Table 1. Public Health Decision Making

Issues	Potential Solutions	Possible Actions*
Explore new surveillance opportunities	Conduct pilot surveillance projects for pathogens that have become practical to monitor due to CIDTs, such as cyclosporiasis, ETEC and other diarrheagenic <i>E. coli</i> , norovirus.	S, F
Case counting	<ul style="list-style-type: none"> <li>Case definitions: Incorporate CIDTs into CSTE case definitions (e.g., use of “confirmed,” “probable,” and “possible” categories depending on type and legal status of test (e.g., FDA-cleared/approved or laboratory-developed test).</li> <li>Data collection: Adapt public health systems to obtain information on test-type used to make diagnosis as part of the reporting process.</li> </ul>	D, W
Adjusting burden and trend estimates	<p>CIDT performance characteristics:</p> <ul style="list-style-type: none"> <li>Conduct collaborative studies to measure real-world performance characteristics of CIDTs</li> <li>Identify methods for monitoring performance (i.e., detect changes due to modification or deletion of DNA target loci in the patient population, or false positives due to off-target amplification)</li> </ul> <p>CIDT use characteristics:</p> <ul style="list-style-type: none"> <li>Develop systems to obtain test denominator data (total tests performed, including those with negative and positive results)</li> <li>Develop systems to track other use characteristics (e.g., who is being tested and the reason for testing)</li> </ul> <p>Develop models to adjust burden and trend estimates for enteric pathogens based on performance and use characteristics.</p>	S, F, D, W
Resolve interpretation issues	<p>Identify knowledge gaps and conduct epidemiological studies to assess the clinical and public health relevance of:</p> <ul style="list-style-type: none"> <li>Newly available target organisms, such as enteropathogenic <i>E. coli</i> (EPEC) and enteroaggregative <i>E. coli</i> (EAEC)</li> <li>Increasingly identified organisms such as <i>Y. enterocolitica</i> and <i>Vibrio</i> spp. (pathogenic vs non-pathogenic strains)</li> <li>Co-detections (multiple positive targets) in symptomatic patients.</li> </ul>	K, S, F
Use of CIDTs in sensitive settings	<ul style="list-style-type: none"> <li>Convene workgroup to identify knowledge gaps and suggest approaches to filling gaps, and develop policies for use of CIDTs in for public health decision making in sensitive settings, such as daycare and food service. Suggest optimal workflow for CIDTs that balances speed and sensitivity.</li> <li>Conduct study to determine how positive CIDT results correlate with transmissibility.</li> <li>Identify approaches for funding public health screening activities using CIDTs, as their use in this setting is not considered medically necessary (for the patient) and therefore may not be reimbursable by insurers.</li> </ul>	K, S, W

\* Possible Action Codes

K = Identify knowledge gaps    S = Conduct studies    F = Identify funding    D = Modify data systems    W = Convene workgroups

**Table 2: Maintaining Isolate-Based Surveillance**

Issues	Potential Solutions	Possible Actions*
<b>Lack of communication and coordination between clinical and public health laboratories (PHLs)</b>	<ul style="list-style-type: none"> <li>• Co-ordinated active engagement clinical laboratories and PHLs as new CIDTs are adopted.</li> <li>• Define a mutually acceptable plan to provide isolates and specimens.</li> <li>• Define a consistent and improved two-way information, data and isolate/sample flow.</li> <li>• Collaboratively develop a communication plan and toolkit that includes stakeholder specific resources with consistent messaging about public health needs, that can be used by multiple stakeholders.</li> <li>• Perform economic modeling to demonstrate population benefit.</li> </ul>	K, F, D, W
<b>Limited awareness among stakeholders of impact of CIDTs on public health surveillance</b>	<ul style="list-style-type: none"> <li>• Establish recommendations for clinical laboratories on how to proceed with required reporting/submission of CIDTs.</li> <li>• Manufacturers should consider modification of specimen collection language to support public health activities.</li> </ul>	K, F, W
<b>Balancing novel collection devices that support industry CIDT development with public health need for isolate recovery</b>	<ul style="list-style-type: none"> <li>• Establish a workgroup between the device industry and users (clinical and PHLs) to ensure compatibility with public health needs as new technologies are developed.</li> <li>• Modify package insert language to ensure a second specimen is collected if the original specimen is destroyed in the testing process.</li> </ul>	K, F, W
<b>Laboratory regulatory requirements</b>	<ul style="list-style-type: none"> <li>• Define the roles of CMS and accreditation organizations in encouraging compliance with state and local reporting and submission rules.</li> <li>• Explore options to modify laboratory accreditation requirements to include current public health mandates that support surveillance.</li> <li>• Explore opportunities for informing CMS state and regional surveyors/inspectors about CIDT challenges.</li> </ul>	K, F, W
<b>Isolate recovery issues for clinical laboratories and PHLs</b>	<ul style="list-style-type: none"> <li>• Establish CPT codes so that reflex culture, isolate recovery and shipment of isolates can be documented and properly reimbursed and/or work credit assigned.</li> <li>• Conduct research to fill gaps in understanding on optimal collection, transport, and processing for CIDT positive specimens.</li> <li>• Develop practice guidelines for public health testing including prioritization and reducing unsuccessful recovery efforts.</li> <li>• Support routine courier services in each state to bring CIDT positive specimens to state PHLs.</li> </ul>	K, F, W
<b>Reimbursement issues – unfunded public health mandates for clinical and PHLs to perform isolate recovery</b>	<ul style="list-style-type: none"> <li>• Define “Diagnostic stool” versus “Isolate recovery stool.”</li> <li>• Establish CPT codes so that reflex culture, isolate recovery and shipment of isolates can be documented and properly reimbursed and/or work credit assigned.</li> <li>• Establish workgroup to explore multiple payment approaches.</li> <li>• Payer engagement is critical in the recognition of value and to ensure payment.</li> </ul>	K, S, F, D, W
<b>Lack of interoperability/ IT challenges</b>	<ul style="list-style-type: none"> <li>• Modernize laboratory data architecture.</li> <li>• Develop user-friendly bioinformatics platforms for data uploading, analysis and reporting.</li> </ul>	K, F, D, W
<b>Developing standard practices for reference laboratories</b>	Develop model reporting and isolate submission language for state reporting rules and encourage their use.	K, F, W

**\* Possible Action Codes**

K = Identify knowledge gaps   S = Conduct studies   F = Identify funding   D = Modify data systems   W = Convene workgroups

**Table 3: Moving Towards Culture-Independent Molecular Characterization**

Issues	Potential Solutions	Possible Actions*
<b>Amplicon sequencing</b>	<ul style="list-style-type: none"> <li>• Accelerate development of highly multiplexed amplicon sequencing panels (HMAS) for near-term implementation in public health laboratories.                             <ul style="list-style-type: none"> <li>○ Continue research to resolve issues of off-target amplification, crosstalk between chambers, performance characteristics, and interpretation.</li> <li>○ Continue to develop pathogen-specific primer panels for core genes, resistance determinants, serotype markers, and virulence genes.</li> <li>○ Research methods for distinguishing pathogen sequences from closely related commensal sequencing (i.e., “phasing”).</li> <li>○ Evaluate assays in public health settings.</li> <li>○ Adapt WGS surveillance infrastructure to assimilate HMAS data.</li> </ul> </li> <li>• Explore other highly multiplexed amplifications technologies when/if they become available.</li> </ul>	K, S, F, D
<b>Shotgun metagenomics</b>	<p>Continue exploration of shotgun metagenomics as a potential long-term approach to directly characterizing pathogens in patient specimens (and food/environmental samples).</p> <ul style="list-style-type: none"> <li>• Evaluate targeted enrichment methods such as bait capture, CRISPR-Cas9-based Finding Low Abundance Sequences by Hybridization (FLASH) and Depletion of Abundant Sequences by Hybridization (DASH) to improve signal-to-noise.</li> <li>• Evaluate phasing approaches such as Hi-C formalin cross-linking, and long-read sequencing.</li> <li>• Adapt or develop improved software for integrating metagenomics into a public health workflow.</li> <li>• Conduct research to fill data gaps.</li> </ul>	K, S, F
<b>Cross-cutting issues</b>	<ul style="list-style-type: none"> <li>• Continue to evaluate optimal specimen/sample collection, storage, and transport methods.</li> <li>• Improve the quantity, diversity, quality, and annotation of genomes populating public databases.</li> <li>• Improve IT infrastructure in the public health system in anticipation of increased needs for bandwidth, compute power, and storage capacity.</li> </ul>	K, F
<b>Other methods</b>	Begin working with partners to explore other methods to characterize pathogens directly from specimens, such as single cell sorting and sequencing.	S, F
<b>Prepare for clinical metagenomics</b>	Begin planning surveillance infrastructure for the metagenomics-based diagnostic era.	S

**\* Possible Action Codes**

K = Identify knowledge gaps   S = Conduct studies   F = Identify funding   D = Modify data systems   W = Convene workgroups

## NOTES

