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MAJOR ARTICLE

Pathogen-Agnostic Advanced Molecular Diagnostic Testing for Difficult-to-Diagnose Clinical Syndromes—Results of an Emerging Infections Network Survey of Frontline US Infectious Disease Clinicians, May 2023

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During routine clinical practice, infectious disease physicians encounter patients with difficult-to-diagnose clinical syndromes and may order advanced molecular testing to detect pathogens. These tests may identify potential infectious causes for illness and allow clinicians to adapt treatments or stop unnecessary antimicrobials. Cases of pathogen-agnostic disease testing also provide an important window into known, emerging, and reemerging pathogens and may be leveraged as part of national sentinel surveillance. A survey of Emerging Infections Network members, a group of infectious disease providers in North America, was conducted in May 2023. The objective of the survey was to gain insight into how and when infectious disease physicians use advanced molecular testing for patients with difficult-to-diagnose infectious diseases, as well as to explore the usefulness of advanced molecular testing and barriers to use. Overall, 643 providers answered at least some of the survey questions; 478 (74%) of those who completed the survey had ordered advanced molecular testing in the last two years, and formed the basis for this study. Respondents indicated that they most often ordered broad-range 16S rRNA gene sequencing, followed by metagenomic next-generation sequencing and whole genome sequencing; and commented that in clinical practice, some, but not all tests were useful. Many physicians also noted several barriers to use, including a lack of national guidelines and cost, while others commented that whole genome sequencing had potential for use in outbreak surveillance. Improving frontline physician access, availability, affordability, and developing clear national guidelines for interpretation and use of advanced molecular testing could potentially support clinical practice and public health surveillance.

Keywords. Advanced molecular diagnostic testing; Emerging infections; Metagenomics; Pathogen agnostic.

Infectious disease (ID) physicians encounter clinical scenarios where available microbiological testing on specimens from patients with clinical infections do not result in pathogen identification during their routine clinical practice. Clinicians managing patients with difficult-to-diagnose clinical syndromes may order a wide range of advanced molecular testing to detect known, emerging, or reemerging pathogens.

Advanced molecular tests that determine precise pathogen identities allow clinicians to distinguish between and identify emerging or reemerging pathogens, evaluate antimicrobial resistance, and detect new pathogen subspecies as potential causes of illness [1–4]. There is a wide range of polymerase chain reaction (PCR)–based assays for specific pathogens and, more recently, advanced molecular testing has included pathogen-agnostic methods, such as 16S ribosomal RNA (rRNA) genomic sequencing, whole genome sequencing (WGS), or metagenomic next-generation sequencing (mNGS), that differ widely in their availability and use [1–10]. In some cases, clinicians may be able to expedite clinical decision-making by reassessing treatment protocols, stopping certain antimicrobials, and prescribing targeted therapeutics to improve patient outcomes, including in vulnerable populations, such as children and immunocompromised hosts, and with certain clinical situations such as meningitis-encephalitis syndromes, intraocular infections, and prosthetic joint infections [5, 11–15]. These instances of difficult-to-diagnose clinical situations also represent an important aperture through which emerging and reemerging pathogens may be detected, monitored, and managed as a critical part of national public health sentinel surveillance [8, 16–19].

Currently, several advanced molecular techniques exist that can directly detect microbes; some of the most common are broad-

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range 16S rRNA genomic sequencing, mNGS, and WGS, among others [10, 20–26]. WGS may be of particular use for outbreak investigation and directing infection control and prevention interventions [27–29]. These advanced molecular tests allow for accurate pathogen identification and are useful for detecting pathogens in critically ill and immunocompromised patients [30–32]. However, advanced molecular tests may be infrequently used due to their specialized technical requirements and high cost [9, 33–35].

Little is known about how and when ID clinicians recommend advanced molecular testing while managing patients with difficult-to-diagnose clinical syndromes. The objective of this assessment was to better understand how and when ID physicians use advanced molecular testing for patients with difficult-to-diagnose syndromes in clinical practice as well as to evaluate barriers to their use that exist at the time of the survey to help inform public health surveillance strategies. Specifically, we examined (1) how often ID physicians in North America encounter such difficult-to-diagnose clinical syndromes; (2) the types of infections for which advanced molecular testing is used; (3) where samples are sent for advanced molecular tests; (4) how those results are used in their clinical practice; and (5) barriers to using advanced molecular testing. This portrait of the domestic terrain, including gaps, may be used to develop systemic solutions to address clinician needs and promote the expansion of effective advanced molecular testing technologies as part of national sentinel surveillance.

METHODS

The Infectious Diseases Society of America (IDSA) established the Emerging Infections Network (EIN) in 1995 through a Cooperative Agreement Program Award from the US Centers for Disease Control and Prevention (CDC) with the goal of building a healthcare provider–based sentinel system to monitor emerging and reemerging infectious diseases [36–38]. This early warning network was designed to provide insight, engagement, and partnership in support of nationwide surveillance of emerging infectious diseases, as well as to curate a space to share knowledge about evolving diagnostic modalities and therapeutic regimens [36]. EIN members are a group of adult and pediatric ID physicians who represent approximately 20% of board-certified ID subspecialists in North America. Most members belong either to the IDSA or the Pediatric Infectious Diseases Society and voluntarily participate in surveys. Geographic and practice characteristics are available for all members.

Survey questions were developed by the EIN in consultation with the CDC. In May 2023, the survey was distributed to 1862 EIN physician members who provide care for adult and/or pediatric patients in the United States or Canada; 174 members who were part of EIN but had never responded to any surveys were excluded.

The survey was composed of introductory text and a 10-item online questionnaire link. Three emailed requests to answer the query were sent; members who did not respond to the initial request received a reminder after two weeks and a final request was sent three weeks after the original query.

Survey questions addressed the use of advanced molecular testing in clinical practice, associated operational logistics, and barriers to use (Appendix 1); for some questions, multiple responses could be selected. Members were asked to first answer two questions that indicated (1) the types of advanced molecular testing they had ever ordered in their clinical practice, with a free-text field that allowed participants to report other types of tests not listed; and (2) how often they had used advanced molecular testing within the past two years. The choices were on a 5-point Likert scale with the following choices: "often (weekly)," "sometimes (monthly)," "rarely (quarterly or less often)," "once," and "not at all." Members who had not ordered or used advanced molecular testing during the last two years were asked to opt out of the rest of the survey at this point, and those who had used advanced molecular testing within two years received additional questions. Symptom/illness duration of patients for whom these tests were ordered and specimen types sent for advanced molecular testing over the past two years were elicited; a free-text field allowed participants to report other specimen types.

Several questions addressed operational logistics, including where specimens were sent, who paid for testing and associated costs, the availability of results for patients, and unacceptable result-turnaround times. Participants indicated the degree of likelihood of coverage by different payors on a 4-point Likert scale with the following choices: "never," "sometimes," "usually," and "not sure;" a free-text field allowed participants to comment on other payors. Participants were asked to determine barriers that have prevented or created difficulty for the use of advanced molecular testing in their practice. They also were able to indicate the degree to which advanced molecular testing was found to be helpful in a variety of situations using a 4-point Likert scale with the following choices: "never used," "rarely," "occasionally," and "often." A free-text field allowed participants to enter any final comments on advanced molecular testing.

Denominators for some questions varied because not all EIN members responded to all questions. The statistical package SAS version 9.4 (SAS Institute, Cary, North Carolina) was used for quantitative analyses. Free-text fields were evaluated using an abbreviated thematic approach.

RESULTS

Utilization of Advanced Molecular Testing

Overall, 643 of 1862 (35%) EIN members started the survey and answered the first two questions (Table 1). They indicated that

Table 1. Advanced Molecular Diagnostic Testing Use, Emerging Infections Network Member Responses to the First 2 Survey Questions—North America, May 2023

Advanced Molecular Testing	No. (%)
Types of advanced molecular tests ordered/used (n = 643)	
Broad-range 16S rRNA gene sequencing	454 (71)
Whole genome sequencing	133 (21)
Metagenomic next-generation sequencing	271 (42)
Other[a]	47 (7)
None of the above	153 (24)
Frequency of ordering advanced molecular tests, past 2 y (n = 643)	
Often (weekly)	48 (7)
Sometimes (monthly)	170 (26)
Rarely (quarterly or less often)	236 (37)
Once	24 (4)
Not at all	165 (26)

[a]Other specified in open text field by 45 participants: Karius testing (by 20), 18S rRNA fungal sequencing (by 9), cell-free DNA (by 7), Biofire (by 3), fungal DNA/sequencing (by 2), internal transcribed spacer, polymerase chain reaction (PCR) and sequencing (by 1), pathogen-specific or multiplex PCR (by 2), and GeneXpert–Respiratory Tuberculosis PCR (by 1).

they most often ordered broad-range 16S rRNA gene sequencing (71%), followed by mNGS (42%) and WGS (21%). Respondents collectively cited additional varieties of tests ordered in the free-text field to specify "Other" test types, some of which may have fallen into the predefined testing categories; these included Karius testing [39], 18S rRNA fungal sequencing, cell-free DNA sequencing, BioFire testing [40], fungal DNA sequencing, and a variety of PCR tests. Frequency of testing within the past two years was most often "rare" (quarterly or less, 37%), followed by "sometimes" (monthly, 26%), and "often" (weekly, 7%). Twenty-six percent (165 of 643 respondents) had not ordered any advanced molecular testing within the past two years and opted out of the remaining questions.

Practice Characteristics of Respondents Who Completed the Full Survey

Of the 478 respondents who completed the survey, 28% had pediatric practices and 72% had adult practices (Table 2). Geographically, respondents practice in all 10 census regions, Canada, and Puerto Rico, with most residing in the South Atlantic and Pacific regions (16% and 22%, respectively). Over a third of respondents had 5–14 years of ID experience (36%) or at least 25 years of ID experience (27%). Fifty percent of respondents worked in university-associated teaching hospitals, 24% worked at non-university teaching hospitals, 18% worked at a community hospital, 5% worked at a Veterans Affairs or US Department of Defense hospital, and 3% worked at a city or county hospital.

Operational Logistics and Payors of Advanced Molecular Testing

Of respondents (n = 478), most provided information on patient symptom or illness duration (n = 465), laboratory specimen type (n = 477), where specimens were sent to (n = 475),

Table 2. Characteristics of Respondents Who Indicated That They Had Ordered Advanced Molecular Testing in the Past 2 Years and Completed the Survey (n = 478)—Emerging Infections Network, North America, May 2023

Characteristic	No. (%)
Specialty	
Adult infectious diseases	344 (72)
Pediatric infectious diseases	134 (28)
Region	
US: New England	28 (6)
US: Mid-Atlantic	60 (13)
US: East North Central	72 (15)
US: West North Central	57 (12)
US: South Atlantic	76 (16)
US: East South Central	23 (5)
US: West South Central	25 (5)
US: Mountain	24 (5)
US: Pacific	106 (22)
Canada and Puerto Rico	7 (1)
Years of experience since infectious diseases fellowship	
<5 y	93 (19)
5–14 y	173 (36)
15–24 y	81 (17)
≥25 y	131 (27)
Primary practice setting (hospital)	
University	238 (50)
Non-university teaching	115 (24)
Community	86 (18)
Veterans Affairs or DOD	23 (5)
City/county	16 (3)

Abbreviations: DOD, Department of Defense; US, United States.

and payors (n = 478) (Table 3). These tests were usually ordered for patients whose symptom or illness duration was subacute (3 weeks to 3 months, 81%), followed by "acute" (<3 weeks, 62%) and "chronic" (>3 months, 50%). Testing was most often ordered from tissue obtained during biopsies or aspiration (81%); blood (plasma), cerebrospinal fluid (CSF), bone, and synovial fluid specimens were also noted, whereas respiratory specimens were reported less often among respondents. Most reported that their institutions sent specimens for advanced molecular testing to commercial/reference laboratories (84%), with a minority forwarding specimens to a state/jurisdictional or federal public health laboratory (14% and 6%, respectively).

With regard to payment for external laboratory testing and associated costs, responses varied. Most respondents were unsure about who pays for advanced molecular testing or did not answer these questions (Table 3, Figure 1). Only 28 respondents (6%) reported that patients usually cover the cost of testing.

Barriers to Utilizing Advanced Molecular Testing

Three-hundred ninety-two respondents (82%) also estimated that a turnaround time of >7 days would be unacceptable (Table 4), and noted that once they received test results, they were usually made available to patients (73%).

Table 3. Patient Symptom Duration, Laboratory Specimen Type, Transport, and Costs Among Respondents Who Indicated That They Had Ordered Advanced Molecular Testing in the Past 2 Years—Emerging Infections Network, North America, May 2023

Characteristic	No. (%)
Symptom or illness duration (n = 465)	
Acute (<3 wk)	288 (62)
Subacute (3 wk to 3 mo)	375 (81)
Chronic (>3 mo)	233 (50)
Types of specimens ordered (n = 477)	
Tissue from biopsy/aspiration	389 (81)
Blood	245 (51)
Cerebrospinal fluid	235 (49)
Bone	177 (37)
Synovial fluid	165 (35)
Respiratory tract	70 (15)
Plasma[a]	65 (14)
Stool	11 (2)
Urine	10 (2)
Other[b]	24 (5)
Laboratory specimens are sent to …. (n = 475)	
Commercial/reference laboratory	398 (84)
Local/institutional laboratory	128 (27)
Research-only laboratory	127 (27)
State/jurisdictional public health laboratory	66 (14)
Federal public health laboratory	29 (6)

Who pays for external laboratory testing and associated costs? (n = 478)	Usually	Sometimes	Never	Unsure, Not Answered
Patient	28 (6)	88 (19)	63 (13)	297 (62)
Health insurance	83 (17)	77 (16)	29 (6)	289 (61)
Hospital/institution	105 (22)	99 (21)	18 (4)	256 (53)
Laboratory	30 (6)	39 (8)	92 (19)	317 (66)
Research funds	4 (0.8)	34 (7)	196 (41)	244 (51)
Other[c]	9 (2)	1 (0.2)	0	468 (98)

[a]Plasma is a derivative of blood and is not a separately collected specimen; this reflects specimens used for some advanced molecular tests.

[b]Other specified in open text field by 26 respondents: heart valve (by 7), pleural fluid (by 5), bronchoalveolar lavage (by 2), ascites (by 2), pericardial fluid (by 2), environmental testing (by 2), outbreak isolates (by 2); tuberculosis isolate whole sequencing, bone marrow, throat, vitreous fluid (by 1 each).

[c]"Other" specified in open text field by 18 respondents: provincial medical services (by 2), Veterans Affairs (by 3), health maintenance organization health plan (by 1), public health laboratory (by 1), state or federal public health (by 2), unknown (by 3), "I think the hospital" (by 1), "research study so no charge to patient" (by 1).

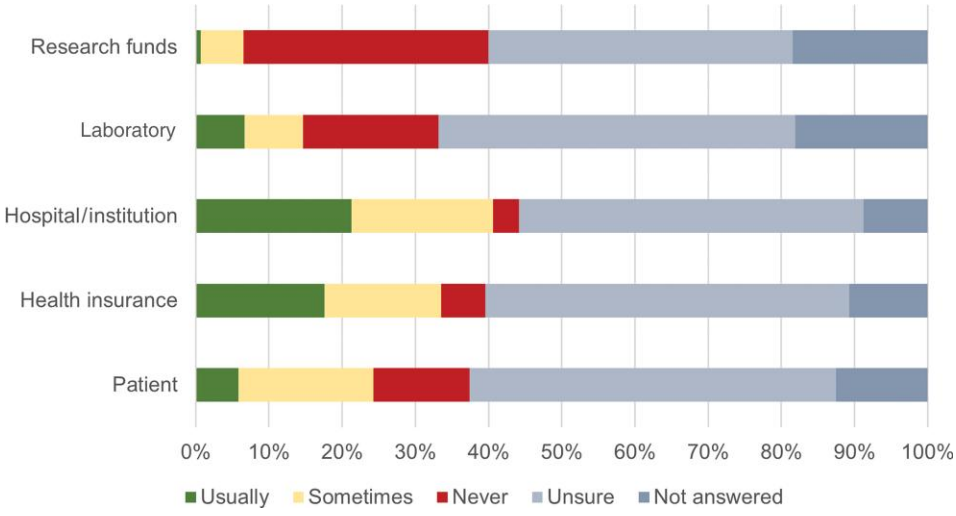

Figure 1. Payors of advanced molecular laboratory testing and associated costs, Emerging Infections Network, North America, May 2023.

Table 4. Strengths and Barriers of Using Advanced Molecular Techniques Identified by Respondents Who Indicated That They Had Ordered Advanced Molecular Testing in the Past 2 Years—Emerging Infections Network, North America, May 2023

Turnaround time (h)	No. (%)
Unacceptable turnaround time to receive results: (n = 478)	
>24 h	1 (0.2)
>48 h	9 (2)
>3 d	57 (12)
>7 d	198 (41)
>10 d	194 (41)
Not answered	19 (4)

Which of the following barriers have … (n = 478)	… prevented you from requesting molecular diagnostic testing	… created difficulty in using molecular diagnostic testing
No barriers selected	212 (44)	64 (13)
Cost or lack of payor coverage	157 (33)	199 (42)
Lack of guidelines	106 (22)	198 (41)
Difficulty interpreting results	81 (17)	179 (37)
Diagnostic stewardship barriers imposed by my institution	73 (15)	123 (26)
Lack of CLIA certification	62 (13)	107 (22)
Difficulty with specimen collection or transportation	60 (13)	179 (37)
Lack of FDA approval	45 (9)	82 (17)
Determining how to order	39 (8)	169 (35)
Determining which laboratory to use	34 (7)	125 (26)
Not sharing test results with patients	2 (0.4)	19 (4)
Other	12 (2)	6 (1)

Found advanced molecular diagnostic tests to be helpful … (n = 478)	Often	Occasionally	Rarely	Never, Not Answered
In clinical decision-making	114 (24)	260 (54)	95 (20)	9 (2)
To assist with stopping antimicrobials	46 (10)	149 (31)	124 (26)	159 (33)
During outbreak investigation	25 (5)	48 (10)	55 (12)	350 (73)
For infection prevention/surveillance	13 (3)	53 (11)	57 (12)	355 (74)
For confirmation of a diagnostic result	30 (6)	136 (28)	117 (24)	195 (41)

Abbreviations: CLIA, Clinical Laboratory Improvement Amendments; FDA, US Food and Drug Administration.

Nearly 9 of 10 respondents (87%) selected barriers that had created difficulty in using this testing (Table 4). Commonly reported barriers were a lack of guidelines (41%), cost or lack of payor coverage (42%), and difficulty interpreting results (37%). Similarly, half of respondents identified barriers that had not only created difficulties, but also prevented them from requesting advanced molecular testing, and these results followed a similar pattern: Cost or lack of payor coverage (33%) and a lack of guidelines (22%) were reported as the most common barriers that prevented respondents from requesting advanced molecular testing (Table 4). Although few respondents (7%) reported that determining which laboratory to use prevented testing, many experienced difficulties (26%) when ordering advanced molecular testing.

Advantages of Advanced Molecular Testing

When asked about the benefit of these tests in various scenarios, respondents affirmed that they were found to be occasionally (54%) or often (24%) helpful in clinical decision-making (Table 4). Respondents also indicated that advanced molecular testing was occasionally (31% and 28%, respectively) or often (10% and 6%, respectively) helpful in stopping antimicrobial agents or confirming a known diagnostic test result at the individual patient level. Most respondents indicated that they did not find advanced molecular testing to be useful or did not answer the question about its usefulness during outbreak investigation or for infection prevention/surveillance (73% and 74%, respectively).

Free-Text Responses

One hundred forty-eight respondents (105 adult and 43 pediatric clinicians) provided comments in an open text field, which yielded rich insight into the use of advanced molecular testing by EIN members. Forty-seven respondents commented on whether advanced molecular testing was helpful; a few clinicians found some but not all tests helpful, and these differences may reflect which tests are utilized by different providers. For example, a respondent wrote, "Our institution uses mNGS frequently and I feel very comfortable using and interpreting this test. It has proven invaluable in multiple scenarios with immunocompromised

hosts. 16S has had less clinical impact." Another clinician noted that "while I have found 16S PCR useful in very specific circumstances, the more sensitive methods such as NGS I (and most of my practice group) mistrust due to risk of overdiagnosis/inappropriate diagnosis." Thirty-two respondents provided additional detail about the clinical situations in which tests are used; common scenarios included infections in transplant and other immunocompromised patients, orthopedic infections, CNS infections, culture-negative infective endocarditis, and patients with sterile cultures due to prior antibiotic exposure. Respondents also raised concerns such as "the potential for patient harm secondary to unnecessary procedures and therapies" based on inappropriate use of test results and cited the need for help with interpretation.

Thirteen respondents described the tests' utility in outbreak responses and for other public health or epidemiologic purposes; WGS was mentioned specifically several times, with one clinician stating that "WGS should be available to all hospitals for outbreak investigation." Many responses also described a need for more research and the development of national guidelines, noting that "it would be very helpful to have more data on when to do these tests so as to achieve the most accurate diagnosis most quickly, minimizing hospitalization duration and needless empirical antimicrobial exposure," and that "a summary of the available evidence and consensus statement from national experts would be extremely helpful."

DISCUSSION

This IDSA EIN survey provides a snapshot of the experiences, challenges, and opportunities with advanced molecular testing from practicing US-based ID physicians on the front line of sentinel surveillance. Physician surveillance relies on the regular and widespread use of cutting-edge technologies, including advanced molecular testing, to identify new and emerging pathogens from specimens that were negative after routine microbiological testing [41, 42]. Advanced molecular testing is available and frequently used by ID physicians to manage patients with difficult-to-diagnose infectious diseases; however, while a wide range of tests, clinical indications, and specimens were reported, 16S rRNA gene sequencing and mNGS were the most frequently ordered tests and among specimens, the most common were tissue biopsy and aspiration specimens. Other tests noted by respondents represent a variety of techniques and clinical indications and were used on different specimen types.

Results of this assessment indicate that only a quarter of responding physicians had never ordered advanced molecular testing, and most respondents order such testing approximately every three months. The interesting finding of a majority of respondents indicating that they ordered these tests for patients whose symptom or illness duration was subacute (3 weeks to 3 months) may be related to availability of testing and turnaround time to impact clinical care in acute illness. Furthermore, often used specimen types such as tissue biopsies and aspirations, bone, synovial fluid, and CSF may be associated with subacute illnesses involving skin, soft tissues, bones, joints, organs, and the central nervous system.

ID physicians highlighted a need for clear guidelines and well-defined criteria for patient selection and interpretation of results; some also raised awareness of the potential for misuse or overuse of tests. In their experience, results were most effective when received with a 1-week turnaround time from specimen shipping to results. Many physicians articulated concerns of ambiguity of test results that limited their usefulness. Although most physicians indicated that the cost of adjunct testing was usually not borne by the patient, cost remained a barrier to advanced molecular testing; this may be due to clinicians' concern and awareness of the high cost of advanced molecular diagnostic testing, and a lack of clarity about payors when ordering tests. Regarding known networks available for advanced molecular testing, clinicians knew the commercial laboratories to which their institution usually sent specimens for advanced molecular testing, and they rarely sent specimens to federal, state, or jurisdictional public health laboratories.

Currently, there is no national coordinated sentinel surveillance network that collates results of advanced molecular testing that are pathogen-agnostic across the country to monitor, detect, and control new and emerging pathogens. Strong sentinel surveillance relies on federal, state, tribal, local, and territorial coordination with public–private entities such as laboratories, academic institutions, and physicians [11, 36, 43, 44]. A recent literature review explored the current landscape of advanced molecular testing in difficult-to-diagnose infectious diseases and revealed the need for coordinated and integrated systems of testing and data exchange among frontline physicians, laboratories, and local, regional, and federal governments [45]. ID physicians and their clinical microbiology colleagues are frontline sentinels in an early warning system to alert public health practitioners of new or resurgent pathogens. To operationalize such a system at a national level, clinician needs must be addressed, and coordination promoted at the state, regional, and national levels. Collaboration between laboratories, professional societies, and federal agencies is needed to develop clear guidelines, patient criteria, and interpretation and analysis of results. To improve accessibility of testing on a national scale, timely, cost-effective, and affordable testing capacities are a worthwhile investment. Additionally, there is potential for improved coordination between healthcare entities, local and state public health jurisdictions and their associated laboratories, and federal agencies to increase availability of advanced molecular testing as part of an effort to build national-level public health surveillance in preparation for future biothreats.

This assessment had several limitations. First, our assessment is subject to possible reporting or recall bias; in addition, participants may have interpreted survey questions in different ways. Second, the results may underestimate intended usage

due to the prevalence of barriers to ordering advanced molecular testing and may overrepresent the views and experiences of ID physicians who order advanced molecular testing and were more likely to respond over those who do not, with or without the institutional capability; furthermore, there may be differences between reported theoretical usefulness and actual utility. Due to the limited nature of the survey, it was not possible to link the test ordered to the specific use case and specimen type, and this merits further study. In addition, barriers being studied may have been the reason for some who were excluded for not ordering or using advanced testing during the last two years, thus limiting the generalizability of the findings. Third, EIN participation is typically strongest in university settings, with results perhaps less representative of small rural or private practice settings. Fourth, the timeframe specified in the survey coincided with the Coronavirus Disease 2019 (COVID-19) pandemic, a period of rapid change and growth in the advanced molecular testing space that clearly demonstrated the need for increased availability and accessibility of advanced molecular testing. As this landscape continues to shift and evolve, a future examination of the advanced molecular testing space will prove valuable. Fifth, the survey participants are primarily based in North America, and the results of this study, particularly barriers and access to testing, may not be generalizable to other countries or healthcare systems.

This survey of ID physicians is meant to explore the experiences of one potential component of a sentinel surveillance network and therefore does not elucidate challenges in or provide visibility of the others at key nodes in clinical specimen flow such as point of collection (hospital and institutional protocols, etc), analysis (clinical microbiologists, laboratory technicians, bioinformaticians, etc), or reporting (public health departments, etc), among others. Further research that comprehensively examines the opportunities and challenges within interconnected elements, such as clinical and research laboratories, data management ecosystems, and local, regional, and federal governments, may provide additional insight. These findings can help inform the development of a national sentinel surveillance system that efficiently monitors for new or resurgent pathogens.

CONCLUSIONS

This assessment enhances our understanding of how and when ID physicians in North America order advanced molecular diagnostic tests for patients with difficult-to-diagnose clinical syndromes, as well as the barriers they face. Improving access, availability, and affordability of advanced molecular testing in difficult-to-diagnose infectious diseases may also help in the development and maintenance of a robust sentinel surveillance system. Although numerous challenges to widespread implementation exist, important next steps could include creating clear guidelines for interpreting test results and use in clinical practice, defining patient selection criteria, improving access and affordability, and improving coordination among clinical, commercial, federal, and jurisdictional public health laboratories. Increased use of advanced molecular diagnostic tests may aid in strengthening sentinel surveillance networks and improve the coordinated and systematic identification and detection of emerging and reemerging pathogens from patients with difficult-to-diagnose clinical syndromes.

Notes

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APPENDIX

Emerging Infections Network Query, Difficult Diagnoses: Pathogen-Agnostic Advanced Molecular Testing for Infectious Disease

Question 1. What types of advanced molecular diagnostic tests have you ordered/used? *[Select all that apply]*

__ Broad-range 16S rRNA gene sequencing
__ Whole genome sequencing
__ Metagenomic next-generation sequencing (mNGS)
__ Other: *[Open text field]*
__ None of the above

Question 2. How often within the past 2 years have you used advanced molecular diagnostic testing?

__ Often (weekly)
__ Sometimes (monthly)
__ Rarely (quarterly or less often)
__ Once
__ Not at all—*please STOP HERE*

Question 3. Within the past 2 years, advanced molecular diagnostic testing was used for: *[Select all that apply]*

Symptom or illness duration:
__ Acute (<3 weeks)
__ Subacute (3 weeks to 3 months)
__ Chronic (>3 months)
Specimen type:
__ Tissue from biopsy/aspiration
__ Blood
__ CSF
__ Bone
__ Synovial fluid
__ Respiratory tract
__ Plasma
__ Stool
__ Urine
__ Other: *[Open text field]*

Question 4. Where have molecular diagnostic specimens in your institution been sent for testing? *[Select all that apply]*

__ Commercial/reference lab
__ Local/institutional lab
__ Research-only lab
__ State/jurisdictional public health lab
__ Federal public health lab

Question 5. Who pays for this external laboratory testing and associated costs?

	Never	Sometimes	Usually	Unsure
Patient				
Health insurance				
Hospital/institution				
Lab				
Research funds				
Other: *[Open text field]*				

Question 6. Are these advanced molecular test results made available to patients in your institution?

__ Yes
__ No
__ Not sure

Question 7. What turnaround time would you find unacceptable and lead to you not using advanced molecular diagnostic testing?

__>24 h
__>48 h
__>3 d
__>7 d
__>10 d

Question 8. Which of the following barriers have …

[Select all that apply]	… prevented you from requesting molecular diagnostic testing	… created difficulty in using molecular diagnostic testing
Cost or lack of payor coverage		
Lack of guidelines		
Difficulty interpreting results (eg, not sure how to interpret a positive test)		
Diagnostic stewardship barriers imposed by my institution		
Lack of CLIA certification (needed for insurance payment)		
Difficulty with specimen collection or transportation		
Lack of FDA approval		
Determining how to order		
Determining which laboratory to use		
Not sharing test results with patients		
Other: *[Open text field]*		

Question 9. In the following situations, have you found advanced molecular diagnostic tests helpful?

	Never used	Rarely	Occasionally	Often
In clinical decision-making				
To assist with stopping antimicrobials				
During outbreak investigation				
For infection prevention/ surveillance				
For confirmation of a diagnostic result				

Question 10. Any final comments about pathogen-agnostic advanced molecular testing: *[Open text field]*