Molecular Testing for Infectious Diseases Should Be Done in the Clinical Microbiology Laboratory

Over the past decade, there has been an explosion in the use of molecular tests to diagnose and manage infectious diseases. HIV is a prime example of an infectious agent whose diagnosis at least in the acute stage, susceptibility testing, and management are all dependent on molecular diagnostics. The ability to accurately diagnose a plethora of respiratory pathogens quickly, simply, and relatively inexpensively compared to traditional methods is becoming a reality. Direct sequencing and microarray analysis holds great promise for directly detecting a wide variety of organisms from clinical specimens. The question is where this testing should be done in the clinical laboratory. There are at least four models that have emerged:

- Molecular infectious disease testing as an arm of the clinical microbiology laboratory
- Molecular infectious disease testing done in a central molecular pathology laboratory under the leadership of a clinical microbiologist
- Molecular infectious disease testing done in a central molecular pathology laboratory under the leadership of an individual whose primary interest is in another area of molecular pathology
- Molecular infectious disease testing sent to a reference laboratory and not done on site or within the institution’s health care system.

We have asked three individuals who have thought about this very complex issue to share their rationale for supporting one of these models. Frederick Nolte is the Director of Clinical Laboratories and Director of Molecular Pathology at the Medical University of South Carolina, is active in and held several positions of responsibility in AMP (Association of Molecular Pathology) and is Chair of the CLSI’s Area Committee for Molecular Methods, Alex McAdam is the Director of the Infectious Diseases Diagnostic Division at Children’s Hospital Boston and an editor of this journal, and his colleague, Nima Mosammaparast, is the Assistant Director of the Infectious Diseases Diagnostic Laboratory at Children’s Hospital Boston.

POINT-COUNTERPOINT

POINT

The recently evolving field of molecular microbiology presents many challenges to the practice of laboratory medicine. One particular challenge is the configuration of molecular microbiology in relation to other divisions of laboratory medicine, particularly the central laboratory and nonmicrobiology molecular diagnostics. Microbiological nucleic acid tests (NAT) can be performed in different areas of the clinical laboratory, and as the editor has described, four common scenarios have emerged. We will consider each of these four models, analyzing their advantages and disadvantages in relation to various considerations. We conclude that high-quality patient care at reasonable cost is most likely to be achieved if NAT for microorganisms are performed in the microbiology laboratory. Other models are also acceptable if they can meet the needs of patient care with reasonable cost.

What is needed for high-quality microbiology NAT? The goal in performing microbiology NAT, like that of other diagnostic tests, is to provide timely results useful for high-quality patient care at a reasonable cost. The steps taken to perform high-quality NAT are the same as those of other clinical microbiology assays (11); however, there are some key issues that are particularly important and challenging in performing NAT, and we will focus on these. They are the availability and quality of expertise for laboratory oversight, turnaround time, and cost containment. We will rely on empirical data to discuss these concerns whenever possible, but to some extent our opinions are simply that, albeit based on experience.

We think it is obvious that expertise in microbiology is needed to oversee microbiological testing, whether by NAT or any other method. The expertise should be provided by a doctoral-level laboratory director qualified in both clinical microbiology and molecular diagnostics. The complexity of the microbial world and the sophistication of NAT mean that expertise is required not only for assay development and performance but also for consultation. Although some FDA-approved NAT are relatively simple to use, many NAT used in microbiology are laboratory-developed assays or use analyte-specific reagents, and the validation and performance of such tests are difficult. A good example of a test requiring expertise is 16S RNA gene amplification and sequencing. 16S RNA sequencing is reasonably accurate for detection of bacterial pathogens in normally sterile sites; however, the results are difficult to interpret when the corresponding culture is negative (when the 16S rRNA test is most useful, but possibly incorrect), when the sequence results indicate a mixed infection, and when the results detect an unusual pathogen. These are not rare occurrences (1, 7, 8, 10). Similarly, evaluations of quantitative NAT for pathogens...
can require considerable expertise, as the results can vary greatly between tests and for many reasons (4, 5).

One advantage of NAT is that these tests can be performed quickly if there are adequate laboratory resources. It is challenging to perform NAT with rapid turnaround times because of the financial advantages of batch testing and the difficulty of staffing the laboratory for frequent testing, but careful evaluation demonstrates that it is worthwhile for some tests. Rapid results obtained by NAT are associated with improvements in patient care in some studies. For example, both empirical data and modeling studies find that faster detection of enteroviral meningitis using NAT is associated with reduced length of stay and duration of antibiotic administration, as well as substantial cost savings (3, 6, 9). Modeling studies suggest that using rapid NAT for detection of methicillin-resistant *Staphylococcus aureus* in bacteremic patients might reduce mortality as well as cost (2). Rapid NAT results do not always have demonstrable patient benefits, however, and so this is another area where expertise is required to determine the best practice (12).

Finally, NAT must be performed with an eye on cost. Laboratory directors are frequently asked to review their expenses and look for ways to squeeze a little more out of the bottom line. We think that NAT can be performed in either the microbiology laboratory or a central laboratory at reasonable cost, as discussed below.

**Who should oversee microbiology NAT, and where should they be performed?** Next, we will analyze how testing under the four models described in the abstract will meet the challenges discussed above (Table 1 offers a summary). We will also raise some other points about how the different models might be appropriate for particular tests or needs.

The first model, performing NAT in microbiology, meets the need for microbiological expertise for assay development, oversight, and interpretation. We think that this model is best for offering rapid results. Many microbiology laboratories are staffed for two or three shifts each day, 7 days a week, and are accustomed to handling requests for rapid results for a variety of tests. The workflow in such a laboratory is flexible and can accommodate NAT as needed or for the occasional, well-justified test performed off the routine schedule. In our microbiology laboratory, an enterovirus NAT (Cepheid Xpert EV) is performed from 7 a.m. to 10 p.m. every day as soon as the workflow reasonably allows, with an average turnaround of under 10 h. Turning to cost, an argument can be made that having microbiology NAT in a central lab would result in lower capital costs because of equipment sharing. However, the requirements of microbiology NAT are often different from those of other types of NAT because of the need for rapid turnaround time, which drives frequent testing of small batches. Thus, for microbiology, the types of nucleic acid extractors and real-time PCR machines that are most suitable are those that have random-access capability, whereas for nonmicrobiology NAT, larger-scale machines can be desirable for larger batches. We use several relatively inexpensive Qiagen Qiacubes for nucleic acid purification and Cepheid Smartcyclers for most real-time PCR in microbiology. These devices are typically running throughout the day shift and so would not be available for nonmicrobiological testing. We doubt that there are savings in staff costs using a central laboratory model compared to performing these tests in the microbiology laboratory; the technologists should be efficient, regardless of where they work. Finally, if there are cost reductions available for purchasing in volume, these can be realized by cooperation between various laboratories performing NAT at an institutional level.

In the second model, microbiology NAT are done with oversight by a microbiology lab director but using shared resources for nonmicrobiology NAT. This meets the need for microbiological expertise. While some costs might be reduced in this model because of shared resources, achieving a rapid turnaround time may prove difficult due to competition for resources. Without rapid turnaround time, cost savings to the institution gained by a shorter length of patient stay or reductions in unnecessary treatments will not be realized. However, if a central molecular laboratory has appropriate microbiological expertise and can achieve the needed turnaround times, this is acceptable. A hybrid model, in which different tests are performed in the microbiology and central laboratories to realize the advantages of each, could also be acceptable.

The third model is to perform microbiology NAT in the central core or molecular diagnostics laboratory, with oversight by a nonmicrobiologist. We believe that this is not acceptable because of a lack of microbiological expertise. Turnaround time and cost concerns would be similar to those discussed for the second model, although perhaps more microbiology NAT would be sent out to a reference lab in the absence of expertise. A variation on this model would be to have joint oversight between a microbiologist and an

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**TABLE 1 Comparison of different models for microbiology molecular testing**

<table>
<thead>
<tr>
<th>Model</th>
<th>Expert oversight and consultation</th>
<th>Turnaround time</th>
<th>Cost</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test in microbiology with microbiologist oversight</td>
<td>Present</td>
<td>Can be rapid</td>
<td>Can be reasonable</td>
<td>Best solution for most institutions</td>
</tr>
<tr>
<td>Test in central laboratory with microbiologist oversight</td>
<td>Present</td>
<td>Can be rapid, although might be compromised by competing needs</td>
<td>Can be reasonable</td>
<td>Acceptable solution if adequate resources are provided to meet clinical needs</td>
</tr>
<tr>
<td>Test in central laboratory with nonmicrobiologist oversight</td>
<td>Absent</td>
<td>Can be rapid, although might be compromised by competing needs</td>
<td>Can be reasonable</td>
<td>Unacceptable due to lack of needed expertise</td>
</tr>
<tr>
<td>Test in reference laboratory (commercial laboratory)</td>
<td>Likely present</td>
<td>Often slow</td>
<td>Variable</td>
<td>Useful for low vol or very difficult tests; usually unacceptable for tests requiring rapid turnaround time</td>
</tr>
</tbody>
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*Staphylococcus aureus*
expert in molecular diagnostics. This could be useful if the available microbiologist is not experienced in molecular diagnostics.

The final model is to perform microbiology NAT at an outside reference laboratory, which we take to mean a commercial laboratory. Although these laboratories are likely to be overseen by someone with appropriate expertise, the expert might not be readily accessible for consultation. Turnaround times can be long due to delays in specimen transport and result reporting, to such a degree that patient care could be compromised. Costs for tests performed at commercial laboratories vary, and this is an area that laboratory directors scrutinize closely. An important advantage of using a reference laboratory is the variety of tests that are available at such reference laboratories. Clearly, hospitals will take advantage of reference laboratories for at least some low-volume or esoteric tests.

In conclusion, we think that the best solution for most hospitals is for microbiology NAT to be done in the microbiology laboratory. The availability of expertise for consultation, test development, and implementation, combined with the resources ideally suited for microbiology NAT, makes this the most reasonable choice.

REFERENCES


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COUNTERPOINT

There is no more exciting aspect of laboratory medicine than molecular diagnostics. In the past 25 years we have witnessed the development of powerful new tools for nucleic acid amplification, detection, and characterization and the rapid transition of these tools from the research to the clinical laboratory. Molecular techniques are of importance in clinical diagnostics in many disciplines in pathology and laboratory medicine, and their applications will continue to grow in all areas. With the advent of more FDA-cleared molecular diagnostic tests and more automated testing platforms, this testing is no longer restricted to academic or reference laboratory settings. Larger community hospitals and private practice pathology groups are adding molecular tests to their locally available test menus to improve turnaround times and the financial health of the laboratory. These considerations raise fundamental questions about how to deliver molecular testing services and how to integrate these services into clinical pathology once the decision has been made to offer them locally. An excellent guidance document on incorporating molecular diagnostics into clinical laboratories where technical knowledge in this area may be limited was recently published by the Clinical and Laboratory Standards Institute (2).

At the heart of the matter is whether each traditional laboratory section or discipline in which molecular diagnostic applications are important should have its own molecular section or whether a multidisciplinary, centralized, molecular laboratory is a more suitable model to deliver these services (1). Over the past 25 years I have had the opportunity to create and direct centralized molecular diagnostic laboratories in two different academic medical centers. I am a medical microbiologist by training, and I currently serve as director of our molecular pathology section and provide professional oversight for all of our clinical laboratories. It is from this perspective that I will provide the rationale and supporting arguments for centralization of molecular diagnostics services, but I fully appreciate that there is no one correct way to deliver these services and that, ultimately, the decision to centralize or decentralize will be driven by locally available expertise and resources and improvements in technology. The major argument for centralization of molecular diagnostics is the efficiency achieved by sharing equipment, reagents, personnel, and space.

One of the major problems in the area of molecular diagnostics is that there is no unifying platform that adequately addresses the medical demand. This is true for infectious diseases as well as the other areas of molecular diagnostics (e.g., hematopathology, genetic diseases, and molecular oncology). Each molecular diagnostic company has one or several platforms, none of which has a broad test menu. As a consequence, many laboratories have several large and expensive platforms to provide the services required. Consolidation of molecular diagnostic activities forces the laboratory to take a broader view of the available test platforms with an eye toward versatility and adaptability. Platforms that have a menu of FDA-cleared tests with “open channels” to accommodate laboratory-developed tests (LDTs) regardless of the clinical applications provide a cost-effective approach to molecular diagnostics.

The reagent costs for molecular diagnostics are among the highest in laboratory medicine. The cost of FDA-cleared tests ranges from $10 to over $80/test, and the analyte-specific reagents...
(ASRs) that serve as the basis of LDTs typically cost $20 to $40 in our laboratory. Although the cost of FDA-cleared tests will not likely be affected by where the testing is performed, the cost of LDTs may be impacted by centralization of testing services within the laboratory through better inventory control and pricing. The same nucleic acid extraction reagents, enzymes, buffers, and nucleotides that are used in an LDT for a microorganism could be used to detect a mutation in a tumor, since only the ASRs differ. In addition, both of the assays could be run on the same real-time PCR platform, providing for more efficient use of the instrument.

In most centralized molecular diagnostic laboratories, infectious disease tests are the highest volume and generate most of the revenue. In many situations medical directors and laboratory administrators are held accountable for their section budgets, with each section being a separate cost center. Infectious disease testing is the financial engine that drives the laboratory and helps cover the costs of the lower-volume tests of high medical value in the other areas of molecular pathology.

One of the biggest problems facing laboratory medicine in general and molecular diagnostics in particular is the lack of adequately trained personnel to perform the tests. Until recently, molecular diagnostics were not part of the core curriculum in clinical laboratory science programs. Consequently, many of the medical technologists in the work force do not have the appropriate knowledge base and skill set. Although credentialing in this area is now available from the American Society for Clinical Pathology (ASCP), the National Credentialing Agency (now merged with ASCP), and the American Board of Bioanalysts, finding staffing for molecular diagnostic laboratories remains a challenge. The most efficient use of this scarce work force is in a centralized molecular diagnostic laboratory.

Another consideration is the special facilities required for molecular diagnostics. Ideally, separate areas for reagent preparation, sample preparation, and amplification and detection should be available and a unidirectional workflow from pre- to postamplification areas maintained to avoid problems with target and amplicon cross-contamination. Although some of the design considerations and work practices are less critical now because of an increased use of closed amplification and detection systems, having multiple areas within different clinical laboratory sections that are appropriate for molecular diagnostics can be problematic in many settings and may lead to significant additional expense.

Professional oversight of a centralized molecular diagnostic laboratory that provides services in areas as diverse as infectious diseases and oncology can represent another challenge that requires a broad range of skills and knowledge. Regardless of the laboratory director’s background, he or she needs to have strong collaborative relationships with the clinical specialists in the other laboratory disciplines to help establish the test menu, testing priorities, and integration with other test methods and to help with the interpretation of molecular test results in his or her areas of expertise.

Molecular pathology should be considered not a separate discipline but a collection of related methods and procedures. Over the past 25 years it became its own area because it required a certain level of knowledge and expertise in molecular biology that was not common among laboratory professionals at the time and because so many of the tests were developed and validated in-house. As more FDA-approved kits become available and technology evolves to more simple, sample-in, answer-out molecular test systems, the testing should migrate back to the laboratory of origin.

A recent example of repatriation of a molecular infectious disease test from our molecular pathology laboratory to the microbiology laboratory was the respiratory virus PCR panel. Up until December 2011, our test of record was the Lumineex respiratory virus panel. This test was one of the more technically demanding molecular diagnostic tests on our test menu, and we made the decision that it was a better fit for our molecular pathology laboratory. With the FDA clearance of the Idaho Technology respiratory virus panel, we made the decision to switch to this much less technically complex test and transferred the testing to our microbiology laboratory. This test requires no special training in molecular biology or modification of space or work practices. In addition, since it is designed for on-demand testing and our microbiology laboratory is staffed around the clock, we have dramatically decreased our turnaround time for respiratory virus results from 48 to 2 h. An added benefit to this transfer of testing is that it has freed up our technologists with advanced skills in molecular biology to develop and validate tests in other areas of molecular pathology and expand our test menu without any additional staff.

Since expertise in molecular diagnostics and resources vary substantially among clinical laboratories, it may be helpful to think about different levels of service regardless of whether the testing is centralized or decentralized. I proposed three different levels of molecular diagnostic services in 2005 based on available expertise and resources (3). Level 1 laboratories have little experience with molecular diagnostics and lack the resources to conduct extensive validation of test performance characteristics. These laboratories should perform only FDA-cleared tests and refer other tests to laboratories with higher levels of service. Level 2 laboratories have more experience with the technologies and greater resources available to document test performance characteristics. These laboratories may perform FDA-cleared tests and use ASRs for LDTs that have been developed and validated by others. Level 3 laboratories have the most experience in this area and additional capabilities and resources to design and validate LDTs.

Currently there are three FDA-cleared molecular diagnostic test platforms available for the detection of microorganisms that represent simple, sample-in, answer-out systems with tests of moderate to low complexity (Cepheid, Idaho Technology, and IQum). Simple test systems such as these can be performed in many different laboratory and near-patient settings. With the availability of systems such as these, point-of-care molecular testing may soon become a reality in many institutions, further complicating the debate about centralization of molecular diagnostic services. In summary, as technology advances and the molecular assays become simpler, more of the testing will return to the laboratories of origin. However, for the near future a centralized molecular laboratory that performs highly complex, laboratory-developed tests for infectious diseases, hematology, oncology, and genetic conditions remains the most efficient model for delivery of molecular diagnostics. Professional oversight of centralized laboratories can be provided by individuals from a wide va-
riety of backgrounds, but the individual charged with this responsibility must work with the subject matter experts in the traditional laboratory medicine disciplines to ensure that these tests are interpreted properly and correlated with other laboratory results.

REFERENCES

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SUMMARY
Points of agreement:

- Molecular testing improves turnaround time and diagnostic accuracy for a variety of infectious agents.
- Regardless of the laboratory setting where molecular testing is performed, expertise in clinical microbiology in the individual overseeing test selection, development, and offering consultative services is essential.
- Random access testing which allows analysis of individual or small-batch specimen testing in real time can improve clinical outcomes.
- Testing in a centralized multidisciplinary laboratory is more efficient than testing in multiple laboratory settings.

Points requiring further consideration:

- Although molecular testing menus are constantly expanding, the number of individuals with the necessary molecular skill set is not keeping pace. This shortage of skilled personnel may dampen rapid test menu expansion at many institutions.
- Molecular testing has a high per-test cost compared to most laboratory tests. The highest-volume molecular tests are those that detect infectious disease agents. Molecular pathology laboratories may look to add these tests to their test menu to help subsidize more expensive and esoteric human genetic and molecular oncology tests.
- Molecular testing is undergoing a rapid evolution with many potential test platforms and testing strategies. Testing platforms for the rapid detection of infectious agents may not be as useful for pharmacogenomic or molecular oncology analyses. It will be incumbent upon members of the clinical microbiology community involved in molecular testing to provide clinical outcome data to justify the use of specialized testing platforms best adapted for infectious disease diagnosis.

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