Commentary

Evaluation of Molecular Diagnostic Assays for Fungal Infections

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The challenges to the laboratory diagnostician continue to increase as medicine becomes more and more complex. This is true in all areas of medicine, but it is arguably most evident in molecular diagnostics. The challenges to the molecular diagnostician range from appropriately recognizing genetic abnormalities in tumor cells that have therapeutic implications (eg, *HER2* gene amplification in breast cancer) to optimizing the strategies for the detection and monitoring of infectious agents. This latter area within molecular diagnostics has grown most rapidly during the past few decades in what some have termed a “molecular microbiology revolution.” Although much of this revolution has depended heavily on advances in chemistry and engineering that have made this testing technically possible, it has been driven largely by the needs of patients with severely compromised immune systems.¹

In this issue of *The Journal of Molecular Diagnostics*, White et al² of the United Kingdom-Ireland Fungal PCR Consensus Group (UKPCG) share the findings of a multicenter evaluation of various homogeneous or real-time polymerase chain reaction (PCR) methods that are used for the detection of *Candida* and *Aspergillus* infections. The UKPCG was formed in 2001 and consists of seven centers; the aim is to reach consensus regarding a robust and reproducible PCR-based test for the diagnosis of invasive fungal infections. Earlier studies from this group have examined optimal methods of nucleic acid extraction from fungi, which is a great place to start given the pivotal importance of nucleic acid extraction for achieving the highest quality result from a nucleic acid amplification test.^{3–5} In this study,² the group proceeds to determine which of several currently used PCR assays may be optimal or suboptimal for the detection of two important fungal pathogenic genera, *Candida* and *Aspergillus*. Herein, they discover the homology of the forward primer of one of the assays examined with the *Homo sapiens* 18S rRNA gene, a fact that significantly limits the utility of this primer set. They also identify matrix issues and interest-

ing variability between some of the real-time PCR instruments, which cannot be readily explained.

This study and numerous previously published studies by this and like-minded researchers are reminiscent of the efforts made regarding the optimization of diagnostics and monitoring for patients with human immunodeficiency virus (HIV) infection. Today, molecular detection and quantitative analysis of several viral pathogens is the standard of care in various clinical scenarios. Quantitative reverse transcriptase-PCR is used routinely to monitor the HIV and hepatitis C virus viral loads of infected patients to evaluate responses to therapy.^{6–8} Quantitative PCR applications are also used to monitor cytomegalovirus (CMV) for patients who are immunosuppressed secondary to transplantation or HIV infection, to monitor Epstein-Barr virus posttransplantation, and more recently, to detect and monitor BK virus for patients with a renal transplant to diagnose and monitor the treatment of BK virus nephropathy.^{9–12}

The AIDS Clinical Trials Group (ACTG) is the largest HIV clinical trials organization in the world and is funded by the National Institutes of Health (<http://www.actg.org>). The Quantitative HIV RNA working group is the group within the ACTG that did much to set the high standards of excellence to which laboratory diagnostics are held today. There are many useful comparisons, I believe, between what has been done in molecular virology and what molecular mycologists would like to achieve. As molecular mycologists, we have the opportunity to learn from the struggles and successes of the ACTG and similar organizations.

The ACTG first addressed the various assays that existed, either laboratory-validated or commercially produced, in an attempt to find which assays had the best performance characteristics, much in the way the UKPCG is examining assays in this study. Additionally, this group and others have examined differences due to specimen type, just as the UKPCG has examined matrix

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issues here. Subsequently, the ACTG went on to investigate quantitation and other viruses important to the HIV-infected patient. These issues, too, have important analogies in the molecular detection of fungal pathogens.

Although the issue of quantitation is not the subject of this manuscript by the UKPCG, it will surely arise in the future and will require systematic and scientifically sound analysis. One of the advantages of real-time PCR is that this technology is innately quantitative when used in conjunction with quantitative standards.^{1,13} Numerous studies have demonstrated significant limitations of qualitative fungal PCR assays, particularly with respect to a lack of specificity and an inability to predict invasive fungal disease; Buchheidt et al¹⁴ have summarized the performance of many of these assays. This lack of specificity, barring contamination of PCR reagents, etc, is likely secondary to transient fungal colonization, mucosal disease (eg, thrush), or fungal spores that may contaminate a clinical specimen. The challenges in molecular mycology are similar to issues regarding the interpretation of qualitative and quantitative CMV PCR results in the clinical setting. Detecting the presence of CMV, a virus that many of us carry as part of our integrated viral microbiota, in a qualitative assay does not provide useful information to the clinician regarding the presence of CMV-associated disease (ie, there is a low positive predictive value regarding CMV disease). However, the information available from a quantitative CMV assay, the CMV viral load, demonstrates not only the presence or absence of the virus but the relative amounts of virus. Although perhaps difficult, with extended clinical experience, one can begin to correlate viral loads, particularly high viral loads, with the definitive presence of CMV disease (ie, the positive predictive value of a high CMV viral load is much higher than that of a positive qualitative CMV assay).^{15,16} A quantitative approach will also likely be the means by which molecular mycologists will distinguish less predictive results (ie, low positives potentially from contamination or colonization) from results with a more powerful positive predictive value (ie, high fungal loads); some researchers have already demonstrated that this approach holds promise.^{17,18}

Finally, as mentioned previously, the ACTG has studied other viruses that are important to patients with HIV. It will also be important, once the testing of the most frequent fungal pathogens are addressed, that attention is given to the numerous non-*Candida* and non-*Aspergillus* species that are important causes of invasive fungal infections in the immunocompromised host.^{19,20}

The time then has come to thoroughly and orderly evaluate molecular diagnostic assays for the assessment of patients at risk for invasive fungal infections, because traditional methods lack the sensitivity to detect early disease.²¹ The authors and participants of the UKPCG study deserve compliments for their efforts and for the systematic approach to challenges in diagnostic molecular mycology. We will learn from the past and look to a future in which well-vetted molecular diagnostic methods will likely be a part of the monitoring of immunocompromised patients for nascent fungal infections and in which deaths from these diseases will be diminished.

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