

Clinical Mycology – Changes to Come

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In 2003, under auspices of American Society for Microbiology, Dr. Glenn Roberts, Prof. Elmer Koneman and Dr. Michael Saubolle came in Romania for an educational course intitled *Mycology without tears – Tools and techniques for demystifying fungal identification* in several university centers.

Clinical Mycology is changing rapidly, not only in regard to the methods that we use to diagnose fungal infections, but the greatest change is the group of patients that we serve. We are observing a dramatic increase in the number of fungal infections in those who are immunocompromised by transplantation, a disease process such as leukemia, the use of potent immunosuppressive drugs, HIV, and the widespread use of corticosteroids. Also, more Opportunistic infections are being seen that are caused by a variety of fungi, including some uncommon species that can present diagnostic challenges to the clinical laboratory.

We are confronted with another challenge. The need for persons with expertise in mycology has increased while the number of trained persons has decreased. Yet, laboratories are expected to utilize rapid tests to shorten turnaround times but are expected to provide accurate results using fewer personnel and a reduced budget. This presents a challenge that is difficult to meet, particularly since the emphasis is on the use of molecular testing in many laboratories.

The greatest problem that we have in Clinical Mycology is traditional testing is not used by many laboratories to diagnose fungal infections and we have newer molecular tests that are available only to those laboratories that have large budgets and highly trained personnel to perform the tests. Currently smaller laboratories cannot afford molecular testing. What do we do about this is the problem? This will be discussed at the end of this editorial.

The direct microscopic examination of clinical specimens for the presence of fungi is one of the oldest and most rapid tests that the clinical laboratory can offer. The simple potassium hydroxide preparation has traditionally been used but during the last years it has been improved by the addition of Calcofluor white or Blankofluor to enhance visualization of fungi. Other stains, including the Gram, periodic acid Schiff, Papanicolaou, H&E and Gomori's methenamine silver stains have been available for years to pathologists to detect fungi in tissue and body fluids. Ironically, there are still many pathologists in practice who do not have the expertise necessary to accurately diagnose a fungal infection.

Media useful for the recovery of fungi from clinical specimens have seen very little change over the years; however, laboratories that offer fungal culture have still been able to provide useful information to the clinician to aid in the diagnosis of a fungal infection. Unfortunately, many laboratories refer the cultures that grow to a reference laboratory for identification since they do not have the experience to identify even the most common fungi.

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This is easily accomplished if one recognizes the characteristic morphologic features of a mold that has been recovered from a clinical specimen.

Yeasts recovered from clinical specimens were previously identified using tests that showed which carbohydrate substrates were utilized, nitrate reduction, urease production, germ tube formation and morphologic features on cornmeal agar. For the past several years commercially prepared yeast identification systems have largely replaced traditional identification methods and simplified the identification process. The old problem of what to identify and how far to go with the identification of an organism makes the process a little more complicated.

Fungal serologic tests for the detection of antibody have been used for many years and include the complement fixation and immunodiffusion tests for histoplasmosis, coccidioidomycosis and blastomycosis. These tests continue to be used although their sensitivity and specificity for diagnosing histoplasmosis and blastomycosis is not optimal. Serologic tests for coccidioidomycosis are sensitive and specific and are the best of those offered. In the 1970's a test to detect the antigen of *Cryptococcus neoformans* was developed and is still used in some clinical laboratories. This latex agglutination test has a high sensitivity and specificity for diagnosing cryptococcal meningitis and disseminated infection. It is particularly useful for diagnosing infections in immunocompromised patients. More recently a test to detect circulating galactomannan in patients with aspergillosis was developed and evaluated by a number of laboratories. Unfortunately, this test has had mixed reviews and is helpful in some cases but is not the answer for making a diagnosis of aspergillosis in transplant patients like we thought that it might be.

Antifungal susceptibility testing has progressed over the past twenty years and is now widely used in many laboratories. Standards for this testing have been developed and clinicians rely on the results. Susceptibility testing of yeasts is better developed than that of molds and the latter is currently only offered in research and reference laboratory settings. *In vitro* to *in vivo* results still have not been well investigated; however, a substantial amount of information is known about which fungi are susceptible to specific antifungal drugs. Newer antifungals continue to be developed and it is critically important to accurately identify the any organism recovered from a patient who has

an active fungal infection so that may be treated with appropriate drug.

The early 1990's offered the first molecular tests available for the identification of dimorphic fungi. These changed the way these fungi were identified and turnaround times decreased from 1-3 weeks to one hour after the fungus is recovered by culture; the use of the fungal nucleic acid probes for the identification of dimorphic fungi continues to impact medical care in institutions where they are used.

Immunocompromised patients make up a large part of the patient population being seen currently due to high dose corticosteroid therapy, severe underlying diseases, long term intravenous catheter placement, HIV, organ transplantation and a number of other factors. These patients require that we make an accurate and rapid diagnosis so that therapy can be started early to increase their chance of survival. The need for more rapid and accurate diagnostic tests is apparent and molecular testing appears to offer promise. Nucleic acid amplified tests have been developed to determine the presence of an organism in clinical specimens from almost every type of fungal infection. However, the sensitivity and specificity of most tests currently are not adequate for patient diagnosis with a few exceptions. Evaluations of testing for the presence of *Histoplasma* and *Coccidioides* have shown that they are useful. Hundreds of tests have been developed for the diagnosis of aspergillosis and no standardized method is available yet. Many are offered on a research basis. The use of real time PCR has shortened testing times to a few minutes and is the most popular type of testing available; however, we still do not have tests that can be used routinely by most laboratories. Nucleic acid sequencing is another tool that has been found useful for the identification of fungi recovered from clinical specimens. Databases are available that contain the sequences of a large number of fungi and results can be made available within 24 hours after recovery. This has provided reference laboratories and large clinical laboratories with the ability to identify and report the identification of fungi in the shortest time possible. The use of microarrays appears to offer promise of detecting and identifying the agents of fungal infections on a chip containing many targets and may be useful for screening for a large number of fungal agents. This requires more development but offers great promise along with other methods being developed. Molecular diagnostic testing is the most rapidly

expanding area within microbiology and many areas of medicine. It is costly, requires extensive development, specific expertise, large-scale clinical evaluations and standardization and commercialization before tests will be routinely used in hospital laboratories.

Currently the number of persons with expertise in clinical mycology has decreased in settings where trained personnel were previously available. Little improvement is expected in the future because training courses simply are not available. It is apparent that many laboratories provide inadequate services due to this lack of trained personnel. Culture currently remains the "gold" standard for the detection of fungi. The best application for molecular methods is for slower growing organisms and for others that are difficult to detect by culture, i.e., the zygomycetes. Newer tests that will be developed will shorten the time to diagnosis in many instances and will lead to better patient outcomes. Molecular methods will someday replace many conventional methods, if the complexity of tests is reduced and costs are reasonable. Methods that will be available may not have the widespread application to smaller laboratories; however, larger clinical laboratories and reference laboratories will be available to offer them.

What do we do in the meantime between now and when molecular tests become routinely available? We must continue to develop, refine, verify, and compare new molecular methods using statistically valid numbers of clinical cases. We must determine how to integrate traditional and molecular methods until the latter become the standard. For example, some tests like the germ tube for the identification will probably always be used because it is a cost effective method.

This is essential to ensure that we provide the best patient care possible. While the need for molecular-based testing is acute and necessary; we must still strengthen our traditional testing until useful and standardized molecular testing becomes available. This can be accomplished by education our current work force and those who are just completing their education; few, if any, will receive training in mycology.

Success can best achieved by sharing information and working together for the needs of the patient; this applies to the teacher and the student and then to the physician.

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