Good-bye Agar-Agar: What's Coming for Infectious Disease (ID) Diagnostic Tests (Acute and Convalescent ID Doctors)

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Thirty years ago, many of us could not spell PCR (polymerase chain reaction). Common and uncommon diagnoses were often made by debate and inaccurate clinical suspicion. And if the patient was fortunate, our selected treatment was of broad enough spectrum to help an immune system effect a cure. Our intentions were no less valiant than those of any era; however, by today's standard, the laboratory support was then at the beginning stages.

YESTERDAY is a quick look at the laboratory "back then": agar-agar was 2 words, most pathogens could not be cultured, and pathogens that grew were often misidentified using visual inspection, smell, and a few chemical reagents. Complement was fixed when it was not broken, and infections were sometimes diagnosed by something called convalescent titers long after the diagnosis was clinically useful. In addition, if no diagnosis was evident by all available means, it was then classified as a "virus" . . . one of those poorly understood DNA-RNA things that can cause an illness for which there was no treatment. Therefore, testing was stopped. We also did not have financial pressures to stop ordering unnecessary tests.

TODAY is our everyday practice. Because of the diligence and expertise of many, we have come a long way. We live it, we use it, and there is no need to review it here for this audience.

TOMORROW we will begin to see some peeks into the immediate future. So let us talk about the laboratory support that is likely to become available to an infectious disease clinician; probably sooner than one might think.

Ideally, a diagnostic test should be accurate, precise, quick, clinically useful, and employ a method (also called a platform) that can be easily performed in most patient care areas. To achieve these goals, we must look at alternatives to cultures of body fluids. Although cultures have served us well, every clinician can quickly list many limitations, and laboratorians know a dozen more.

TOMORROW our alternative to cultures is to determine the presence of an infecting organism by identifying a tiny molecular piece of the pathogen (or even a unique metabolic product) that is so specific that the diagnosis is almost certain. Nucleic acid-based amplification technologies (NAAT) are already in use. Nucleic acid-based amplification technologies, using PCR to amplify a specific piece of a nucleic acid, have already increased the sensitivity and specificity of bacterial identification and simultaneously improved turnaround time. In addition, commercially available NAATs can identify sequences that underlie drug resistance and detect microorganisms the require special consideration for therapy and infection prevention and control.

One limitation of most currently available molecular tests is that the clinician has to know what organism is being sought. For example, requesting herpes PCR tells you whether herpes is present but no additional information. Likewise, a rapid test for Group A streptococcus or methicillin-resistant *Staphylococcus aureus* identifies the presence of only what is requested. In contrast, a body fluid culture can recover hundreds of potential pathogens with just 1 set of media.

Therefore, the burgeoning technology will be an attempt to merge these features by using panels of molecular reagents with tens, hundreds, or even thousands of different substrates. Imagine putting a drop of blood into a test system and learning in minutes that a patient is bacteremic for *Klebsiella pneumoniae* carbapenemase, *Escherichia coli*, and bacteroides. In addition, envision panel testing

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spinal fluid that would identify all enteroviruses, arboviruses, herpes simplex, and common bacterial pathogens with a turnaround time that is clinically meaningful.

We already have a few commercial molecular diagnostic products that "multiplex" (identify more than 1 target), such as 1 test for gonorrhea and/or chlamydia and another single assay for a multitude of different respiratory viruses. Without a doubt, more tests are on the way.

As a quick overview, a simplified glossary for the laboratory in our future might include the following.

NUCLEIC ACID AMPLIFICATION-BASED TECHNOLOGIES

Traditional labor-intensive nucleic acid isolation and purification methods are now replaced by automated platforms or incorporated directly into 1-step cartridge-based devices. Newer methods such as loop-mediated amplification (LAMP), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), and strand displacement amplification (SDA) require less equipment and time.

ELECTROSPRAY IONIZATION TIME-OF-FLIGHT MASS SPECTROSCOPY

Electrospray ionization time-of-flight mass spectroscopy (ESI-TOF) charges nucleic acid strands that move through a flight tube. Nucleic acids from clinical samples are first amplified by PCR then entered into the test system. Lower mass amplicons travel faster and reach the detector before higher mass amplicons. Then, the ESI-TOF software analyzes the amplicon weight to determine the base compositions of complementary DNA strands from multiple sequences. A major benefit is the ability to identify many diverse pathogens without having to target each analyte specifically.

PROTEOMICS AND MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is based on charged molecules moving through an electric field, which then create a unique spectrogram. This method is already being used in some clinical laboratories for rapid identification of culture results. The method is surprisingly simple and under exploration for direct use on clinical specimens, blood, and body fluids.

SEQUENCING

Sequencing is process of determining the precise order of all or a portion of the nucleotides within a molecule of RNA or DNA. Selected nucleic acid sequencing can establish a microbial etiology in cases in which no pathogen was identified by traditional culture methods, including those microorganisms that grow poorly or are inhibited in vitro by antibiotic therapy (eg, culture-negative endocarditis). Selected or targeted sequencing can provide genus identification for approximately 90% of bacterial strains, species level identification in 65%–85% of the isolates tested, and also identify known and novel mutations relating to drug resistance. Whole genome sequencing (WGS) provides a different approach and takes the concept of identification and pedigree to a whole new level. By identifying the entire genome, WGS could potentially identify complex mechanisms of resistance, virulence capabilities, and relatedness of isolates. Currently, these are tests are costly, but over time they may be more cost-effective.

DNA MICROARRAY: (DNA CHIP)

A microarray is typically a single slide or solid surface chip that can have tens of thousands of different probe dots on the surface. Flooding the slide with the sample to be tested can screen for a large number of DNA sequences. Microarrays are already being used for research, and a few laboratory developed clinical assays. The introduction of this method for the mainstream, commercial microbiology laboratory is not quite ready for widespread implementation, but there are obvious reasons to continue pursuit.

OVERALL

So what is the downside to abandoning our visible, countertop agar plates? Currently, state, federal, and some private agencies collect and save frozen or freeze-dried isolates for epidemiology and research. In addition, there has been value in resurrecting to life old, banked cultures to test for newer assays, such as reviving streptococci to test for new virulence factors. Unless or until molecular analysis can accomplish these and similar practices, we may need to use designated surveillance laboratories similar to our current practice for influenza.

Because the cost of some nonculture platforms remains steep, hybrid laboratories with cultures and molecular tests will likely lead us into the next generation. The laboratory of the very near future is likely to have 1 or more table top machines into which the user can insert a patient sample, select a test panel, and walk away. Results will be sent to an electronic health record and the clinician's smart phone at the patient bedside. Most importantly, there are aligned incentives to keep costs low and quality high. Therefore, the successful test systems will likely be efficient, affordable, and widely available in hospitals and at point of care.

The best is yet to come and it is coming quickly. Good-bye, agar-agar.