The Excitement of Clinical Microbiology

A nostalgia-prompted visit to a diagnostic lab brings insights into the disparate challenges clinical microbiologists face on a daily basis

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Clinical microbiology, one of the major branches of microbiology, goes largely unnoticed by academic microbiology researchers, in part perhaps because diagnostic activities are done in other places such as hospital and commercial labs. I suspect that many researchers have never set foot in one of these diagnostic labs. They are missing out on an exciting and rewarding experience.

This rift casts aside the historical roots of microbiology. At the beginning, soon after its pioneers recognized that bacteria cause infections, there was a huge rush to develop methods for determining the identity of agents responsible for patient illnesses. Ingenious laboratory media were designed to favor the growth of certain organisms and to reveal their distinguishing properties, while serological techniques were also being developed. In those early days, there was no gap between diagnostic and basic microbiology. In time, however, basic research diverged more and more from diagnostic microbiology.

Unfortunate Gap between Diagnostic and Basic Microbiology

I find it unfortunate that such a cleft exists. From a practical standpoint, clinical diagnostic laboratories continue to make most of the diagnoses of infectious diseases in hospitalized patients and thus play a key role in medicine and public health. Further, clinical microbiology can teach the rest of us a great deal.

For example, consider the story told during one of the presentations at the General Meeting of ASM in 2011, featuring medical microbiologist Andreas Baümler of the University of California, Davis (http://www.microbeworld.org/index.php?option=com_content&view=article&id=950:twim-8-live-in-nola&catid=107:this-week-in-microbiology&Itemid=275). He lifted the curtain on how tetrathionate broth, a once-standard laboratory medium, selectively enriches for salmonellae from stool specimens. It was developed for diagnostic use, taking advantage of how *Salmonella* carries out anaerobic respiration using tetrathionate generated in the colon and thereby outcompetes the normal flora.

In addition to diagnosing infections caused by well-established pathogens, clinical microbiologists uncover new pathogens, acting as sentinels for possible epidemics (http://blogs.cdc.gov/publichealthmatters/2012/09/laboratories/). They also provide statistical and clinical information regarding pathogens on the scene and spur demands on research to create novel diagnostic tools. In fact, the development of such tools is taking place so swiftly that, in not too many years, the practice of clinical microbiology may well become unrecognizable. Not only is the use of nucleic acid-based techniques expected to expand, but other sophisticated techniques such as mass spectrometry will make microbiological diagnoses ever more rapid and accurate (see p. 15).

A Personal Look at a Diagnostic Lab in Action

Let me share with you highlights and reflections from my recent nostalgic visit to the clinical microbiology lab of the VA Medical Center in San

- **Although closely aligned when microbiology emerged as a discipline, diagnostic and basic microbiology diverged years ago and are still mainly separate.**
- **In addition to their diagnostic work, clinical microbiologists uncover new pathogens and act as sentinels for possible epidemics.**
- **Although much testing in modern diagnostic labs is automated, technologists rely heavily on old-time skills and individual know-how.**
- **Despite trends for automated instruments to replace personnel in diagnostic labs, some clinical microbiologists remain confident that their skills will continue to be needed in this workplace.**

FIGURE 1

Members of the San Diego VA Clinical Microbiology lab. (l-r) Romelia Quinonez, Raymond Samson, Carlo Basallo, Monica Beach, Icela Gonzalez, Dr. Joshua Fierer, Juan Ybarra, Jasmine Estrada, Tracey Grosser, and Laura Gomez.

Diego, where I followed staff members during their normal course of work while asking them a bunch of questions (Fig. 1).

Nostalgia arose because this experience took me back to my early years. As a youngster, I had the good fortune to work in a pharmaceutical company in Quito, Ecuador (for an account, see http://schaechter.asmblog.org/elios_memoirs/, chapter 8). Then the only local facility equipped for microbiological work, the company also served as the diagnostic lab for physicians in Quito. For several years, I was more or less in charge of doing this diagnostic work. And I loved it. To a kid in his late teens, what could be more exciting than finding out that a specimen contained notorious bacterial pathogens such as staphylococci, typhoid bacilli, pneumococci, or shigellae?

I well remember the exquisite pleasure it gave me to isolate one unusual and difficult-to-cultivate organism, the agent of chancroid, *Haemophilus ducreyi* (http://microbewiki.kenyon.edu/index.php/Haemophilus_ducreyi). I also isolated a strain of *Klebsiella pneumoniae* var. *rhinoscleromatis* (http://en.wikipedia.org/wiki/Rhinoscleroma) that led to my first publication and which made it into the American Type Culture Collection, and it's still there (http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=9436&Template=bacteria). I felt a personal relationship to all these exciting bugs.

With such memories in my head, I stepped into the San Diego VA laboratory to learn what, if anything, was preserved from the old days (Fig. 1). Sure enough, much in this lab is automated. Biochemical tests for sugar fermentations and other physiological attributes, once done in test tubes, are now performed several dozen tests at a time by inoculating multisectored plastic plates. The readout is electronic, and the identities of bacteria are revealed by comparisons to databases. Blood samples are placed in a special incubator and monitored continuously for anything that grows and produces carbon dioxide. PCR-based tests detect gonococci, chlamydiae, and many viruses, with more such applications on the horizon. For some specialized purposes, the material is outsourced to a central laboratory facility.

Despite all the new gadgetry, however, most of the lab transactions require old-time skills, and abundantly so. Here is one example: Crucial to the work is "reading" the Petri dishes 24 hours after inoculation with clinical specimens, including sputum, feces, and abscess aspirates. Each specimen is inoculated onto several selective and differential media designed to favor the growth of specific species and to disclose some of their characteristics such as colonial morphology, sugar fermentations, and whether they cause hemolysis (Fig. 2).

FIGURE 2

Blood agar plate inoculated with a throat swab and incubated for 24 hours. Note the great variety of colonial types. The areas of clearing of the blood cells reveal that the bacteria are hemolytic. (Image from http://www.haloarchaea.com/teaching/molec_prac_course/classical.html.)

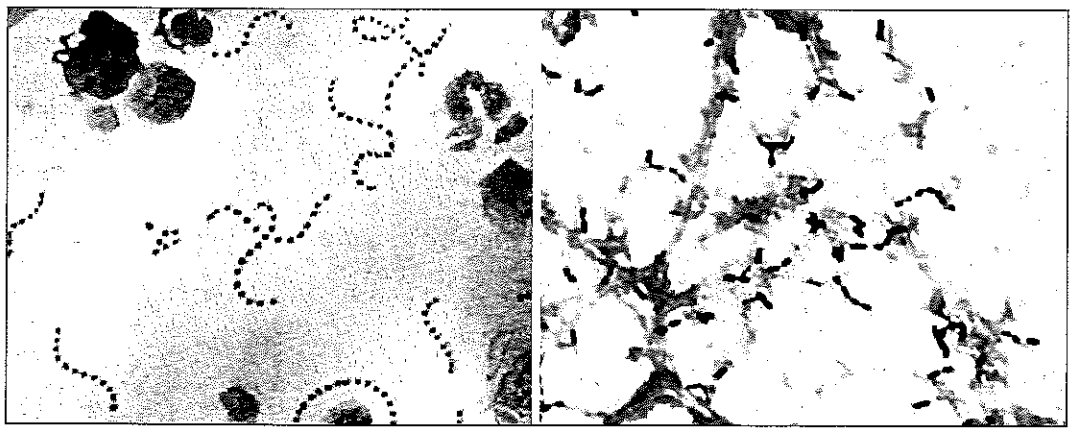
Nothing Simple about Choices facing Diagnostic Lab Technologists

Though "reading" the plates may sound simple, it surely is not. Every set of plates presents a different challenge about what to do with each bacterial colony, and it takes considerable experience to get it right. In fact, laboratory technologists—a title not to be confused with *laboratory technician*—go to school for five years but are not considered to be fully trained until they have three more years of practice! I could see why it takes so long. The information that can be gleaned from just looking at these dishes and smelling some of them seems endless. I could almost see the wheels turning in the brain of an experienced technologist, Tracy, as she navigated through a set of decision trees. She had to choose among many options, such as picking a colony for biochemical tests, making a smear for a Gram stain, and carrying out antibiotic sensitivity tests. I could hardly believe how many judgments she made in front of me in a matter of seconds, all while thoughtfully answering my questions.

One key challenge confronting technologists is to tell if the bacteria growing on plates are relevant to a diagnosis or are adventitious contaminants. This evaluation is seldom easy. A specimen of sputum may be badly tainted by the contents of the pharynx, a urine sample may come from a "bad catch," or bacteria may be picked from the skin while drawing blood. Making this judgment may require looking at a Gram-stained smear of a specimen as well as the colonies that grew from it.

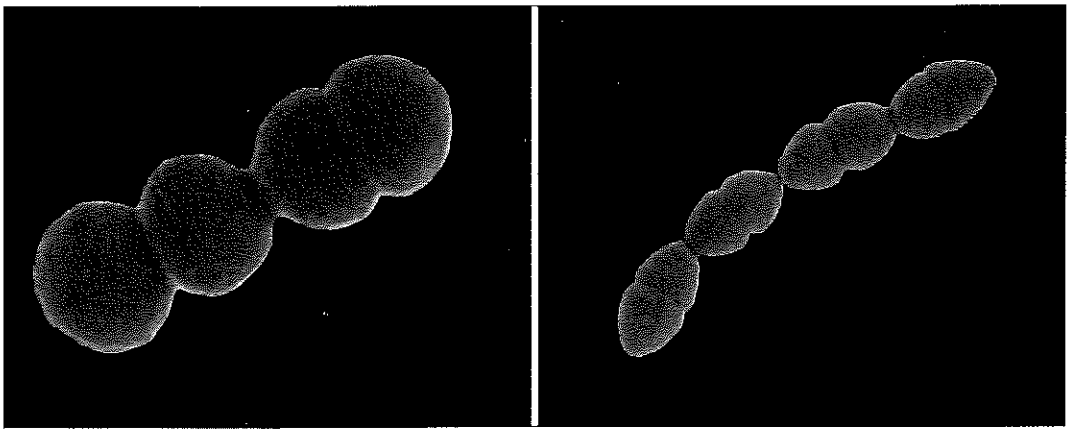
Jasmine, one of the newer technologists, showed me how looking at the smear helps her decide how to proceed. For example, if characteristic epithelial cells are present and neutrophils are absent in a sputum specimen, it probably contains mainly material from the throat and not

FIGURE 3



In a Gram-stained preparation, the β-hemolytic pneumococci (right) appear slightly elongated as compared to the α-hemolytic *S. pyogenes* (left). You'll appreciate that it takes an expert to tell the difference by the shape of the cells alone. (CDC images.)

FIGURE 4



The difference in shape between *S. pyogenes* (left) and *S. pneumoniae* (right) becomes obvious under the electron microscope. (Images © Dennis Kunkel Microscopy, Inc.)

deeper in the respiratory tract. And always there is the challenge of distinguishing potential pathogens from normal flora, especially in samples that contain large numbers of different organisms, such as throat swabs and feces. In addition, technologists who run PCR and immunodiagnostic tests must be familiar with such technology and keep up with frequent advances. Proficiency with other special techniques is required to diagnose fungal and animal parasites. Such an impressive array of essential skills would be hard to duplicate with machines (Fig. 3 and Fig. 4.).

Not surprisingly, basic science continually interfaces with both the established and the newer techniques. My friend Josh Fierer, who is an infectious disease physician and microbiologist affiliated with this VA and also the University of California, San Diego, explains these matters to interns and research fellows as follows: It is important to glean as much information as possible from microscopic examinations of Gram stains.

Basic microbiology knowledge often enters the picture. For instance, an experienced person can often tell α-hemolytic from β-hemolytic streptococci by looking at them in a smear. The former add new cell wall material from the septum outward (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2108156/), and thus will look elongated just after they divide. The β-hemolytics, on the other hand, make this material mainly at the septum; after division, they eventually become spherical. There are many such tricks. It is amazing how much information of great value can be gathered from simple procedures combined with keen observation.

Some of the challenges that these labs face are distinctly new. From time to time, for example, public health agencies that are responsible for detecting agents of bioterrorism send the labs "unknowns," asking them to determine the identity of the microorganisms in those samples. Depending on the organism, this task can be a big challenge or relatively straightforward. But either way, they had better get it right. Juan, the technical specialist in the VA lab, allowed that having to identify these "unknowns" is a good thing for the lab because "it keeps us on our toes!"

I was curious about how the people in the lab felt about future changes. All the signs point to continuing technological developments geared toward increasing the speed of diagnosis but likely displacing laboratory personnel in the process. But when talking to Monica, the supervisor of the lab, I appreciated her assurance that even in a very high-tech world, human skills will still be required. She felt that though technologies will change, the need for astute and experienced people will continue, even if the particular complement of skills required will somehow shift. She sounded confident. I was mighty glad.

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