The Laboratory Diagnosis of Infectious Disease — From Pasteur to PCR

By Paul A. Granato, Ph.D., Director of Microbiology

The medical specialty of infectious disease and the scientific discipline of microbiology were founded in the late 19th century when the great French scientist, Dr. Louis Pasteur, along with others established the Germ Theory of Disease. Prior to that time, even though human infections were known and epidemics of disease decimated large populations from several European countries, the scientific community was unwilling to accept that anything as small as a microscopic-sized “animalcule” or microbe could cause such human devastation and death. Fortunately, soon after Pasteur proposed the Germ Theory of Disease, Dr. Robert Koch validated Pasteur’s theory by establishing Koch’s Postulates and proving that a disease, known as anthrax, was indeed caused by a bacterium or “animalcule”, that he named Bacillus anthracis.

Since the great discoveries of Pasteur and Koch, the science of microbiology has continued to evolve which is now represented by the disciplines of bacteriology, virology, mycology, and protozoology/parasitology. Since the 1870s, when the Germ Theory of Disease was proposed to the present, many hundreds of different microorganisms have been discovered as the cause of human infections with new microbial causes of infectious disease discovered every year.

Traditionally, the laboratory diagnosis of most infectious diseases has been dependent upon the direct microscopic detection or cultural recovery of the microorganism from a clinical specimen. Microscopic detection is an inexpensive approach that can be performed in a matter of minutes but it suffers from poor sensitivity because large numbers of microorganism must be present in the specimen for its visualization.

Alternatively, culture-based methods are more commonly used to establish the diagnosis of most infectious disease, particularly those infections caused by bacteria, viruses, mycobacteria, yeast, and fungi.

Cultural methods offer improved sensitivities compared to microscopy but culture is a time-dependent process. Even though many bacteria can be recovered and identified from clinical specimens within 24 to 72 hours, other groups of microorganisms may take considerably longer. For example, the traditional cultural isolation of influenza virus from a respiratory specimen might take 7 to 14 days with other viruses, like cytomegalovirus, taking many more days. For mycobacteria, such as Mycobacterium tuberculosis, the cause of tuberculosis, the cultural isolation may take 4 to 6 weeks with another several weeks required for its biochemical identification. Comparable periods of time are required for the isolation and identification of fungi from clinical specimens. Clearly, these inordinate time delays are not commensurate with the needs of clinical practice and could potentially have an adverse affect on patient care by delaying the confirmed laboratory diagnosis of an infectious disease and the institution of appropriate therapeutic management.

For over a century since Pasteur’s and Koch’s great discoveries and despite the remarkable advances in the medical sciences, the laboratory methods, namely microscopy and/or culture, used to establish the diagnosis of an infectious disease remained unchanged. However, in the 1950s, this began to change when Drs. James Watson and Francis Crick published a scientific paper that described the structure of the DNA double helix, the molecule that carries genetic information from one generation to the next. Nine years later, in 1962, Watson and Crick shared the Nobel Prize in Physiology or Medicine with Maurice Wilkins, for their momentous discovery in solving one of the most important of all biological riddles – the structure of DNA.

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Laboratory Alliance’s fourth site for implementing the principles of Lean Management was our Rapid Response Lab (RRL) at Upstate University Hospital - Community Campus (UUH-CC).

The project commenced in October 2012. With each new Lean initiative, our implementation of Lean is enhanced. The physical improvements that were made at UUH-CC are very apparent to all visitors.

The laboratory has been transformed into a brighter one with better utilization of space. The new design better promotes visual and verbal communication. Some managers from a regional laboratory recently visited the UUH-CC lab and they were immediately impressed. They indicated that they may consult with Laboratory Alliance in the future when they design their new laboratory.

The lab at UUH-CC has a spectacular layout for many reasons. First, Lab Manager Rita Romano helped her staff embrace, with a positive spirit, the changes that Lean brings. Second, all laboratory employees were encouraged to offer input on how to improve the lab, and all ideas were considered. Approximately 95 percent of the suggestions were implemented.

Last, but not least, the success of the UUH-CC project can be attributed to the Lean Team members. Medical Technologists Diana Signore and Sara Grimshaw worked hard to plan and implement the new lab design. Thanks to their creativity and their scientific backgrounds, this RRL has become a more efficient area — one that can more easily adapt to future changes.

Although the design phase has been completed at UUH-CC, Rita and her staff continue to look for ways to increase productivity and to improve their processes. Indeed, they prove the words of Henry Ford to be true: “If you think you can do something, you can!”
Laboratory Alliance Acquires a GC/MS System

Laboratory Alliance is pleased to announce the acquisition of a Gas Chromatograph Mass Spectrometer (GC/MS) system to expand the menu of tests that can be performed at its Operations Center.

The GC/MS platform will enable measurement of target molecules, such as drugs, with greater specificity than is possible with current clinical chemistry / immunoassay-based analyzers. This instrument system is based on the combination of two popular laboratory technologies: gas chromatography and mass spectrometry. The GC/MS platform has been used for many years in chemical, pharmaceutical, forensic, and clinical laboratories.

Gas chromatography is a very effective way to rapidly separate compounds in biological samples (i.e.: blood serum or urine) based on parameters such as molecular charge, polarity, and other molecular properties. Mass spectrometry is a highly sophisticated methodology for the identification and quantitation of chemical compounds. It involves subjecting molecules within the sample to fragmentation, separation of these fragments based on charge-to-mass ratio, and their subsequent identification. The fragments produced are unique to the structure of the parent compound and can serve as a fingerprint to identify the parent compound.

The identification and quantitation of a particular compound in a biological sample by GC/MS involves an initial sample preparation step to isolate the compound of interest and chemically transform it into a volatile derivative. The volatile derivative is injected into the GC where it is heated and introduced into the gas phase, and further purified from the mixture of molecules in the original biological sample. The partially purified derivatized target molecules are then automatically introduced into the mass spectrometer where they are identified and quantitated.

The GC/MS platform will enable Laboratory Alliance to perform tests that require a particularly high degree of sensitivity and specificity, such as confirmatory tests for cannabinoids and other drugs.

Typically drug screening is performed with immunoassay technology, which utilizes antibodies that recognize the target drugs. This technology is cost effective and efficient, but the antibodies may cross react with other substances to produce false positive test results. Samples found positive for a particular drug by the initial screening test must be tested by more specific methods, such as GC/MS, to confirm the presence of the drug.

A confirmatory test for urinary cannabinoids is currently under development at Laboratory Alliance and will be the first test released on our new GC/MS platform.

For more information, contact Dr. Huchzermeier at 315-453-7200, or by email to royhuchzermeier@lacny.com.

Serum Testosterone in Women/Children by Liquid Chromatography Tandem Mass Spectrometry

The testosterone molecule is a steroid comprised of 19 carbon atoms arranged in four rings. Testosterone, like other steroid hormones, is derived from cholesterol. It is produced by the adrenal cortex, reproductive glands, and to a lesser extent by peripheral tissues. Testosterone in serum is largely protein-bound to either SHBG (sex hormone binding globulin) or weakly to albumin. A small fraction of testosterone in serum remains as the free hormone.

Although testosterone is most notable for its androgenic properties, and its importance for male health, it is also important for female health. The primary function of testosterone in the male is as a sex hormone; it is responsible for the development of male sex organs and secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair. The primary role of testosterone in females is as an estrogen precursor. In both males and females testosterone is important for health and well-being and plays an important role in bone metabolism, bone remodeling and prevention of osteoporosis.

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Other great scientific achievements followed the pioneering work of Watson and Crick but one of the most notable was the discoveries by Dr. Marshall Nirenberg. In brief, Nirenberg and others discovered that DNA consists of a code language comprising four letters or base pairs — adenine, cytosine, thymine and guanine or ACTG — which make up what are known as codons or words. Each codon is three base pairs long. A specific sequence of base pairs in the codon codes for one of the 22 amino acids that are used to make proteins.

Nirenberg’s seminal work, for which he was the co-recipient of the Nobel Prize in 1968, deciphered the genetic code, known as the “code of life,” and described how the genetic message of DNA was translated into proteins. These findings, along with those of many others, helped establish the scientific specialty of molecular biology that eventually led to the genetic sequencing of microorganisms and eventually to the sequencing of the entire human genome, which was achieved in 2003 through the combined efforts of an international research project called the Human Genome Project.

Knowing the nucleic acid sequence of each microorganism was an extremely important scientific advance as it allowed scientists to identify specific regions of nucleic acid sequences that were unique to that microorganism. This knowledge led to the development of non-cultural based, molecular methods that detected the presence of these unique sequences of nucleic acid that thereby identified the presence of the microorganism directly in a clinical specimen. The detection of these unique nucleic acid sequences in a clinical specimen confirmed the diagnosis of an infectious disease caused by a certain microorganism without having to use the less sensitive microscopic and/or cultural methods. These discoveries led to the use of rapid and highly sensitive molecular assays for establishing the diagnosis of an infectious disease.

Perhaps the greatest scientific advance in the use of molecular technology for the diagnosis of an infectious disease was the discovery of the polymerase chain reaction (PCR) by Dr. Kary Mullis for which he won the Nobel Prize in 1993. Mullis developed a thermal cycling reaction which allowed for the chemical polymerization of a complimentary sequence of nucleic acid that was unique to a specific microorganism. One cycle of the polymerase chain reaction could be completed in a short period of time which resulted in a doubling in number of the specific nucleic acid sequences that were present in the specimen. The product of this nucleic acid amplification test (NAAT) is called an amplicon. Since there was a doubling of amplicons every PCR cycle, ten PCR cycles would result in a 1,000-fold increase of amplicons while 30 PCR cycles would result in a one billion-fold increase. In a typical PCR assay, 40 thermal cycles are performed, which, using the instruments available today, can be completed within 70 to 90 minutes on average.

The use of molecular technologies, such as PCR and many others like it, is replacing microscopy and culture for the laboratory diagnosis of an increasing number of infectious diseases. The assays can be completed in “real-time” with final results generally available within 90 minutes of specimen receipt. Scientific evaluations of these NAATs have found that they are considerably more sensitive than standard culture. Importantly, highly reliable test results are now available in a time period that is commensurate with clinical practice (e.g. 1 or 2 hours instead of days, weeks or months).

Laboratory Alliance’s Microbiology Department has been using NAATs for the rapid and reliable diagnosis of a number of infectious diseases for the last decade. Some of these assays include the direct detection of: a) methicillin-resistant *Staphylococcus aureus* (MRSA) in anterior nares; b) MRSA, methicillin-susceptible *S. aureus*, and coagulase-negative staphylococci from blood cultures; c) group B streptococci in cervical/rectal specimens; d) epidemic and non-epidemic strains of *Clostridium difficile* in stool; e) enterovirus in cerebrospinal fluid; f) influenza A and B viruses in nasopharyngeal specimens; and, g) *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from genital/urine specimens.

Other NAATs have been developed that screen for up to seven to 15 microorganisms in a single specimen. These tests are called multiplex assays and Laboratory Alliance also uses these assays for the detection of a wide variety of microorganisms from respiratory specimens as well as the characterization of seven different gram-positive bacteria directly from blood cultures. Within the next 12 to 24 months, many more multiplex NAATs will become commercially available for the rapid and reliable diagnosis of many other infectious disease pathogens, particularly those that cause diarrheal disease.

Pasteur made many great discoveries during his lifetime not the least of which was the Germ Theory of Disease. No doubt, not even his brilliant mind could have imagined the possibilities of using NAAT molecular technologies for the diagnosis of not only infectious diseases but also certain genetic disorders and cancers. One of Pasteur’s most famous quotes was “Chance only favors the prepared mind.” Certainly, some discoveries are by chance but all discoveries are the product of having a prepared mind. Today, the technologic and therapeutic advances in modern medicine that we now enjoy have been the combined result of scientific and clinical investigations from prepared minds, in fact, very well prepared minds. Dr. Pasteur would be proud of their scientific achievements and the impact these discoveries have had on modern medicine and our every day lives.
Globally, tuberculosis (TB) is a common and, in many cases, fatal infectious disease that can be caused by several species of mycobacteria, most commonly *Mycobacterium tuberculosis*. The World Health Organization has estimated that 33% (over 2 billion people) of the world's population is infected with *M. tuberculosis*. TB is second only to human immunodeficiency virus (HIV) as an infectious cause of death worldwide. On average, a new TB infection occurs every second with a person dying every 20 seconds or about 4,400 people per day.

Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze or otherwise transmit respiratory droplets through the air. Over 90% of infections are asymptomatic or latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected.

Because over 90% of people who are infected with TB have asymptomatic or latent infection, it is important to identify these people by using effective screening tests before they reactivate their infection to symptomatic and potentially fatal disease which they may also transmit to others.

The diagnosis of latent or asymptomatic infection relies on the use of the tuberculin skin test (TST) or a blood test, called the QuantiFERON–TB Gold In-Tube (QFT-GIT) test. The TST was first developed in 1908 and is often called the Mantoux test in honor of the French physician, Dr. Charles Mantoux, who refined the use of the test. Even though the TST has been in use for over a century to identify individuals who may have latent TB, there have been limitations in its use in certain patient populations and problems with subjective test interpretation.

In 2001, the Food and Drug Administration approved the use of a blood test that detects latent TB infection by quantifying the amount of interferon-gamma released from sensitized lymphocytes in a patient’s blood following exposure with purified protein derivative from *M. tuberculosis*. Because patients with latent TB develop a cell-mediated immune response to protein antigens of *M. tuberculosis*, the production of interferon-gamma is a surrogate marker for the patient having latent *M. tuberculosis* infection.

Since its introduction in 2001, the QuantiFERON assay has undergone several modifications to improve test performance, the most recent being the QuantiFERON-TB Gold In-Tube (QFN-GIT) test. The test has greatest application for identifying individuals (mainly immigrants and refugees) with latent TB who have been previously vaccinated with Bacille Calmette-Guerin (BCG) vaccine. The TST cannot be used to screen BCG vaccinated individuals due to false positive reactions, which are not encountered with QFN-GIT assay.

In 2010, the CDC published updated guidelines (MMWR. 2010, 59 [No.RR-5]:1-25) on the use of the QFN-GIT assay. Aside from its recommended use in BCG vaccinated individuals, the QFN-GIT assay is recommended for all situations in which the TST was used. In addition, the QFN-GIT assay has been reported to have higher sensitivity and specificity than the TST.

A limitation in the more widespread use of the QFN-GIT test is its cost. However, when one considers the improved test performance of the QFN-GIT assay compared to the TST, an overall cost savings may be realized by the elimination of needless chest x-rays and the administration of unnecessary medications in individuals who have false-positive TSTs. In addition, patients need not return to their healthcare provider for the subjective interpretation of their TST within 48 to 72 hours of administration which can sometimes be interpreted incorrectly.

Laboratory Alliance has been offering the QFN-GIT test since 2007 and is the only laboratory in Central New York that provides this service. The QFN-GIT test can only be performed using blood collected in three special tubes that are provided by Laboratory Alliance. Any healthcare provider who is interested in having this test performed on his/her patient should contact Laboratory Alliance’s Client Services at 315-461-3008 to obtain specimen collection instructions and the three specimen collection vacutainer tubes.

This poster highlighting testing performed by Laboratory Alliance for Quidel Molecular was presented by Marcia Degilio, left, and Brenda Alkins at the 29th annual Clinical Virology Symposium held April 28 to May 1 in Daytona Beach, Fla.
Recognizing Outstanding Service to United Way

Senior Vice President Anne Marie Mullin, newly elected member of United Way of Central New York’s board of directors, presented Dr. Michael R. O’Leary with United Way’s Gold Award, recognizing his outstanding service to the board of directors and the Central New York community from 2009 to 2013. Dr. O’Leary and his wife, Colleen O’Leary, M.D., were co-chairs of the United Way’s Alexis de Tocqueville Society, a leadership giving program that promotes the importance of voluntary community service and personal giving. Anne Marie joined United Way’s board of directors in April 2013. She also serves on the operating board of Hospice of Central New York.

Great Response to United Way Book Drive

By Brenda Alkins, Device Trial Specialist, Microbiology Department

Our “Bring on the Books” book drive which collected children’s books for United Way’s Success By 6 saw overwhelming success when Laboratory Alliance employees filled 15 boxes with more than 450 books.

In addition Laboratory Alliance is purchasing and donating books on behalf of patients who participate in our postcard campaign. We mailed postcards to residents in Camillus promoting our Patient Service Center at Medical Center West and to residents in Cazenovia promoting our new center on Albany Street and when they bring the card with them to their next visit, a book is donated on their behalf.

The Success By 6 campaign is a children’s initiative of the United Way that promotes literacy and works to ensure that children in Onondaga County are ready for success in both school and life. The books will be donated to childcare centers and other programs that serve the youth in Onondaga County.

Serum Testosterone in Women/Children

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The circulating concentration of testosterone in women is only approximately 5-10% of that in men. The circulating concentration of testosterone in children is highly age and sex dependent with reference ranges extending down to less than 5% of the adult male testosterone concentration.

The sensitive and specific measurement of circulating levels of testosterone in women is essential for the investigation of androgen disorders such as alopecia, acne, and hirsutism; and for the detection of androgen-secreting tumors. In children, the measurement of circulating levels of testosterone is used for diagnosis, treatment and gender assignment of newborns and young infants with ambiguous genitalia. It is also used for pubertal stage determination and follow-up of children with precocious or delayed puberty.

Automated immunoassays have become widely used for routine testosterone testing because of their relatively low cost and potential for high throughput. Immunoassays are capable of accurately and precisely measuring the testosterone concentrations found in males. However, they lack the sensitivity and specificity to reliably measure the low testosterone concentrations found in women and children.

Liquid chromatography tandem mass spectrometry (LC/MS/MS) testosterone methods have gained popularity in recent years because they can achieve the required sensitivity and specificity for the low concentrations typical of females and children; these methods also require low sample volumes (particularly important for children).

In the second quarter of 2013 Laboratory Alliance will offer in-house LC/MS/MS testing for serum testosterone in women and children. The LC/MS/MS assay was developed by Laboratory Alliance and approved by the New York State DOH CLEP. This new in-house assay represents state-of-the-art methodology utilized by major reference laboratories and offers comparable sensitivity and specificity.
Hands-ONLY CPR

In the case of sudden cardiac arrest, the two steps everyone needs to know are:

1) Call 911
2) Push hard and fast in the center of the chest

How can you help?
Download the free app today!

The American Heart Association has developed a FREE Hands-Only CPR app for smartphone users. The app literally assists you in dialing 911 for emergency responders to be notified and then returns to show you exactly how to perform Hands-Only CPR. The app is available for iPhone or Android phone users. Help us get this information into the community!

Laboratory Alliance is promoting our local presence with a marketing campaign that supports local community groups, and is currently running advertising on YNN News, public radio stations, Syracuse.com, The Post-Standard and weekly and monthly newspapers, including The Catholic Sun and Eagle Newspapers.

As part of Laboratory Professionals Week April 22-26, employees who participated in Jean Day contributed $300 to benefit Hospice of CNY.

New Employees
Please welcome our new employees
At our Operations Center
Ann Adams – Courier
Alison Alsweiler – Technical Processing Assistant

At our Rapid Response Laboratory at Upstate University Hospital - Community Campus
Maria Abbate – Medical Technologist
Mickey Muscolino, Jr. – Medical Technologist

At our Rapid Response Laboratory at St. Joseph’s Hospital
Sandra Gunn – Laboratory Office Assistant
Karina Lenartowicz – Technical Processing Assistant
Jonathan Papazides – Laboratory Office Assistant
Linda Taylor – Laboratory Office Assistant

Employee Anniversaries

March, 10 years:
Malinda Desjardins
Carol Freitas

April, 5 years:
Diane Hall
Kathleen Shumway

April, 15 years:
Deborah Cullen
Lorna Dewitt
Olga Farrell
Susan Hayes
Claire Huchzermeier
Cathy Husted
Martha Stewart

May, 5 years:
Juliane Breh
Mary Maher

May, 10 years:
Patricia McKeigue
Joan Rusin

May, 15 years:
Lori Martin
Paul Suits
Pam Swierczek

June, 5 years:
Kelly Allport
Michelle Emerson
Nico Lyon
William Mammone
Janis Nolan
Sally Riggall

June, 10 years:
Linda Stallcup

June, 15 years:
Ian Crossett
Dawn Doviak
Christina Essig
Sheryl Hamilton
Ronald Hart
Laryl Hludzenski
Janice Munnett
Lori Post
Jill Rudnick
Theresa Tirabassi

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For five years in a row, we’ve been named the largest licensed laboratory in Central New York. Doctors and patients depend on us to provide reliable laboratory results vital to medical diagnosis and treatment.

Locally owned and employing more than 430 Central New York professionals, Laboratory Alliance performs more than 10 million tests annually. When you need laboratory tests, we’re in your neighborhood!

laboratoryalliance.com

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laboratoryalliance.com
CALENDAR OF EVENTS

Friday, May 31  St. Joseph’s Hospital Health Center Gala, Turning Stone Resort. **Laboratory Alliance is a sponsor.**

Tuesday, June 11  **United Way of CNY**, Leadership Recognition Reception, The M.O.S.T. **Laboratory Alliance is a sponsor.**

Tuesday, June 18  **JPMorgan Chase Corporate Challenge.** Laboratory Alliance employees who want to participate need to register online by Tuesday, May 21.

Wednesday, June 19  **Oneida Healthcare Foundation Golf Classic**, Kanon Valley Country Club. **Laboratory Alliance is a participant and a sponsor.**

Friday, June 28  **Foundation for Upstate Towsley Pro-Am**, Shenendoah Golf Club at Turning Stone Resort. **Laboratory Alliance is a participant and a sponsor.**

Monday, July 15  **Crouse Health Foundation Classic Golf Tournament** at Bellevue Country Club. **Laboratory Alliance is a sponsor.**

Nearly 100 co-workers, friends and family of Barb Gonnella participated in the American Heart Association Heart Walk on April 6 in Barb’s memory, following her sudden and unexpected death on Feb. 14. Barb was an advocate for a healthy lifestyle and encouraged physical fitness. The event was held on a sunny morning at Onondaga Community College.

J.P.Morgan

**Corporate Challenge**

Laboratory Alliance will be “Teaming Up For A Greener Tomorrow” with JPMorgan Chase for the **2013 Corporate Challenge on Tuesday, June 18**. Join the Laboratory Team and run, jog or walk the 3.5 mile course at Onondaga Lake Parkway starting at 6:25 p.m. The JPMorgan Chase Foundation will make a donation on behalf of all participants to the YMCA of Greater Syracuse.

To register go to [www.jpmorganchasecc.com](http://www.jpmorganchasecc.com)

- On the left side of the screen, under “Schedule and Registration” click on “Syracuse.”
- Again, on the left side, under “About Syracuse” click on “Registration.”
- Under “Online Registration” click on “Register.”
- Click on “Laboratory Alliance of CNY”, then “Register For This Company.”
- Fill in the bold fields and then agree to the “Agreement and Release” at the bottom of the form.
- Laboratory Alliance will be paying the $32 entrance fee for each participant; for payment method click on “Pay later (payment being covered by Company Captain).”
- You will receive a confirmation email from J.P.Morgan Corporate Challenge.

If you have any questions please go to [www.jpmorganchasecc.com](http://www.jpmorganchasecc.com) or call Team Captain Becky Reynolds, Microbiology Department, at 410-7067 (days).

**LABlines**

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Comments, suggestions or inquiries should be directed to Anne Marie Mullin Senior Vice President 315-461-3036, or by email to annemariemullin@lacny.com