Identification using MALDI-TOF

Protein fingerprinting-based bacterial identification using MALDI-TOF

By Jennifer Bibbey

Residual Host Cell DNA and Host Cell Protein

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By Sadettin Ozturk, PhD

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By Mark S. Klempner, M.D. and Keith Reimann, DVM

The recruitment of Dr. Keith Reimann, faculty and staff in his laboratory, and the National Non-human Primate Reagent Resource marks an important milestone for MassBiologics of UMMS. Not only does this represent the largest recruitment of outstanding scientists with stellar international stature to MassBiologics, but the expertise of the Reimann group in primate models of human diseases, immunoglobulin evolution and biology and academic collaborations around the world broadens our horizon and our pipeline. Dr. Reimann’s laboratory has been on the forefront of immunology studies in primates. His research has focused on the pathogenesis and treatment of simian immunodeficiency virus (SIV) infections in nonhuman primates, the roles of co-stimulatory molecules in transplantation and autoimmunity, and the evolution of primate immunoglobulins. In collaboration with the Letvin laboratory in the late 1980’s and 1990’s Dr. Reimann published a series of seminal articles exploring the pathogenesis SIV infection in non-human primates. Using monoclonal antibodies to specific cell subsets, these studies clarified the roles and dynamics of CD4/CD8 cells in SIV infection. These studies also pointed the way toward passive and active immunotherapy for SIV/HIV infections. As an outgrowth of his own research Dr. Reimann established the NIH Nonhuman Primate Reagent Resource, a resource laboratory that has been continuously funded by the NIH for last 12 years. This unique resource laboratory supports over 400 academic and industry investigators world-wide. Novel reagents developed by this lab are administered to animals to deplete specific lymphocyte populations, to block the action of signaling molecules such as cytokines, or to interfere with specific immune pathways in vivo. These new reagents have enhanced the utility of animal models for studying pathogenesis and immunoprophylaxis of AIDS and other infectious diseases, in studies of developmental biology and in drug development.

In his letter of recommendation for appointment of Keith to Professor of Medicine at UMMS, R. Paul Johnson, M.D., Professor of Medicine at Harvard Medical School wrote “many of the most important observations in the SIV-macaque model [of HIV/AIDS] came from the group led by Drs. Letvin and Reimann where Dr. Reimann played a critical role. Many of the most important observations during this phase included the application of flow cytometry to identify specific subsets of lymphocytes that were involved in AIDS immunopathogenesis. Flow cytometry was in its infancy then, and techniques for the immunophenotyping of rhesus macaques were not well-described. Dr. Reimann’s rigorous, careful work quickly became the standard in the field. His national and international stature in this area has continued to this day.

Protein fingerprinting-based bacterial identification using MALDI-TOF, contd.

genotyping in the industry, where not explicitly required, is largely due to the absence of reliable and reproducible phenotypic methods.

Recently, proteotypic identification has emerged as a rapid method for species level identification of bacteria, (it does not yet include yeasts and molds), and it is positioned between the standard genotypic and less reproducible phenotypic methods. The ability to make a taxonomic assignment at the species level, using relatively unprocessed cells has emerged as a tool for fast and reliable identification of microorganisms. The use of Matrix-Assisted Laser Desorption/Ionization (MALDI) based on Time of Flight (TOF) Mass Spectrometry for the identification of large bio-molecules has expanded to microbiology by identifying patterns of bacterial proteins. Using MALDI-TOF, analysis of bacteria from a single colony is possible, allowing for the screening of mixed cultures. In this process, a small number of viable cells from the unknown bacterium are transferred to an analysis plate covered with a matrix crystallization mix. The target then undergoes laser pulses that desorb, ionize/charge, fractionate and displace the proteins at speeds dependent on their mass. Spectra of proteins in the 2 to 20 KDalton range are compared to a reference standard and a bacterial database of isolates.

MassBiologics’ vendor’s database for the genotypic method contains about 2700 entries while the proteotypic database contains fewer entries but continues to grow. To expand their service and database, the vendor has encouraged clients to select the proteotypic test method for all bacterial samples, with the undertaking that where a sample cannot be definitively identified using the MALDI-TOF triage, the genotypic test method would be used to confirm the identity. A proteotypic identification report is characterized by a score value based on the presence/absence of about 2000 different size fragments. A genotypic report measures less than 500 base pairs in the traditional 16S sequence, largely invariant but, with one of four nucleotides a possibility at each base position. Again, the top 10 matches are reported and interpretation rules require minimum scores for a species identification and minimum difference in score between the first and second closest match to substantively discriminate between the two.

Historically, MassBiologics has exceeded regulatory requirements by using 16S genotypic identification methods for all isolates requiring species level identifications. Since 2010, MassBiologics has adopted the proteotypic technology for routine bacterial isolate identifications. During a qualification period, samples were sent to the vendor for testing using both methods. The proteotypic method
produced data consistent with the genotypic method. In some cases, library and taxonomy limit clear species identification; for example, the proteotypic method returned *Bacillus subtilis* while the genotypic method reported the superspecies complex *Bacillus amyloliquefaciens/ atrophaeus/ mojavensis/ subtilis/ vallismortis*. While the 16S method attributed the isolate to a group of highly related species, the closest match was to *Bacillus subtilis spizizenii*. Minor variations in results yielded by the two methods are not unexpected given the differences in libraries searched and incompletely agreed taxonomy.

In 2012, MassBiologics submitted 65 isolates for MALDI bacterial species identification. Of these, MALDI identification was achieved for 59. The samples not definitively identified by the proteotypic method were Gram positive rods: *Bacillus amyloliquefaciens, Bacillus thuringiensis, Corynebacterium tuberculosis*, *Paenibacillus glycanilyticus* and Gram negative rods: *Pelomonas saccharophilia*, and *Acidovorax soli* which were identified by the genotypic method. The success rate for Gram positive cocci was 100% while limitations for gram positive and negative rods are still encountered. Some limitations may be inherent to the current method capabilities but others may be impacted by phenotypic differences in the protein profile expressed by the isolate. Different proteins will be expressed under different conditions so the method still depends on growing fresh colonies on a standard medium to achieve consistent results.

Proteotypic identification by MALDI-TOF is a cost effective and time saving method of identifying facility and process isolates. While genotyping remains an important tool for critical investigations and problem samples, the majority of organisms that MassBiologics encounters in our GMP microbiology program are reliably identified to the species level by proteotypic identification.

**Residual Host Cell DNA and Host Cell Protein, contd.**

Contaminating mammalian host cell DNA is potentially dangerous to patients in two ways – by carrying genes that may increase the risk of cancer or by carrying viral DNA. In 1986 the WHO guidelines limited host cell DNA to no more than 100 pg per dose of drug. It is technically difficult to achieve this level of purity given the doses required for many monoclonal antibodies, and this amount of DNA can be difficult to measure. In addition, the DNA present in our antibody products is broken into small pieces, decreasing the chance that an intact gene or virus is present. For these reasons the WHO guidelines –(1997) call for an upper limit of 10 ng host cell DNA per dose. For example, the amount of DNA initially present in the volume of cell culture for a 10mg/kg dose of MAb X is about 500ug. To achieve 10 ng per dose, at least 99.95% of the DNA has to be removed by the purification process. Our purification processes at MBL routinely achieve much greater purity.

How large a risk is contaminating DNA to patients? Direct evidence is limited, but scientists at the FDA have done experiments to address this question. Mice were injected subcutaneously with different amounts of DNA encoding two genes associated with cancer, H-ras and c-myc. They found that injection of 12.5 mg of both genes into mice increased the incidence of tumors. The incidence of tumors did not increase when less DNA was injected, or when mice were injected with DNA of just one of the two genes (1). The same laboratory also found that transfection of cells (introduction of DNA directly by chemical means) with 2 mg of DNA isolated from cells infected with HIV could generate infectious HIV (2). These results can be interpreted as showing that the risk from host cell DNA is real, but when these amounts are compared with the FDA guidelines it can be seen that the margin of safety is large.

Contaminating host cell proteins present a different risk. These proteins are potential immunogens and allergens. The regulatory guidelines for residual host cell proteins are less specific than for host cell DNA because of the heterogeneity of the potential contaminating proteins and lack of specific information about their identities. a concentration of <100 ng/mg antibody is typically considered acceptable as long as it can be demonstrated that the assay detects a majority of the proteins that may be present. Even though the risk is small, it is a real risk, as demonstrated by a recent FDA clinical hold being placed on a product that was found to have a host cell protein contaminant that caused an immune response in patients (3). A sensitive ELISA was developed at MassBiologics to assay residual host cell proteins at concentrations as low as 0.8ng/mg host cell protein at a 25 mg/mL product concentration, well below the specification of 100 ng/mg.

Measurements of these impurities and their removal throughout the development of our products are some of the many ways we insure a safe, consistent product.


Former Massachusetts Gov. Argeo Paul Cellucci, a devoted public servant who dedicated the final chapter of his life to raising funds to support the University of Massachusetts Medical School’s research into a cure for amyotrophic lateral sclerosis (ALS), died June 8, 2013 at his home in Hudson, surrounded by family, from complications of the disease. The announcement was made by UMass Medical School Chancellor Michael F. Collins, MD, on behalf of the Cellucci family. Gov. Cellucci was 65.

The 69th governor of Massachusetts and former U.S. Ambassador to Canada announced in January 2011 that he had been diagnosed with ALS, or Lou Gehrig’s disease. Soon after, he joined Chancellor Collins and renowned UMMS physician-researcher Robert H. Brown Jr, DPhil, MD, in launching the UMass ALS Champion Fund, supporting ALS research in Dr. Brown’s lab at the medical school. The campaign has raised nearly $2 million under Gov. Cellucci’s leadership.

“It is with deep sorrow that we acknowledge the passing of Governor Cellucci as a consequence of ALS. From the beginning, the Governor refused to allow his challenging diagnosis to prevent him from continuing his lifetime’s work of serving and helping others. Indeed, he made a conscious and inspiring decision to use his illness to raise awareness, galvanize action and spread hope,” said Collins. “In launching the Champion Fund, the Governor’s primary motivation and greatest satisfaction came from knowing that others, now and far into the future, could win the gift of more days as a result of the breakthroughs enabled through the support of his fundraising efforts.”

“Our sincere condolences are with Governor Cellucci’s family,” Collins said. “To his many friends and colleagues who were touched by his humanity — before and during his illness — the passing of Governor Cellucci is a moment of great sorrow, but also of inspiration. Because of him, many were inspired to support research into possible cures for ALS. Many more were inspired by his willingness to invest time and energy, which became more precious with each passing day, to help others.”

“The entire University of Massachusetts community is saddened to learn of the death of Governor Cellucci,” said University of Massachusetts President Robert L. Caret. “Governor Cellucci devoted his life to the cause of public service and worked to improve the quality of life for every citizen of the commonwealth.”
Cellucci is survived by his wife, Jan, their two daughters, Kate, and Anne, and four grandchildren, Rhys, Gabriel, Francesca, and Lucia. The Hudson native served more than three decades as an elected official and was proud of having never lost an election. After graduating from Boston College in 1970, he became involved in local government. He served as a selectman in Hudson while attending Boston College Law School. He was a member of the U.S. Army Reserves. He graduated from law school in 1973 and continued to serve in the Reserves, earning an honorable discharge at the captain's rank in 1978. In 1976, Cellucci was elected a member of the Massachusetts House of Representatives, where he served until 1984, when he began three terms in the state senate.

Cellucci was elected as Gov. William Weld’s lieutenant governor in 1990 and was sworn in as governor to complete the remainder of Gov. Weld’s term of office in 1997. He was elected governor in his own right in 1998. In 2001, President George W. Bush named Cellucci U.S. Ambassador to Canada. In his role as ambassador, Cellucci served for four years to strengthen and grow the economic trading relationship between the United States and Canada, expedite border crossings for commercial and passenger vehicles, continue the integration of the North American energy market, and help resolve trade disputes. These responsibilities were particularly challenging to address following the September 11, 2001, terrorist attacks, when security immediately became the ambassador’s top priority.

When Cellucci left his post at the U.S. Embassy in Ottawa, he returned to work in the private sector focusing on U.S.—Canada initiatives that continued to strengthen the economies of these two nations, the largest bi-lateral economic relationship in the world. Gov. Cellucci joined the government relations consulting group ML Strategies in March of this year, following his longtime political ally, Gov. Weld, into the firm led by their former Secretary of Economic Affairs Steve Tocco. At the time of his passing, Cellucci was working on developing Canadian energy clients for the firm.

In launching the Champion Fund in 2011, Gov. Cellucci oversaw an effort that had immediate success and made important impacts on the ALS research being conducted in Brown’s laboratory at UMass Medical School. Gov. Cellucci enjoyed the support of prominent business and political leaders including Massachusetts Gov. Deval Patrick, all of the former governors of the commonwealth, former Lieutenant Gov. Timothy Murray and former White House Chief of Staff Andrew Card. Gov. Cellucci launched the Champion Fund during an on-field event at Fenway Park in May 2011. He played a role in securing countless major gifts, including a $500,000 donation from Biogen Idec.

“I commend Chancellor Collins and Dr. Brown for developing this important partnership with Governor Cellucci and know that the ALS research taking place at UMass Medical School — work that has already led to important breakthroughs — will continue and that the progress and advances we expect in the years ahead will serve as a fitting memorial to Governor Cellucci’s career and service,” President Caret said. “While we at the University of Massachusetts mourn the loss of a great friend, we are proud of our association with Paul Cellucci and will be proud to continue this vital work in his name.”

Additional information on the UMass ALS Champion Fund, as well as video, is available at www.umassALS.com.

and is probably best well-recognized in Dr. Reimann’s leadership of the Nonhuman Primate Reagent Resource. This resource laboratory remains the standard in the field as the gold-standard clearinghouse for information on the ability of various antibodies to be used for immunophenotyping of nonhuman primates and has provided an invaluable service in its efficient distribution of reagents for both in vitro and in vivo use.”

Primates are relatively new animal species evolving only 60 million years ago, well after dinosaurs became extinct. The nonhuman primates’ phylogenetic proximity to man make them unique and valuable animal models for studying diseases that cannot be adequately modeled in the common laboratory animals. Of more than 300 different species of primates alive today, only about a dozen species—those which are plentiful in nature, easily reared in colonies and whose genetics are defined—are utilized in biomedical research.

Nonhuman primates serve as important models for many infectious agents that do not infect rodents or other common laboratory animal species. These animal models are used to study disease pathogenesis, test new therapeutics and evaluate vaccine candidates whenever the studies cannot be performed in humans. Using the reagent “tools” developed by the Nonhuman Primate Reagent Resource, investigators can determine which components of the immune response are most effective in controlling many infections. Recently the Reimann lab collaborated with two laboratories developing novel Ebola virus vaccines and helped them define whether antibodies or cellular immune responses were responsible for vaccine protection. Studies such as these are critical for understanding the immunopathogenesis of many infections. More importantly, they help to improve or design new vaccines. [ref 1,2, ]

Primates also serve an important role in preclinical evaluation and safety testing of new drugs. Many therapeutic biologics bind only to primate receptors. Thus, testing for efficacy and safety must be performed in a primate species. The Reimann laboratory’s work with transplantation biologists has led to a better understanding of the immunologic pathways that result in rejection of transplanted organs. They have also identified novel immunosuppressive antibodies that are being explored as human therapeutics [ref 3]. The Nonhuman Primate Reagent Resource currently supports studies of allogeneic kidney, pancreatic islet, bone marrow and spermatogonial stem cell transplantation. They also assist investigators utilizing porcine hearts and islets in primate xenotransplantation models.

All nine members of Dr. Reimann’s Beth Israel Deaconess laboratory have relocated with him to MassBiologics of UMMS, including his lab’s key leadership. Rijian Wang, MD, PhD, Associate Director, directs all molecular biology and protein chemistry. He completed a post-doctoral fellowship at Dana Farber Cancer Institute and has industry experience at Geron and Aveo Pharmaceutical. Pamela Chatis, PhD, Project Manager, completed a postdoctoral fellowship at MIT’s Center for Cancer Research and continued work in molecular virology and infectious diseases. She, too, has prior industry experience at DuPont Medical Products and PerkinElmer Life Sciences. Jichu Li, MS, Research Associate III, has had responsibility for upstream and downstream processing in the Reimann lab for the past 7 years and has worked with other lab members to develop their current expression platform. As new members of the MassBiologics community, they will continue operating the Nonhuman Primate Reagent Resource (http://www.NHPReagents.org) and utilize MassBiologics facilities for development and manufacturing of primate-specific antibodies. The group will also participate in development and validation of MassBiologics products using their experience in antibody engineering and characterization, and in preclinical models of infectious diseases.

We are excited to welcome the entire Reimann laboratory to MassBiologics of UMMS. Keith and his group bring a stellar new chapter to our 120 year history of exploring and exploiting the immune system to discover, develop and manufacture medicines that improve people’s lives around the world.

References:


Lyme Research Study

A Research Study of Antibodies to the Lyme disease bacteria

MassBiologics of UMMS is sponsoring a research study of antibodies to *Borrelia burgdorferi*, the bacteria that causes Lyme disease.

We are seeking healthy volunteers who:
- Are at least 18 years old
- Have previously received the licensed Lyme disease vaccine (LymeRx) or prior immunization with vaccine containing *B. burgdorferi* antigens

After valid consent volunteers will fill out a questionnaire and provide a blood sample.

Compensation for time will be provided.

Please contact 617-474-4444 or LymeAntibody@umassmed.edu

UMASS Medical School

IRB #H00000760

Approved UMass Medical School IRB
Status of Purification Suite Renovation (2nd in construction /renovation series)

By Frank Fazio

On March 11, 2013 a project was started in the MassBiologics Manufacturing area to renovate rooms 1072, 1073, and 1074 to make them appropriate to perform purification of tetanus toxoid and diphtheria toxoid. Historically these processes were performed in the MassBiologics Jamaica Plain Facility.

During the months of March and April the existing rooms 1072, 1073, and 1074 went through a complete transformation in order to increase their respective classifications from ISO 8 to ISO 7, as well as providing the necessary infrastructure for the purification process. The ceilings were removed and replaced with hard gypsum ceilings appropriate for ISO 7 environments. New doors and entry ways were designed to provide the appropriate gowning capabilities for an ISO 7 area. The air supply ducting and exhaust ducting were rerouted and modified to supply the necessary air volume to achieve the air changes required by ISO 7. Room 1073 was converted into a 400ft² ISO 7 1°C-5°C cold room. The 1073 cold room is used as a processing cold room where manufacturing operations take place inside the cold room. The existing Mattapan hot WFI loop was extended to include a port inside of the purification room 1072. In order to complete the modification to the WFI loop the 3" stainless steel WFI piping was cut in two places in the Mattapan 1 basement and 3" stainless steel pipe had to be run across the basement, up into room 1072, back down into the basement and orbital welded in place in two places. The interior of the pipe then had to be passivated before WFI could be reintroduced into the system. Lastly stainless steel case work was moved from Jamaica Plain and installed in the new ISO 7 1072.

All of this work was completed on schedule by April 26, 2013. The next steps include moving the specific process equipment into their final positions and completing the required validations.