

CMPT's 2014 Annual General Meeting

This year's Annual General Meeting was held for the first time close to our new location at the University of British Columbia campus.

We were fortunate to hold our meeting at the Liu Institute for Global Issues, located at the edge of a densely wooded site, and the participants were impressed.

After Dr. Noble's welcoming remarks, CMPT's coordinator, Esther Kwok, reviewed the year's events the challenges, successes, and the road ahead.

Next, editor Veronica Restelli, presented data on proficiency testing results on throat swab cultures over a period of 15 years.

Our senior technologist, Caleb Lee, introduced us to a new type of sample designed at CMPT to address the need of laboratories to screen for multi-resistant organisms. The program is now in its testing phase with the Clinical Bacteriology program participants.

CMPT's technologist, Suhanya Bhuvanendran, spoke about the usefulness of heterotrophic plate count (HPC) in water microbiology and the possibility of adding this component to the Water Microbiology program.

Our guest speaker, Dr. Jennifer Won from the Canadian Immunohistochemistry Quality Control (cIQc) program, spoke about the new developments in their immunohistochemistry proficiency testing program.

The AGM is also CMPT's opportunity to reflect on the past year and review the goals and objectives set during the previous meeting.



Liu Institute for Global Issues - Case room.

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Dr. M. Noble presents at the CMPT's AGM

Dr. Noble's presentation focused on the year in review. As usual, Dr. Noble highlighted the constant and strategic presence of CMPT in Quality meetings, international activities, partnerships, and educational initiatives.

Our Annual Satisfaction Survey targeted CMPT's website on two fronts: the data entry and the site navigation's friendliness.

The ease of data entry lagged behind other measures such as quality, usefulness, and ease of navigation. This was not surprising because some participants feel the data entry process is not typical of in-laboratory standard data entry. We have committed to address this issue and CMPT will work on improving the data entry process.

CMPT'S ANNUAL GENERAL MEETING

CMPT's website is a content rich, high-utilization site loaded with both current and archived critiques and newsletters, and is also the corner-stone access point for entering EQA information and challenge results. It is important that this content is accessed in the most effective way and thus, we have also committed to improve the users' navigation experience of our site.

Finally, Dr. Noble presented the Goals and Objectives for 2014—2015 (Table 1) which reflect our commitment to our Quality Policy and Mission Statement.

You can read the complete "Year in Review" report by Dr. Noble in our [Annual Report](#)



Right: CMPT's Clinical Bacteriology expert Committee

Table 1. GOALS and OBJECTIVES 2014-2015

P14_1	Begin revamp of www.cmpt.ca to improve data entry and navigation
P14_2	Purchase new microscope photography apparatus to improve time and focus issues
P14_3	Pursue new Antibiotic Resistance Screen program
P14_4	Generate at least one manuscript preparation and publication
Q14_1	Continue with ISO 9001:2008 certification with view to prepare for ISO 9001:2015
Q14_2	Prepare for ISO 17043:2010 accreditation by American Association for Laboratory Accreditation (A2LA)

Dr. Michael Noble's note:

"As you know, one of my aspirations is that we start to get some staff from CMPT's participating laboratories attend the meeting. I understand that travel restraints may prevent people from outside the lower mainland from attending, and that work expectations could interfere with some local people attending. Nonetheless, we look forward to overcoming these barriers and reaching our participant constituency. Any and all suggestions are welcome."

CMPT AND ISO 17043

There are a variety of reasons for an organization to seek an external review such as accreditation or certification. Sometimes, it is simply because it is a regulated requirement; they have no choice. Of more interest, some organizations do it as a voluntary activity. Perhaps it is done as a business opportunity or as a way of demonstrating and assuring customers of organizational quality; sometimes it is done because of the organization's internal and best practice reasons. For CMPT, it is a combination of the three, and more.

CMPT started down the path of external evaluation in 2002 when it became evident that some participants in the laboratory community saw a disconnect: while medical laboratories were expected to demonstrate their quality through external review, there was no expectation or requirement of those same external organizations, who were pronouncing on laboratory quality, to undergo the same level of discipline or rigour. CMPT's decision to undergo certification to ISO9001:2000 at the time was an in direct response to this disconnect. We were one of the first medical quality partner organizations in North America to take that step.

For us, it was a major success. We have maintained our certification for annual review ever since and have found the process of established quality management critical to our very existence. We are financially more stable, make fewer errors, pick up errors faster, and respond faster than we ever would have before. The laboratories that work with us comment, in almost uniformity (greater than 90 percent in a recent survey), that knowing that we maintain our external international certification gives them more confidence in our processes and credibility.

Starting in 2015, we begin a new path by taking on a new external review to the document ISO/IEC 17043:2010 (*Conformity assessment -- General requirements for proficiency testing*). 17043 (for short) was first developed by the International Organization for Standardization in conjunction with the International Laboratory Accreditation Cooperation (ILAC) in 2008 and CMPT was one of the organizations that sat at the meeting table as the document was created.



Similar to ISO 9001, ISO/IEC 17043 is a document with a broad audience in mind, not just the medical laboratory or water testing laboratory proficiency testing (PT) bodies.

One would be astounded by the range of PT providers in existence, including PT for food testers, cement makers, bridge designers, textile makers, dye makers, and electrical impedance testers. If there is an industry that measures its quality and consistence through a laboratory activity, there is usually a group of PT providers to help ensure that the quality of work is documented and credible. We are just a small part of that process.

ISO/IEC 17043 became available as an assessment tool in 2010-2011. While we were interested in the document and we could have sought assessment sooner, we decided to wait until the ISO/IEC 17043 accreditation bodies had some experience with the document.

We wanted the confidence to know we were working with a competent assessment body. We have now decided to go forward using the American Association on Laboratory Accreditation (A2LA), an international organization that has now assessed a number of medical laboratory PT programs, including another within Canada.

Our decision to seek accreditation to ISO/IEC 17043 does not imply in any way that we are dissatisfied with ISO9001:2008 as a credible measure of quality, nor of Standards Australia International (SAI Global) as an assessor. Indeed, it is our intent to continue on with both assessments.

ISO 9001 is still the single most important assessment tool for measuring management quality for the broad range of service providers and manufacturers. 17043 is more of a drill-down assessment of specific aspects of proficiency testing, such as our organizational structure, advisory committees, how challenges are selected and created, quality control, and the type of information that we provide. We see the two standards as complementary, each in its own zone of expertise.

We will keep CMPT laboratories informed as the process progresses.



Dr. Michael Noble
CMPT Director and Chair

Part II: MALDI-TOF in the Clinical Microbiology Laboratory

These series of articles on Mass Spectrometry (MS) are intended as teaching materials for laboratory staff without a physics background. The purpose of these articles is to assist with the understanding of the application of MS in microbiology and is not intended to be an extensive or comprehensive review of the different MS techniques. The reader is encouraged to check the numerous reviews on the topic, some of which are listed at the end of each article.

Timely and accurate identification of microorganisms is important for clinical laboratories and ultimately for patient care.

Bacterial identification is normally based on Gram staining and phenotypic testing. Although some phenotypic tests are performed within minutes, most require 18 hours after the initial culture is obtained; in addition, the wide range of testing associated with the identification of a wider variety of organisms presents a significant challenge for microbiology laboratories.

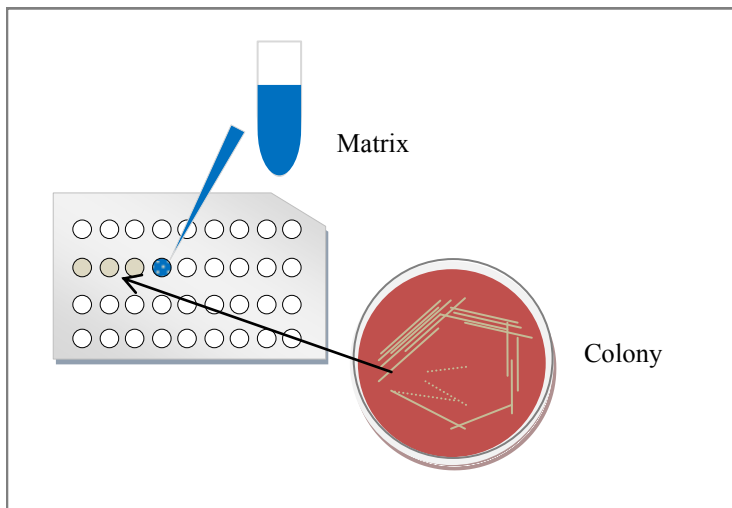
Biochemical test panels or automated instruments for microorganism identification help with the identification of routine and more complex organisms, however, these methodologies still have long turnaround times and require costly consumables.

Matrix-Assisted Laser Desorption – Time of Flight Mass Spectrometry (MALDI-TOF MS) is a very promising technology because of its ability to identify bacteria with virtually no sample preparation, in a very short period of time, and starting from a single colony.

Sample preparation

In MALDI-TOF bacteria are typically removed from the agar using a culture loop and suspended in a solution to make a cloudy suspension that is spotted on the test plate. Samples are overlaid with matrix and allowed to air dry and the plate is then inserted into the MS for analysis.^{1,2}

Intact cells (IC) can be directly processed by MALDI-TOF without pre-treatment because most vegetative bacteria are lysed following exposure to water, organic solvent and/or strong acids in the MALDI matrix.³



Sample preparation in MALDI-TOF MS

The IC method has shown to be sensitive enough to differentiate between closely related organisms however, it may not always be appropriate for all types of microorganisms. In addition, biosafety concerns have led different groups to add an inactivation step prior the addition of the matrix solution. Most often a strong organic acid (acetic, formic) or alcohols are added.^{2,4}

The inactivation step not only tackles the biosafety concerns but also improves spectral generation. Some viruses, bacterial spores, and yeast cells usually require pre-treatment steps to release biomarkers for MS analysis. These steps may involve the simple addition of strong organic acids and/or alcohols as mentioned before or a full scale protein extraction.^{2,4,5}

A large number of bacteria are required for identification as reducing the cell number analyzed by MS reduces the number of peaks generated.⁶ This limits the ability to rapidly identify microorganisms directly from biological fluids where the bacterial count is expected to be relatively low.¹¹ It is also important that a well isolated colony is used as the MS technique is not able to resolve mixtures.

After the sample is spotted on the test plate, the chemical matrix is mixed in excess with the sample. This matrix accomplishes two very important tasks. First, it provides a means for coupling the laser's energy with the sample triggering a sublimation of the matrix and the sample into a gas phase.² Second, the matrix provides a gentle mechanism for transfer of charge to the analyte (avoiding fragmentation) creating intact ions for analysis.¹

The generated ions are separated by the mass analyzer and the spectrum obtained is then compared with spectra contained in a database.

Sample preparation is crucial to optimize sensitivity, reproducibility, and mass accuracy as spectra of the same bacterial species have been shown to vary when different culture conditions, matrix composition, or sample treatment are used.⁷

Biomarkers

Because only singly charged ions are formed of intact proteins, the m/z ratio can be associated to the molecular weight of those proteins. The entire spectrum of protein biomarkers is needed for organism identification and this process is often referred to as mass profiling or fingerprinting.⁸

Good biomarker proteins for MALDI-TOF need to be conserved in spectra obtained from genetically identical bacteria; they must be highly abundant and ionize readily.

Several studies have shown that ribosomal proteins are unique to their respective bacterial groups or species.^{2,7} Because ribosomal proteins are part of the cellular translational machinery, they are present in all living cells and are not significantly influenced by variability in environmental or growth conditions.⁹ In light of these findings, the pre-treatment methods for cell lysis and the matrix used have been optimized for the extraction and ionization of ribosomal proteins.

MASS SPECTROMETRY AND CLINICAL MICROBIOLOGY

Time-of-Flight (TOF) analyzer

Ions generated from both the matrix and sample must be separated and analyzed to obtain the characteristic spectra.

In MALDI, a pulse of ions from the sample is produced by an instantaneous exposure to the laser beam and these ions enter the flight tube simultaneously. The ions then move into a field-free drift region, where the only force affecting their movement is the kinetic energy from the acceleration step which is directly related to the m/z ratio of the ion.

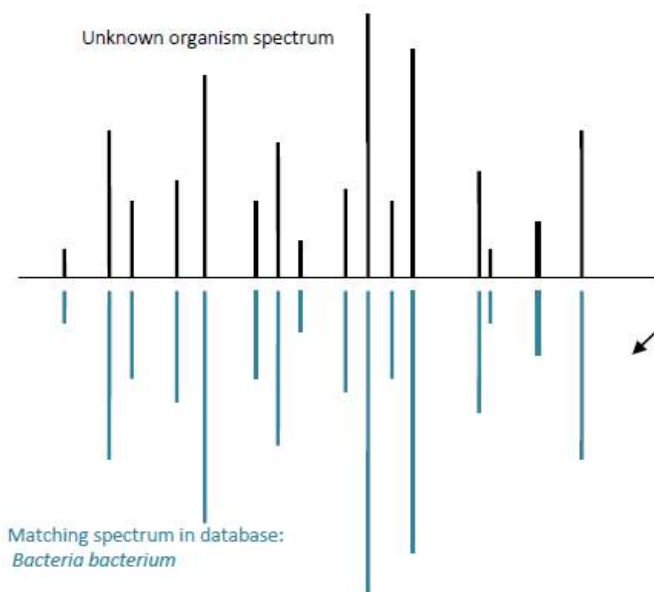
Larger ions will have a longer drift time and smaller molecules will have a shorter drift time allowing for their separation.²

Ion detection and creation of spectrum

The ions, then collide with the ion detector, which measures their charge and time to impact. The time of flight required to strike the detector is used to calculate the masses of the ions and to create a mass spectrum. The spectrum obtained is then compared against a database of reference spectra.¹⁰⁻¹²

The construction of reliable and quality databases that allow experimental data to be characterized based on matching profiles is the key part in the MALDI-TOF MS technology.^{11, 13}

Veronica Restelli, Editor



Database

Representation of a spectrum obtained from an unknown organism and comparison with a spectrum in the database.

Interesting

Use of MALDI-TOF MS is not the first application to analyze disrupted bacteria by Mass Spectrometry. Forty years ago bacteria were put on a iron wire and rapidly disrupted by a surge of electricity and heat (Pyrolysis-MS). MALDI-TOF MS requires less preparation and less material, and the computer support is much more modern.¹⁴

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SAFETY IN THE CLINICAL LABORATORY

Ultraviolet rays and laboratory safety

By Suhanya Bhuvanendran—CMPT's Safety Officer



Disinfection and decontamination is pivotal to practicing laboratory safety. While there are many products available to decontaminate, Ultraviolet rays can be one of the quickest and easiest methods used.

Ultraviolet (UV) rays' sterilizing effect on microorganisms was discovered in 1878 and its damaging effect on DNA was established by 1960. Although it is not quite clear when the use of UV rays in laboratories started, it can be said that it has been used for more than 100 years as a decontaminant method.

This method of decontamination uses a lamp source that radiates UV-C band of UV rays at wavelengths 250nm to 270nm and destroys most microorganisms on surfaces. A lot of biological safety cabinets and some laboratory rooms are equipped with UV light lamps to help decontaminate the interior surfaces. UV radiation is divided into UV-A, UV-B and UV-C radiations depending on their wavelength ranges.

Because it stimulates production of Vitamin D, some exposure to UV-B rays is considered normal and good for well-being. However, excessive exposure can result in severe health consequences.

The shortwave, high frequency UV-C band of light is used for decontamination even though it poses the maximum health risk to humans. Exposure to lower wavelengths (265nm – 275nm) of UV-C band can cause skin and eye damage. Overexposure to UV light can cause severe eye damage, chronic skin health effects including premature skin aging, wrinkles and skin cancer, and can also affect the immune system in healthy individuals. Eyes are most sensitive to UV-C rays and although short exposures can cause reversible damage, extended exposures can cause cataracts, conjunctival degeneration of the eye (pinguecula). Permanent damage leading to blindness can occur in people whose eye lenses have been removed.

Institutions and facilities should have guidelines to limit workers from being exposed to UV light. Below is a list of few precautionary steps that can be undertaken when working with UV light:

Elimination or Substitution

- Substitute alcohol or bleach where possible if UV light is only used for decontamination.

Engineering controls

- Limit access by closing the room's door or the biosafety cabinet sash completely when decontaminating with UV-light.
- Interlock UV lamps with general light so that the UV lamp turns off when the general light is switched on.
- Limit UV light to designated areas using plastic shielding and UV radiation absorbing glass.
- Paint reflective surfaces or place bench covers to reduce reflection of UV light.
- Proper maintenance and monitoring of the lamp's output should be part of preventative measures.

Administrative controls

- Place warning signs, labels, or visible light to warn workers of potential risk.
- Provide adequate training to all workers.
- Allow only authorized users to access rooms or equipment that use UV radiation. Authorization can be provided when the worker has been trained properly.

Personal Protection Equipment

- Lab coats and long pants cover most of the skin and reduce skin exposure.
- Proper goggles that cover the sides of the face and/or face shield can offer protection for eyes and skin on face.
- Gloves can protect the employee's hands.

First Aid

Laboratory workers should be aware that most UV-exposure related health effects do not show symptoms immediately. It may take up to 24 to 48 hours or sometimes years before symptoms arise. Sometimes, photokeratitis can occur in exposed workers hours after exposure.

When UV exposure is suspected:

For the eye – Place a sterile dressing over the eye and get medical help immediately.

For the skin – Wash with cold water, place an ice pack or a towel soaked with cold water to the skin burns, and get medical help immediately.

After an incident, an assessment of the accident should be done and measures taken to prevent such circumstances from recurring.

Ultraviolet rays produce no contaminants or waste and can be a very effective decontaminant when used appropriately. However, inadequate training, ineffective engineering or administrative controls, and ineffective personal protective equipment can all contribute to hazardous situations that can put the worker at risk.

Many institutions do not recommend or support using UV radiation in laboratories. But for those workers who may have to use UV light, a good understanding of its abilities and limitations can help them with making safe practices more effective.

For more information:

http://www.ccohs.ca/oshanswers/phys_agents/ultravioletradiation.html

www.labour.gov.on.ca/english/hs/pdf/uv_radiation_workplace.pdf

Upcoming Events

APRIL 2015

European Congress of Clinical Microbiology and Infectious Diseases

April 25—28, Copenhagen, Denmark

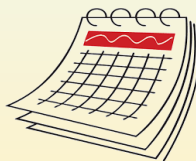
More info: http://2014.eccmid.org/eccmid_2015/#c11979

JUNE 2015

2nd Annual Microbiology and Infectious Diseases Asia Congress

June 2015, Singapore

More info: <http://www.microbiologyasia-congress.com/>



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Become a contributor

We would like you to write on any topic within the spectrum of Quality or Microbiology.

Is your laboratory working on a new project?

Send us your article, approximately 500 words, preferably with a picture.

We want to hear from you

Please follow the link to submit questions, suggestions, information about events, etc.

www.cmpt.ca/newsletter_bulletin/news_submissions.htm

ABOUT CONNECTIONS

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