Summer 2014

Connections

ISSN 1496-3876

www.cmpt.ca

CMPT QUARTERLY ON-LINE NEWSLETTER

Volume 18 Number 2

IN THIS ISSUE

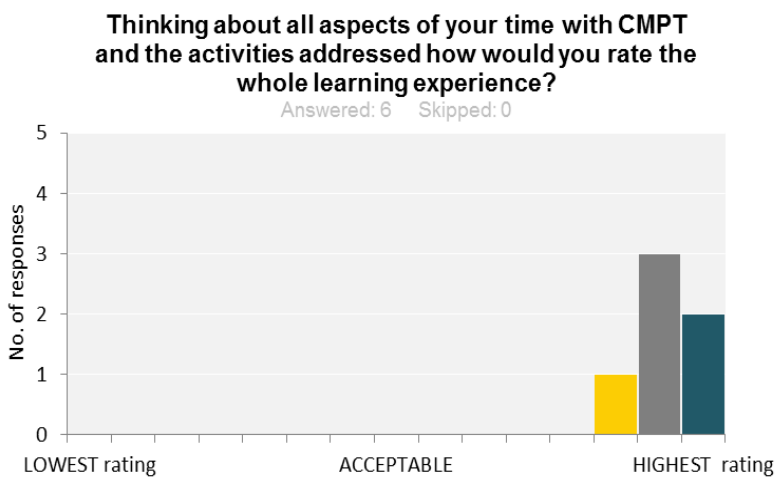
Customer satisfaction survey - CMPT's International EQA Program

Many years ago, CMPT decided that if we were going to find our own niche in Proficiency Testing / External Quality Assessment, it was going to be the production of challenge samples that closely mimic actual human samples received and tested in clinical microbiology laboratories.

In order for that to occur, we had to create samples that were stable over a certain period of time without resorting to lyophilisation. It was important that we could control, not only the specific bacteria in samples, but also their concentrations, both alone and in mixtures. Moreover, some samples needed to contain relatively low concentrations of "normal flora" with or without recognized pathogens.

Sample simulation came with a certain cost. Although stable for local transport, some samples would not remain stable when shipped over very long distances and prolonged transport times. As a result, even when asked, it would not be possible for us to send our typical challenges samples to laboratories in Africa or China or the Middle East.

Figure 1. Overall experience



What might seem like a limitation at first has turned to be an opportunity for both CMPT and other countries. It opened the doors to invite laboratorians from other countries to come to Canada and learn how to make challenge samples so that they could develop proficiency testing programs similar to ours in their own region. That was the start of our International Proficiency Testing Training Program.

Over the years, we have trained visitors representing national proficiency testing organizations from Europe, the Middle East, Africa, and Asia; nearly 30 in total. Readers of our newsletter 'Connections' have read comments from many of them.

We recently developed an electronic survey to capture the collective thoughts of the most recent participants on their experiences at CMPT during training. Unfortunately, this has not been as easy. Many have changed positions and moved on to other organizations or even other countries, making the follow-up and contact very difficult.

Although we did not get as many responses as we would have liked, we received opinions from about 40 per cent of those delegates training at CMPT within the last four years. Since the responses were all anonymous and independent, we felt there was statistical value in looking at their comments and survey results.

When asked about the on-site activities, all participants rated highly the number and variety of topics included, the discussions on management, and the support they received from CMPT staff. The highest ratings went to having questions answered, being kept fully active, and getting lots of hands-on training.

Regarding the value of their CMPT experience, all, but one, reported they had been successful in setting up some form of PT program in their own country. While all participants were confronted with different barriers, none were directly related to their CMPT training.

CMPT's International EQA Training Program

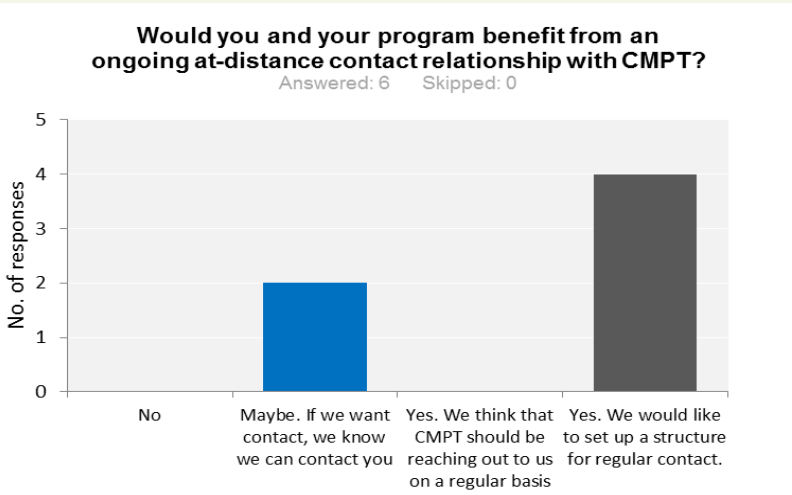
All participants found their overall time spent training at CMPT a valuable experience (average rating greater than 98 percent) and would recommend the program to others (also rated greater than 98 percent) (Figure 1). Likewise, they all saw some value in continuing an ongoing relationship with CMPT and even including some form of active collaboration (Figure 2).

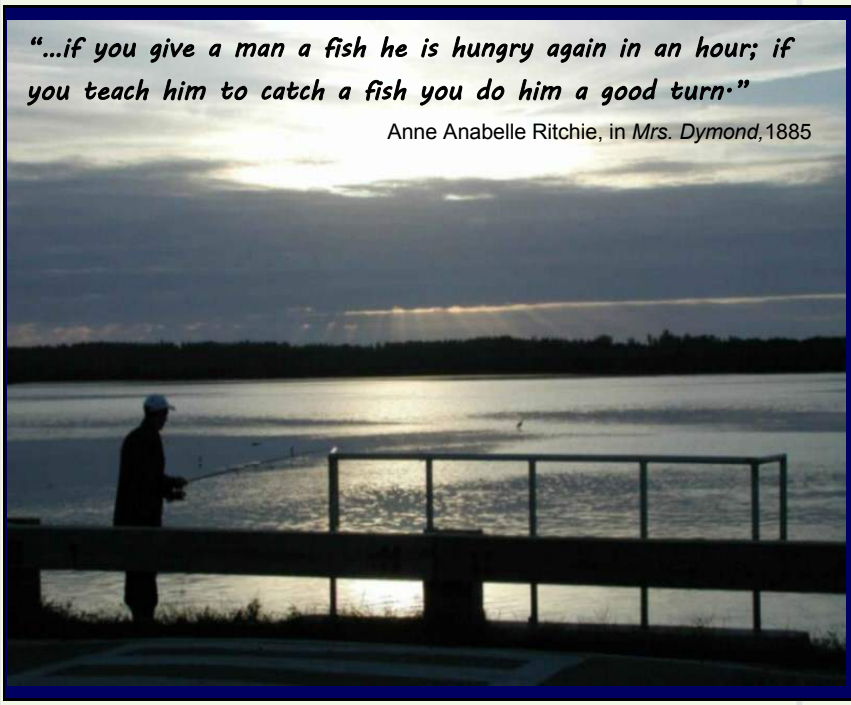
In line with this ongoing relationship, we look forward to the return visit in August 2014 of one of our previous participants from Oman for supplemental training in creating slides for analysis of acid fact bacteria.

We have learned some valuable lessons through this exercise; first and foremost, we create a disservice to ourselves and to others when we wait too long before making follow-up contact. Still, from those that were available, it appears that we have created a useful program that indeed contributes to laboratory improvement around the world.

It is our intent and goal to continue with our International Proficiency Testing Training Program as long as countries are interested.

Figure 2. Post training contact with CMPT





"For many [laboratories], the solution is to purchase PT samples from a larger country, which is always more than happy to provide them. [However,] the samples were never designed to address the issues of developing countries, and ... to get the samples to the laboratories at considerable distance they [may] have to compromise their quality by freeze-drying the samples ...; the informative sheets ... are written from the perspective of the large parent country and without any perspective for the developing region."

Extracted from Dr. M. Noble's blog: Making Medical Lab Quality Relevant , April 2014

For information on CMPT's International EQA Training Program, please visit CMPT's website:

http://www.cmpt.ca/education/education_international.html

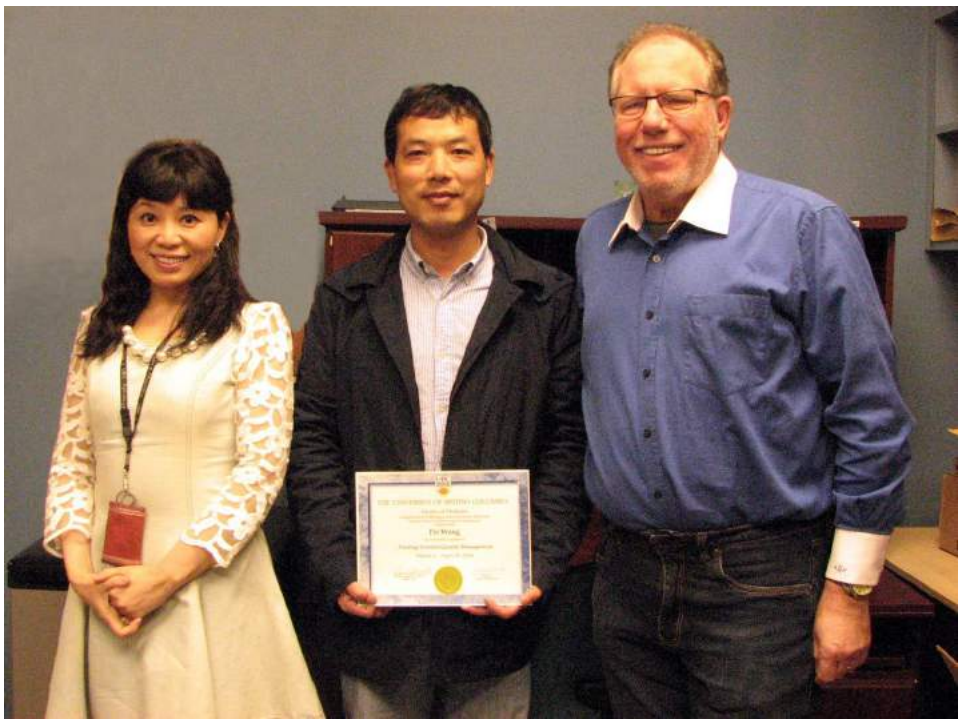
International visiting Scientist

We recently received the visit of Dr. Pei Wang, Director of the Department of Laboratory Medicine of The First People's Hospital of Jingmen, P. R. China.

Dr. Wang's main purpose for the visit was to gain experience in ISO certification and Risk Management Assessment as many hospitals in China are now applying for ISO 15189 "*Medical laboratories — Particular requirements for quality and competence"* certification.

"Everybody can read and understand the documents", says Dr. Wang, "but when you are faced with real situations, how to apply the requirements into practice, most [people] are confused."

For Dr. Wang, Dr. Michael Noble's involvement in ISO accreditation and experience with Quality Control is the perfect combination to be able to discuss the documents together and learn from experiences how to deal with different situations encountered within the laboratory.



From left to right: Maggie Ma, POLQM coordinator, Dr. Pei Wang, Dr, Michael Noble.

As part of his visit, Dr. Wang had the opportunity to attend the AMMI, CACMID Quality Seminar in Victoria, BC , join Risk Management teams at UBC for laboratory audits, and discuss Quality Assurance with the Vancouver General Hospital Clinical Microbiology Laboratory staff.

The task now, explains Dr. Wang, is to improve the Quality system in his hospital's laboratory. First thing to modify is the Proficiency Testing process; right now, testing is not consistent with the processing of regular clinical samples and failures are frequently followed by punitive actions from the accreditation bodies. Second issue to attend is continuing education; many staff members now don't have much sense of the importance of quality assurance and quality improvement. Third point is to implement an incident reporting system to record the problems in the laboratory process and detect the areas of weaknesses than need attention.

Overall, the goal is to create a culture that understands the importance of Quality Assurance and Quality improvement as a main requisite for patient safety.

> Everybody can read and understand the documents but when you are faced with real situations, how to apply the requirements into practice, most [people] are confused.

Photo credit: Dr. Pei Wang

Mass Spectrometry in Clinical Microbiology - Part I: Introduction to Mass Spectrometry

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.

Mass Spectrometry (MS) is an analytical chemistry technique that detects the mass-to-charge (*m/z*) ratio of a bioanalyte. Although the principles of the MS technique have been established for over a century, thanks to the contributions of Eugene Goldstein, Wilhelm Wien, and J.J. Thomson, the first modern techniques of MS were developed in the early 1900s.

In a MS procedure, a sample is ionized; the ions are separated according to their mass-to-charge ratio and then detected by a detector of charged particles which results in a spectrum of relative abundance versus mass-to-charge ratio.

There are three main components of a mass spectrometer:

Ionization source

Mass analyzer

Detector

Ionization source

Ionization is the process that converts analytes of interest into gas phase ions. There are different ionization techniques and the ionization energy and the physical state of the analyte are key to determining what types of samples can be analyzed by MS.

The ionization energy controls the amount of fragmentation an analyte undergoes after ionization. Some ionization techniques are very energetic (**hard**) and cause ions to undergo extensive fragmentation producing unpredictable spectra in the case of biomolecules. Other techniques impart little residual energy (**soft)** onto the molecule and thus, only produce molecular ions.

The ionization of the sample molecules can be generally achieved by exciting the neutral analyte molecule which then ejects an electron to form a radical cation (M+•) or by ion molecule reactions that produce adduct ions (MH+).

For many years, only volatile analytes were able to be analyzed by MS as the sample had to be first volatilized and then ionized. This approach limited the analytes to relatively low-molecular weight compounds that are thermally stable.

As most biomolecules have relatively high MW and high polarity severely limiting their volatility, the study of these molecules by MS was not possible without breaking them down to monomers and derivatization processes.

Two new ionization techniques have emerged for the analysis of non-volatile and thermally labile compound: Electrospray Ionization (ESI) and Matrix Assisted Laser Desorption Ionization (MALDI).

Electron impact ionization (EI)

In an electron impact mass spectrometer, a high energy beam of electrons is used to form a radical cation known as the molecular ion (M+•). The high energy imparted to the molecular ion makes it too unstable therefore it fragments to produce other smaller ions (Figure 1).

1) M: + e^- —> M+• + $2e^-$

2) M+• —> m1+• + R1

3) M+• —> m2+• + R2

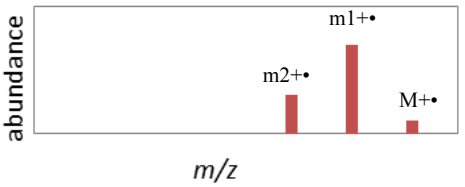


Figure 1. Schematic representation of EI spectrum of molecule M. *Abundance of the different ions will depend on how favoured one fragmentation is over the other. Fragmentation reaction 2) in this case is the most favoured one thus, more m1+• will be detected.*

EI ionization is an example of a hard ionization technique and it is suitable for gas phase ionization only. Since it produces high degree of fragmentation, it is valuable to study the molecular structure of the analyte.

Chemical ionization (CI)

In the CI spectrometer a reagent gas (GH) is ionized with an electron beam to produce a cloud of ions. The reagent gas ions in this cloud react with each other and produce adduct ions which are excellent proton donors.

1) GH + e^- —> GH+• + $2e^-$

2) GH+• + GH —> G• + GH_2^+

When analyte molecules (M) are introduced to this cloud of ions, the reagent gas ions donate a proton to the analyte molecule and produce MH+ ions.

3) GH_2^+ + M —> GH + MH^+

Because the adduct ions have little excess energy and are relatively stable, CI is very useful for molecular mass determination as mostly the molecular ion is produced (Figure 2)

CI ionization is an example of soft ionization technique and it is suitable for gas phase ionization only.

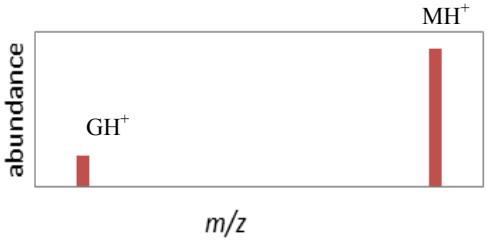


Figure 2. Schematic representation of CI spectrum of molecule M.

Matrix Assisted Laser Desorption Ionization (MALDI)

In MALDI, the sample is mixed with a low-mass organic compound, called matrix, and both co-crystallize to form a solid deposit.

This deposit is irradiated with a UV laser beam. The energy from the beam is absorbed by the matrix molecules triggering a sublimation of the matrix into a gas phase, forming a cloud which is directly followed by the ionization of the sample.

The exact mechanism of ionization is not well understood, but can be explained by a two-step mechanism:

1) Excited-state matrix molecules produce one matrix radical cations. The absorbed photon (hv) from the excited-state matrix molecule (X*) is transferred to the second excited matrix molecule, resulting in the formation of a cationic matrix radical (X+·).

$X\ X + 2hv \rightarrow X^*X^* \rightarrow X + X+\bullet + e^-$

2) The second ionization involves a proton transfer from the excited matrix molecule to the clinical sample (M), resulting in ionization of the sample molecule.

$X+ + M \rightarrow (X - H) + MH^+$

Additional ions of the clinical specimen are formed by secondary ion-molecule reactions between matrix-matrix and matrix-specimen interactions.

$X+\bullet + X \rightarrow XH^+ + (X - H)\bullet$ and $XH^+ + M \rightarrow X + MH^+$

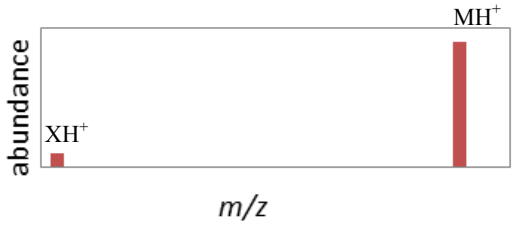


Figure 3. Schematic representation of MALDI spectrum of molecule M

Matrix Assisted Laser Desorption/Ionization (MALDI) is a soft ionization technique and produces one molecular ion and thus is suitable for analyzing large biomolecules.

Electrospray ionization (ESI)

In electrospray ionization (ESI) analysis, the sample is dissolved in a solvent (S) and then guided through a capillary with a high voltage applied; this results in the emission of an aerosol of charged droplets. As the droplets evaporate, charges are transferred to molecules present within the droplet.

Droplet solvent evaporation Ion formation

$(n)SH+ + (n)M \longrightarrow MH+ , MH_2++, MH_3+++, MH_4+++$

Protonation/de-protonation is the main mechanism of producing biologically relevant ions in ESI. Because electrospray produces multiply charged ions, high molecular weight compounds are observed at lower m/z value. Multiple charging enables mass spectrometers with limited m/z ranges to analyse higher molecular weight molecules. This increases the mass range of the analyzer so that higher molecular weight compounds may be analyzed.

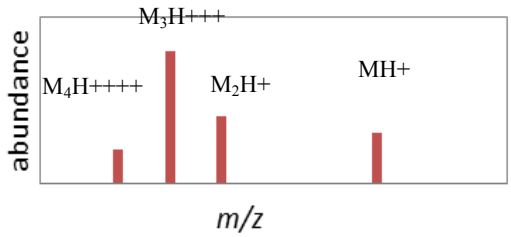


Figure 4. Schematic representation of ESI spectrum of molecule M

Mass Analyzers

Mass analyzers separate the ions according to their mass-to-charge ratio and they can use different mechanisms. Time-of-flight (TOF) analyzers separate ions in space according to their m/z and detect all or most of the ions formed. Quadrupole analyzers can detect one particular ion at a time by filtering out those that are of no interest.

Time-of-Flight Analyzers

In a TOF analyzer, the ionized sample is exposed to an electrostatic field causing the ions to accelerate. The ions then move into a field-free drift region where their traveling speed depends only on their m/z ratio. Ions with low m/z ratio will travel faster than those with high m/z (Figure 5). This allows for the separation of ions in the sample.

Quadrupole Analyzers

In a quadrupole analyzer, 4 parallel, positively and negatively charged rods alternate their charge at a set frequency causing attraction and repulsion of the sample ions. By selecting the fre

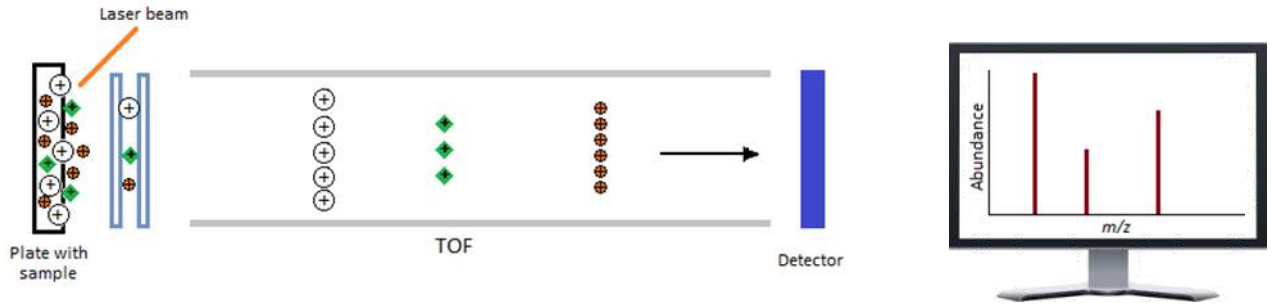


Figure 5. Schematic representation of a MALDI-TOF mass spectrometer. The analytes in the sample are ionized and accelerated; the ions will reach the detector at different times, depending on their m/z. On the right, a representation of the spectrum obtained.

quency at which the charges alternate, a desired analyte maintains a stable flight path to the detector (Figure 6). Sequential detection of different analytes can be done by changing the frequency at which charges alternate.

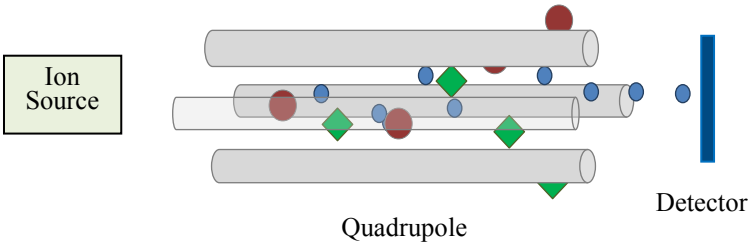


Figure 6. Schematic representation of a quadrupole mass analyzer with a charge change frequency optimized to detect the blue particles.

Detector

The detector records either the charge induced or the current produced when an ion passes by or hits a surface. Typically, some type of electron multiplier is used.

The information gathered by the detector is used to create a mass spectrum, which displays not only the m/z values but also the abundance of ions.

Suggested readings

1. Van Bramer SE. 1998. An Introduction to Mass Spectrometry Widener University, Department of Chemistry. Available at: http://science.widener.edu/svb/massspec/massspec.pdf
2. Strathmann FG, Hoofnagle AN. Current and future applications of mass spectrometry to the clinical laboratory. *Am J Clin Pathol*. 2011;136:609-616.
3. Glish GL, Vachet RW. The basics of mass spectrometry in the twenty-first century. *Nat Rev Drug Discov*. 2003;2:140-150.
4. Fox A. Mass Spectrometry for Species or Strain Identification after Culture or without Culture: Past, Present, and Future. *J Clin Microbiol*. 2006;44:2677-2680.
5. Demirev PA, Fenselau C. Mass spectrometry for rapid characterization of microorganisms. *Annu Rev Anal Chem (Palo Alto Calif)*. 2008;1:71-93.
6. Sauer S, Kliem M. Mass spectrometry tools for the classification and identification of bacteria. *Nat Rev Microbiol*. 2010;8:74-82.

Safety in the Clinical Laboratory

A simple guide to assessing risks in your workplace

By Suhanya Bhuvanendran—CMPT's Safety Officer

Risk assessment is an important step towards preventing injuries or accidents in the workplace because it creates awareness of hazards and risks associated with work and space.

A thorough assessment allows for determining if the existing control and hazard-preventative measures are adequately protecting the workers and their work.

The proper risk and hazard assessments are the responsibility of the person performing the lab work, but the responsibility is shared by the laboratory supervisor(s) and/or lead hand(s). It is recommended that the actual risk assessment should be done by a designated safety officer or trained personnel, who are familiar with the workspace, work flow and machinery. Some institutions have risk management services or health and safety offices that have experts who can guide laboratories through their risk assessments. In order to be effective, all groups involved in upholding a safe work environment would have to work cooperatively to ensure that all hazards are identified and proper risk management procedures are implemented prior to beginning work.

How do you do a risk assessment?

Identify space, equipment, or procedure to be assessed.

- **Identify hazards** by asking laboratory workers about the risks/hazards that they notice. Instruction manuals may have lists of hazards that can aid in risk assessment of equipment. Accident, incident and near-miss reports can also help identify hazards.
- **Determine who will be affected and how.** Many laboratories have a slew of people working days and nights. New and inexperienced workers, experienced workers, pregnant workers, housekeeping, workers with disabilities; these maybe just the first tier of people affected by a certain hazard. Sometimes, maintenance workers, visitors, people who use or work in other parts of the building may also be harmed.

  Example: A mercury thermometer broke and mercury has spilled onto the floor. A new and inexperienced worker could panic and run for help before cordoning off the area. Mercury vapors can cause tremors, impairment of cognitive skills and sleep disturbances in healthy individuals and neurodevelopmental problems in unborn fetuses in pregnant workers. Housekeeping staff, unaware of the spill, may vacuum or mop the area and increase exposure to themselves and others. Mercury aerosol, created by the vacuum cleaner or mop, can be carried by air current or attach itself to clothing and be carried home by housekeeping staff, maintenance workers, visitors and/or other laboratory staff.

- **Assess the severity and the frequency** of hazards under normal operational conditions, as well as, under unusual conditions (i.e. flood, earthquake, power outage)
  - A risk management matrix (Figure 1) can aid in evaluating the severity of risk if the likelihood of a risk varies under different parameters

Accidents, Incidents, and Near-Misses

Different facilities have slightly varying definitions of what constitutes Accidents, Incidents or Near-misses. Employees must adhere to their own workplace definitions and policies to avoid discrepancies in risk management by their respective institutions. The definitions below are provided to give a general understanding of these terms.

An **Incident** represents an unexpected event that has occurred that has the potential to harm person(s) or property.

Example: Broken mercury thermometer causing mercury spill inside a closed thermometer holder.

An **Accident** represents an unexpected event that resulted in injury to person(s) or damage to property.

Example: Broken mercury thermometer causing mercury spill on an employee.

A **Near-miss** event represents an unexpected event that given a slightly different set of circumstance (such as time, distance or physical health, etc.) could have caused injury to person(s) or damage to property.

Example: A mercury thermometer falls to the ground while being handled, but doesn't break as it fell on a rubber carpet in the area. This could have been an incident/accident if circumstances were different such as falling outside the carpet area (thermometer would have probably been broken).

- Determine new or existing **control measures** and how effective they are in controlling identified risks. If it is not possible to eliminate the risk completely, then examine newer, alternative ways to reduce risks.
  - Personal protective equipment (PPE) may be effective in preventing percutaneous exposures, but not chemical burns
  - Administrative controls: For example, only workers trained to operate the autoclave are allowed to operate the equipment
  - Engineering controls: For example, using incinerators instead of open flame to sterilize loops alleviate fire hazards
  - Elimination or substitution: Substitute glassware with plastic ware where possible
- Decide which control measure to use and **implement** for each hazard
- Decide on a **Plan of action** for when a risk cannot be completely eliminated. The plan of action should include logs to record regular checks of the control measures, list of persons responsible for controlling or monitoring risks, and procedures to remind workers of control measures in place. The plan of action should be concise and clear so it is easy to follow by all workers.

- **Review** with an expert where necessary
- **Re-evaluate risks** after control measures have been implemented to measure residual risk
- **Store** all documents relating to risk assessment including the process, the evaluations and the conclusions until the equipment or procedure is completely eliminated or if the work space is changed to a new location

When is an assessment done?

- At the start of a new project
- Before a change in the work flow or procedure is implemented
- When a new equipment, machinery, protocol or chemical is introduced
- When there is change in safety guidelines (MSDS, manufacturers' instructions)
- Moving into a new space

Risk assessment can be a daunting and a time-consuming process, however, a proper risk assessment can highlight other issues that may not have been obvious prior to the assessment.

Along with protecting workers, this process helps understand the different sources of weaknesses in the system. Therefore, this process can be the first step towards a better quality control as it can help in eliminating errors as well as make the working space safer.

Consequence	Improbable	Remote	Occasional	Probable	Frequent
Catastrophic	**Medium**	**High**	**High**	**Extreme**	**Extreme**
Significant	**Medium**	**Medium**	**High**	**High**	**Extreme**
Moderate	**Low**	**Medium**	**Medium**	**High**	**Extreme**
Low	**Low**	**Medium**	**Medium**	**Medium**	**High**
Negligible	**Low**	**Low**	**Medium**	**Medium**	**High**

Likelihood

Figure 1. Risk Management Matrix

How to use a risk management matrix (example)

Situation: A supplier provides a free glass mercury thermometer with the purchase of a water bath.

MSDS classifies elemental mercury (present in most mercury thermometers) as Class D1 (Toxic). Mercury exposure can lead to potential health effects relating to blood, kidneys, central nervous system, liver and brain.

***Risk:** Potential for accidental breakage is high if the thermometer is handled frequently. i.e. If the thermometer is removed from the water bath on a daily basis to monitor and record temperatures, then the likelihood of risk of accidentally breaking the thermometer is Probable. The consequence of such a break is Moderate to Catastrophic. This puts the severity of the risk at High to Extreme levels.*

Substituting the mercury thermometer with an alcohol thermometer reduces the consequence (resulting from a broken alcohol thermometer) to Negligible to Low levels. This reduces the severity of the risk to Medium.

Get Connected

Upcoming Events

September 2014

54th ICAAC

September 6 - 9, 2014 Washington, DC
More info: http://www.icaac.org/index.php/meeting/icaac-2014

International Conference on Infections and their Prevention,

19-20 Sept 2014, Beijing, People's Republic of China
More info: Link

October 2014

III International Conference on Antimicrobial Research

October 1-3 Madrid, Spain
More info: http://www.icar-2014.org

Point-of-Care Testing Workshop: Today and Tomorrow, IQMH

October 14 Toronto, Ontario
More info: Link

IMED 2014

October 31—November 3, 2014 Vienna, Austria
More info: http://imed.isid.org/

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/

ABOUT CONNECTIONS

"Connections" is published quarterly by CMPT and is aimed at the Microbiology staff.

Editor: Veronica Restelli

Contact Connections

By mail
Room G408, 2211 Wesbrook Mall,
Vancouver, BC V6T 2B5
Canada

By phone: 604– 827-1754
By fax: 604-827-1338
By email: restelli@mail.ubc.ca

Connections is available online:
www.cmpt.ca/newsletter_connections.html

We want to hear from you. Please follow the link to submit questions, suggestions, articles, information about events, etc.
www.cmpt.ca/newsletter_bulletin/news_submissions.htm