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Connections

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Changes in CMPT's Clinical Bacteriology Advisory Committee

CHANGING OF THE GUARD

Dr. Robert Rennie will be retiring as Chair of the Clinical Bacteriology Committee, a position he held for the last 10 years.

Throughout this time, Dr. Rennie has managed to keep the Committee on topic and focused, a service that he has undertaken with enthusiasm, effort, and energy. CMPT is immensely grateful to him for his dedication. THANK YOU!

Dr. Rennie joined the Clinical Bacteriology Advisory Committee in 1992 and has been an invaluable help to CMPT. Also a member of the Mycology Advisory Committee, Dr. Rennie has been collaborating with CMPT in numerous ways. He has written numerous articles for our newsletter 'Connections', has answered letters from the participants, has written and reviewed challenge critiques, and has given advice to CMPT in a variety of topics.

More importantly, Dr. Rennie has done and continues to do all this with warmth, dedication, patience, and a great sense of humour.

Thank you!

CMPT is pleased that Dr. Rennie will remain a member of the Clinical Bacteriology and Mycology Committee.

WELCOME TO OUR NEW CHAIRMAN: DR. DAVID HALDANE

CMPT is pleased to announce the appointment of Dr. David Haldane as the new Chair of the Clinical Bacteriology Committee.

Dr. David Haldane is a Medical Microbiologist and Director of Bacteriology and Special Pathogens at the Queen Elizabeth II Health Sciences Centre, Halifax, NS.

Dr. Haldane has been a member of the CMPT's Clinical Bacteriology Committee since 2003.

I would like to invite the participants to join CMPT staff in welcoming Dr. Haldane as the new Committee Chair and support him as he takes on this responsibility.

WINDS OF CHANGE

It's been a time of change at CMPT. After many years of incredible service to CMPT, Dr. Deirdre Church, Ms. Beverley Borgford, and Dr. Michelle Alfa have stepped down from their roles as members of the Clinical Bacteriology Advisory Committee.

Dr. Church joined CMPT in 1992. Besides her vital work as an advisor for the Clinical Bacteriology program, she has been an invaluable contributor to our newsletter, writing numerous articles and comments on challenges. Thank you Dr. Church!

A big thanks also to Ms. Beverly Borgford who, since 2003, has been sharing her technical expertise with CMPT and has dedicated her time to review, grade, and improve CMPT's challenges and critiques. Thank you Ms. Borgford!

Lastly, many thanks to Dr. Michelle Alfa for her incredible energy, continuous input, and suggestions during the Committee meetings. Dr. Alfa has collaborated greatly to the work of CMPT by suggesting new programs, offering her expertise on many issues, and regularly contributing to our newsletter. Thank you Dr. Alfa!

CMPT's Clinical Bacteriology Committee

Welcome new members of the Clinical Bacteriology Advisory Committee

Dr. Titus Wong

Dr. Titus Wong completed his degree in Medicine and residency in Medical Microbiology at the University of British Columbia and currently works in the division of Medical Microbiology and Infection Control at Vancouver General Hospital. He is the assistant program director for the UBC Medical Microbiology residency training program and co-director of Pathology 722, a UBC course for residents and fellows. Dr. Wong is completing a master's degree in clinical epidemiology and is focusing on research to prevent hospital acquired infections.

Dr. Wilson Chan

Dr. Wilson Chan graduated medical school from the University of Alberta where he completed his residency in Medical Microbiology. Dr. Chan obtained his Master's of Science and Diploma in Tropical Medicine & Hygiene at the London School of Hygiene & Tropical Medicine, London, UK and has been working at Calgary Laboratory Services as a Medical Microbiologist since 2011.

Dr. James Karlowsky

Dr. James Karlowsky is a Professor for the Department of Medical Microbiology and Infectious Disease and the Director of the Clinical Microbiology Fellowship Training Program of the Faculty of Medicine, University of Manitoba. Dr. Karlowsky also works as a Clinical Microbiologist at the Diagnostic Services of Manitoba, St. Boniface Hospital, Winnipeg, MB.

CMPT is delighted to add Dr. Chan, Dr. Wong, and Dr. Karlowsky to the Clinical Bacteriology Committee.

Quality Management Conference

Organized by the Program Office for Laboratory Quality Management (POLQM) Department of Pathology and Laboratory Medicine, University of British Columbia

Marriot Renaissance Hotel
Vancouver, British Columbia
October 16—18, 2013

Conference's website

Methods for Microbiological Monitoring of Water Quality

Most of the diseases associated with water are infectious in nature, and more than half of these are intestinal infections.

Hence, the microbiological examination of drinking water emphasizes assessment of the microbiologic quality of the supply. This requires the isolation and enumeration of organisms that indicate the presence of fecal contamination.

Microbiologic analysis may also be used to assess the efficiency of drinking water treatment plants, an important element of quality control.

Monitoring the presence of faecal indicator organisms within the distribution system allows the quick detection and correction of any problems arising to ensure that water reaching consumers is clean and safe to drink.

The number of samples to collect and test should reflect the size of the population being served by the distribution system and be representative of the water source, treatment plant, storage facilities, distribution network, and points at which water is delivered to the consumer.

According to Health Canada, daily tests for residual disinfectant and turbidity should be performed and *E. coli* presence should be tested at least weakly to confirm microbiological safety.

In order to obtain reliable estimates of the number of organisms present, a minimum volume of 100 mL should be examined and samples should be processed as soon as possible.

Figure 1. Simple filtration system using a vacuum pump.

Figure 2. A filtration membrane is placed in a filtration cup.

Although *E. coli* is the only reliable indicator of faecal contamination, some laboratories have procedures to test total coliforms based on the following rationale: 1) *E. coli* and faecal coliforms will give a positive total coliform count; 2) total coliforms are easily and cheaply assayed in waters. If a total coliform test comes back positive, then the examination for *E. coli* is carried out.

Water samples can be analyzed by a range of techniques. The most common techniques used include the most probable number (MPN), presence/absence (PA), and membrane filtration (MF).

Most Probable Number (MPN)

The most probable number depends on the separate analysis of a number of volumes of the same sample or different dilutions (usually 10-fold dilutions) of the water to be tested.

Each volume/dilution is mixed with culture medium and incubated. These samples are then incubated at 35°C, and examined for growth, gas and acid production after 24 and 48 hours.

Bacterial density and the 95% confidence limits can be estimated with the use of MPN tables for the volumes/dilutions and numbers of aliquots used and reported as "most probable number" per 100 mL of the original sample.

The MPN technique is highly recommended for high-turbidity waters, but it has longer turnaround times for results and high densities of non-coliform bacteria may have an adverse influence in *E. coli* detection.

Presence Absence (PA)

The PA test is a qualitative procedure. The procedure consists of inoculating the water sample into a bottle containing the appropriate concentration of PA medium and a fermentation tube for gas entrapment. The bottle is incubated for 24 to 48 hours at 35°C and inspected for acid and gas production. If gas is noted in the fermentation tube or an acid reaction (color change of the indicator dye) is observed, a small inoculum of the culture is transferred to a tube of brilliant green lactose broth for confirmation. The production of gas in the confirmatory medium is related to coliform occurrence.

Because this approach fails to provide data on the magnitude of contamination, presence/absence tests may be most appropriate for monitoring good-quality drinking water where positive results are known to be rare.

Membrane Filtration (MF)

In the MF method, the water sample is passed through a pore size of 0.45 um membrane filter that retains bacteria. The membrane is then placed on an appropriate medium and incubated. Discrete colonies with typical appearance are counted after 24 to 48 hours.

The advantages of this technique are its simplicity and the possibility of analyzing larger volumes of water. The greatest limitation of the MF test is that it is useful only for low-turbidity waters.

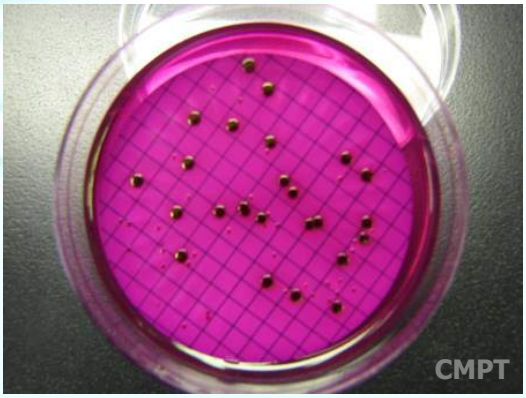

Figure 3. Endo agar plate with two types of colonies: small, pink colonies and larger, with green-metallic sheen colonies (coliforms).

Detection of *E. coli* using MF method

CMPT uses the MF method for the quality control of the samples of its Water Microbiology PT program. The preparation begins with 200 mls of water, which is aliquoted to two 100 mls of water. Each of the two 100 mL aliquots of water are passed through filtration membranes (0.45 μm pore size) using a simple vacuum system (Figure 1).

The membranes, with the trapped bacteria, are then placed on selective media. One membrane is placed on mEndo agar and incubated at 35°C for 24 hours (total coliforms). The second membrane is placed on mFC agar and incubated at 44.5°C (thermotolerant or faecal coliforms). See "Selective Media" for a description and composition of the media.

At 24 hours, colonies growing on the mEndo agar (Figure 3) and on the mFC agar (Figure 4) are counted and the amount of total coliforms and faecal coliforms per 100 mL of water is calculated.

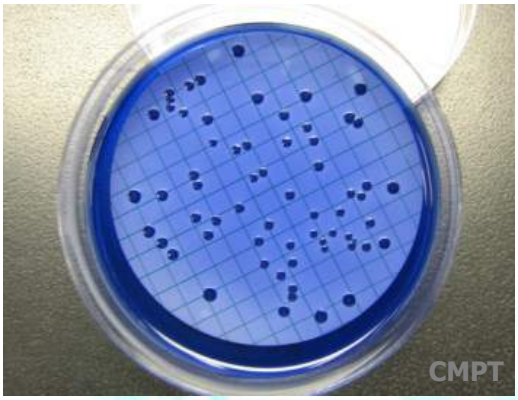

Figure 4. mFC agar plate with blue colonies (incubated at 44.5°C) indicating fecal coliforms present.

Confirmation of *E. coli* colonies is done by placing the membrane on the mEndo agar on a NA-MUG agar plate and incubating for 4 hours at 35°C. The plate is then observed under a long wavelength UV light and the fluorescent colonies (*E. coli*) are counted (Figure 5).

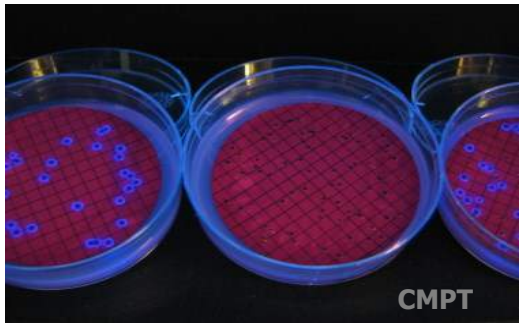

Figure 5. NA MUG plates under UV light. Fluorescent colonies (left and right plates) demonstrating *E. coli* colonies and non-fluorescent colonies (centre) demonstrating non *E. coli* coliforms.

The main purpose of the microbiological monitoring or drinking water is to protect consumers from illness due to consumption of water that may contain pathogens.

Although in the past, microbiological controls have relied on the analysis of faecal indicators in the finished drinking water, the World Health Organization (WHO) recommends a more preventative approach where water safety is ensured by monitoring critical points from the water source to the consumer's tap.

This approach not only provides a better understanding of the risks of contamination by pathogens at each step along the system, but helps detect water quality deterioration before it reaches the consumer.

Selective Media

mEndo agar: the agar contains sodium lauryl sulfate and sodium desoxycholate as selective agents. These components inhibit gram positive cocci and endospore-forming bacteria. Basic fuchsin and sodium sulfite serve as differential agents. Organisms that are able to ferment lactose to acetaldehyde form a green metallic sheen on colonies and are counted as total coliforms.

mFC agar: the agar contains rosolic acid that inhibits bacterial growth in general, except for fecal coliforms and bile salts that inhibit non-enteric bacteria. Aniline blue is used as an indicator. Faecal coliforms ferment lactose to acid, which causes a pH change in the medium (blue colonies). Thermotolerant coliforms are further selected by incubating the plates at 44.5°C.

NA MUG agar: the agar contains a fluorogen, 4-methylumbelliferyl-β-D-glucuronide (MUG). The fluorescence is caused by the breakdown of MUG which is cleaved by an enzyme, β-glucoronidase, specific to *E. coli*. Fluorescent halo colonies are observed with a long-wave (366 nm) ultraviolet lamp.

Photo credits: Suhanya Bhuvanendran

Veronica Restelli - Editor, CMPT

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Chicago Sky Blue Stain for Mycology KOH slides

Direct microscopic examination of KOH-prepared material for fungal elements is important as it provides rapid diagnostic information, whereas culture results may take days or weeks.

Although KOH direct microscopic examination is simple and inexpensive, the lack of contrast presented by this method requires an experienced observer. Moreover, the KOH test has been reported to have 5–15% of false-negative results.

Calcofluor white (CW) stain offers a more sensitive and specific alternative, but it requires a fluorescent microscope, which is not available in many mycology labs.

Chicago sky blue (CSB) stain is a new contrast stain that shows promise as a rapid diagnostic method for dermatomycoses. It contains 1% CSB 6B and is used together with KOH as the clearing agent. Fungal filaments stain a distinct blue against a pale or purple background. Unlike the CW stain, it requires a light microscope for reading.

As part of the Mycology Proficiency Testing program, CMPT provides laboratories with slides for direct examination of fungal elements. The slides are prepared by adding epithelial cells alone (negative samples) or with dermatophytes (positive samples).

Quality control of the fungal slides at CMPT requires the examination of KOH preparations to verify the presence/absence of fungal elements. We recently incorporated the CSB stain as an additional stain for the fungal slide preparation.

We found that the addition of CSB to the fungal slide provided a color contrast that makes reading and interpretation of the slides easier. Cells remained colourless while the fungal elements stained blue (figure 1). Examination of the slides after 24 hours revealed a clearer background (due to the digestion of epithelial cells), but no deterioration of the stained elements.

Veronica Restelli, CMPT Editor

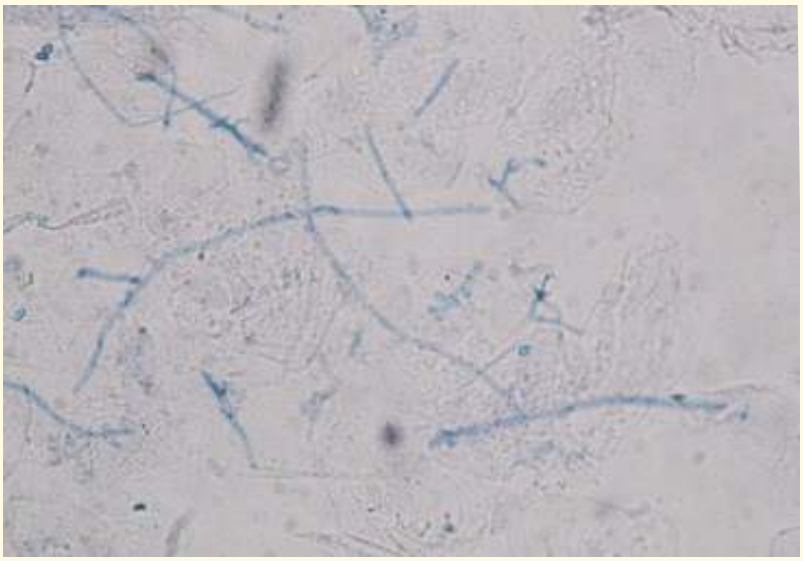

Figure 1. CSB stain of a KOH slide revealing blue fungal hyphae against a clear cellular background (original magnification ×400).

You can find more information on the CSB stain in the following articles:

- Lim C, Lim S. New Contrast Stain for the Rapid Diagnosis of Onychomycosis. *Arch Dermatol.* 2011;147(8):981-982. .
- Lim S, Lim C. New Contrast Stain for the Rapid Diagnosis of Pityriasis Versicolor. *Arch Dermatol.* 2008;144(8):1058-1059.
- Tambosis, E. and Lim, C. A comparison of the contrast stains, Chicago blue, chlorazole black, and Parker ink, for the rapid diagnosis of skin and nail infections. Int. J. Derm. 2012. 51: 935–938

CMPT's OPEN HOUSE

CMPT would like to invite participants to its annual general meeting (AGM).

Every year, CMPT rounds up the year with an annual meeting during which we evaluate our performance during the year, receive suggestions from our committee members and accreditation bodies, present new ideas and programs, and discuss future directions.

For the first time, CMPT would like to extend the invitation to one representative from each participant laboratory to attend the AGM. We think that it would be advantageous for both parties to increase communication and participation.

CMPT Annual General Meeting
Monday October 7, 2013
Holiday Inn Vancouver-Centre
711 W. Broadway, Vancouver, BC.

For more information, please contact CMPT at cmpt.path@ubc.ca.

Please RSVP by September 20, 2013 as space is limited.

Get Connected

Upcoming Events

October

6th Trends in Medical Mycology

October 11—14, 2013 Copenhagen, Denmark
More info: http://www.timm2013.org/en/Home_10_6_12.html

CMPT Annual General Meeting

October 7, 2013 Vancouver, BC
Holiday Inn Vancouver-Centre; 711 W. Broadway
More info: cmpt.path@ubc.ca

POLQM—Quality Management Conference

October 16—18, 2013 Vancouver, BC
More info: http://polqm.ca/conference_2013/conference_2013/conference_home.html

April 2014

16th International Congress on Infectious Diseases

April 2-5, 2014 Cape Town, South Africa
More info: http://www.isid.org/icid/

July 2014

89th Annual Meeting of the American Society of Parasitologists

July 24-27, 2014 New Orleans, Louisiana
More info: http://amsocparasit.org/node/79

IUMS—International Union of Microbiological Societies Congresses

July 27 – August 1, 2014 Montreal, Canada
XIVth International Congress of Bacteriology and Applied Microbiology
XIVth International Congress of Mycology
XVIth International Congress of Virology
More info: http://www.montrealiums2014.org

ABOUT CONNECTIONS

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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

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