Microbiology Rotation for Medical Laboratory Technology Students

Welcome to Microbiology!

Microbiology is an interesting and dynamic science. Your time spent in our lab should be an enjoyable and successful learning experience.

The main objective of this training program is to produce a technologist who has the ability to apply theoretical knowledge to the practical aspects of the Microbiology laboratory, efficiently and with a caring attitude.

During your rotation you will be exposed to all aspects of the department. You will handle specimens from their receipt in the department through to the reporting of results. You will develop the ability to make a presumptive identification of common pathogens and be able to select the correct tests to fully identify them and perform antimicrobial sensitivity testing.

Department Information

UNIVERSITY HEALTH NETWORK/Mount Sinai Hospital Department of Microbiology

14th Floor - Mount Sinai Hospital
600 University Ave.
Toronto, Ontario
(416) 586-4432

Regular Lab Hours

7:45am to 16:00pm **or** 8:00am to 16:15pm

Coffee and lunch schedules should be worked out with the bench tech you are working with.

If you are sick or cannot report to the lab please call the Senior Technologist's at (416)586-4800 ext 3822 and leave a message.

Schedule for MLT Students Rotation

1. Orientation

i. Introduction to Microbiologist-in-Chief Dr. Mazzulli and Microbiologist Dr. Poutanen. Introduction to Administrative Director - Katherine Wong, Supervisor - Pauline Lo and Charge Technologists - John Ng and Lilian Law.

ii. Tour of lab
- where to get clean lab coats, put dirty ones and wash up area
- various benches, incubators, fridges, store room
- bathroom and lunchroom facilities, including Food Hall on main floor
- bulletin board with schedules, notices, lectures
- conference room where journals and reference books are kept
- where main lab manual, CLSI guidelines, Safety Manual, WHMIS Manual and other reference books are kept

iii. Safety talk by Safety Officer
- overview of lab safety procedures e.g. no open toe or open heel shoes, fire alarm pulls, stairway exits, fire extinguishers, eye wash / shower stations, WHMIS, HPTR etc

2. Competencies and Objectives

Please refer to objectives given to you by your individual teaching institutions. Objectives are also available with the senior teaching technologist.
Note: Competencies and Objectives are actually set by CSMLS.

3. Training

We have students from different institutions with variations in their length of rotation therefore, students will rotate differently on the benches. Some will be doing all and some will be doing specific benches. The benches include Specimen Processing, Urines, Respiratory, Enterics/Gynae., Wounds/Miscellaneous, Bloods, Infection Control, Quality Control, Virology/Serology and perhaps mycology. A schedule will be given to you on your first day.

4. **Performance Assessments**

Each student will be evaluated by the technologist working the bench that the student is assigned to that week. Students will also evaluate the benches on their rotation every week. Final evaluation will be done by the Senior Teaching Technologist at the end of rotation. Each student (except Mohawk) will be given "unknowns" throughout the microbiology rotation.

St Lawrence College students do weekly quizzes and final exam online. These students will also have a practical exam week 8.

Michener students do weekly quizzes online and submit competencies for sign-off via CompTracker.

UOIT students do weekly quizzes online. These students will also have a written and practical exam week 8. These students submit competencies for sign-off via CompTracker.

Mohawk students will have a quiz given by the teaching technologist at the end of Week 3.

5. **Meetings and Rounds**

There are several tutorials/rounds you may attend:

- Departmental meetings and/or seminars are every Wednesday from 2:00-3:00 p.m. They are held in the 14th floor or 11th floor classroom.
- Other relevant seminars or infectious disease/micro rounds will be announced on an individual basis.

Bench Technologist – Teaching Guidelines

The bench technologist teaching the students should:

1. Discuss the nature of the specimens and the organisms (both pathogens and commensals) likely to be found in them. The student should be told how to identify these organisms and how the media is used for this purpose.

2. Make sure the organism(s) listed in each section are seen and worked up. If the organisms have not been seen in actual specimens, then the students should subculture the stock cultures and do the appropriate tests if time permits.

3. Demonstrate all procedures used in identification of pathogens on the bench, guide the students to perform all the procedures on their own.
   Due to the use of Vitek MS, students should be made familiar with classical methods as well.

4. Allow the students to work on the specimens independently for some (or all) of the time on the bench.

5. Read specimen gram smears. Correlate grams smear with culture results. The respiratory bench should show the students how to screen sputum smears by interpreting oropharyngeal contamination using epithelial cells in gram stains. The gynecological bench should show students bacterial vaginosis slides and also wet preps.

6. Complete bench evaluation on each student they work with.

Canadian Society for Medical Laboratory Science (CSMLS) - Code of Professional Conduct

1. Medical laboratory professionals are dedicated to serving the health-care needs of the public. The welfare of the patient and respect for the dignity of the individual shall be paramount at all times.

2. Medical laboratory professionals work with other health care professionals, to provide effective patient care.

3. Medical laboratory professionals shall promote the image and status of their profession by maintaining high standards in their professional practice and through active support of their professional bodies.

4. Medical laboratory professionals shall protect the confidentiality of all patient information.

5. Medical laboratory professionals shall take responsibility for their professional acts.

6. Medical laboratory professionals shall practice within the scope of their professional competence.

7. Medical laboratory professionals shall endeavour to maintain and improve their skills and knowledge and keep current with scientific advances. They will uphold academic integrity in all matters of professional certification and continuing education.

8. Medical laboratory professionals shall share their knowledge with colleagues and promote learning.

9. Medical laboratory professionals shall be aware of the laws and regulations governing medical laboratory technology and shall apply them in the practice of their profession.

10. Medical laboratory professionals shall practice safe work procedures at all times to ensure the safety of patients and co-workers and the protection of the environment.

Microbiology Rotation for Residents

Welcome to Microbiology!

Microbiology is an interesting and dynamic science. Your time spent in the Microbiology Laboratory should be an enjoyable and successful learning experience.

During your rotation you will be exposed to all aspects of the department. You will handle specimens from their receipt in the department through to the reporting of results. You will develop the ability to make a presumptive identification of common pathogens and be able to select the correct tests to fully identify them.

In order to make your rotation a positive experience for both you and the other members of the department following these guidelines will be useful:

1. Your rotation in the clinical laboratory is first priority.

2. Don't change or delay your rotation without first discussing this with Dr. Poutanen.

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Coffee and lunch schedules should be worked out with the bench tech you are working with.

If you are sick or cannot report to the lab please email Dr. Poutanen or "microbiologyspecialqueries@sinaihealthsystem.ca".

Schedule for Resident's Microbiology Rotation

Orientation

1. Introduction to Dr's Mazzulli, Poutanen, and McGeer. Introduction to Administrative Director Katherine Wong, Supervisor Pauline Lo and Charge Technologist Lilian Law & John Ng.

2. Tour of lab:
   - where to get clean lab coats, put dirty ones and wash up area
   - various benches, incubators, fridges, store room
   - bathroom and lunchroom facilities, including cafeteria location
   - bulletin board with schedules, notices, lectures
   - library where journals and reference books are kept
   - where main lab manual is kept and CLSI guidelines Safety Manual, WHMIS Manual and other reference books (QA bench)

3. Full safety talk by Safety Officer - Judith Cunningham,
   - short overview of safety rules e.g. no open toe or open heel shoes, fire alarm pulls, stairway exits, fire extinguishers, eye wash / shower stations, re: WHMIS, HPTR etc.

4. Arrange time for Quality Management talk by Quality Assurance technologist.

5. Arrange time for IC talk by an Infection Control Educator.

Training

One to two weeks on the following benches – Specimen Processing, Urines, Respiratory, Enterics/Gynae., Wounds/Miscellaneous, Bloods, Infection Control, Quality Control and Virology/Serology. Extra weeks in certain areas if desired and time permitted.

A schedule will be given to you on your first day.

Additional Training

One month with Infection Control Department.
One week at Public Health Lab (Optional)

Performance Assessments

1. Bench evaluations will be done by both the bench technologist and the student weekly.

2. Unknowns: series of unknown cultures to be given on the second week of the bench rotation.

3. Weekly Teaching Sessions / Questions
   - one per week that will cover the area of the laboratory that you have been exposed to.
   - Consists of review and discussion with staff microbiologists.

4. Examinations (For Microbiology Residents Only)
   - One practical exam will be given consisting of specimens to work up, and smears to read.
   - An oral exam will also be given by the Microbiologists

Meetings and Rounds

1. Infectious Diseases/Microbiology Rounds

- 1300 - 1400 every Tuesday, at TGH Radiology Conference Room, East South, 1-452.

- see bulletin board for list of topics

2. Departmental Seminars

- 1400 - 1500 every Wednesday

- you may be required to present a topic and/or fascionoma rounds.

3. Infection Control Meetings

- 1430 - 1530 every Thursday

4. Lab Management Meetings

- every Wednesday

5. Quality Team Meetings

- every Monday

6. Other Rounds / Seminars

- Please refer to Academic Events Schedule

Projects

- you may be given a project to work on with guidance from the Microbiologist
- plans for the project should be made halfway through the diagnostic rotation and media etc. ordered. (Please check with Pauline)

University of Toronto Training Objectives
Postgraduate Training Program in Infectious Diseases

Training Objectives for Adult Infectious Diseases: Medical Microbiology Rotation

The department provides a six month rotation for infectious diseases trainees. The objectives of this rotation are:

1. To familiarize the resident with all microbiology services provided by the department including routine bacteriology, mycology, parasitology, virology, serology, and quality assurance.

2. To familiarize the resident with both the laboratory and clinical aspects of infection by having them rotate on the infection control bench as well as actively participating on the Infection Control Team, assisting with the Surveillance Program, taking part in the investigation of any relevant problems that may arise during their rotation, attend the weekly infection control team meetings and the monthly infection control team meetings.

3. To expose the resident to clinical and applied research by having them carry out a research project over the six months of their rotation. The candidate is expected to submit an abstract to a major national or international meeting and prepare a manuscript for submission for publication.

4. To allow the resident to attend ongoing microbiology and infectious diseases rounds/seminars as well as participate in, and present at, the departmental laboratory rounds and journal club.

5. To familiarize the resident with common clinical problems presented to the medical microbiologist by participating in a discussion of a pre-assigned weekly "question-of-the-week" regarding clinical microbiology and/or laboratory management issues.

6. To familiarize the resident with aspects of laboratory management and quality assurance by having the resident participate in management meetings and as member of the quality committee.

Resident Objectives at MSH

SPECIMENS

Reference: Cumitech 9. Collection and Processing of Bacterilogical Specimens

The resident shall:

1.0 Identify properly collected and transported specimens

2.0 Recognize and state problems in poor or unsuitable specimens

3.0 Demonstrate proper and safe handling of all specimens

4.0 Demonstrate proper use of the biological safety cabinet and how it works

CULTURAL TECHNIQUES

The resident shall:

1.0 Select suitable primary media for all types of specimens

2.0 Recognize specimens that should be set up for anaerobes and understand the importance of handling anaerobic specimens promptly and planting them on pre-reduced media

3.0 Demonstrate proficiency inoculating and streaking a specimen on agar media

4.0 Be able to set up cultures in an anaerobic atmosphere and a 5-10% CO_2 atmosphere

5.0 Perform quantitative cultures and report the colony count

6.0 Prepare and read a simple wet mount for *Trichomonas vaginalis* specimen

7.0 Examine and report on the suitability of a sputum for culture (screening of a sputum)

STERILIZATION

Reference: Disinfection, Sterilization, and Preservation, Block, Fourth Edition.

The resident shall:

1.0 Demonstrate aseptic technique

2.0 Select and perform correct sterilization for all types of media and objects

3.0 Demonstrate an understanding of these methods of sterilization and how to quality control them
- 3.1 Dry heat
- 3.2 Moist heat
- 3.3 Filtration
- 3.4 Ultra violet irradiation
- 3.5 Ethylene oxide
- 3.6 Gamma radiation

4.0 Demonstrate an understanding of disinfection
- 4.1 Select proper disinfection for cleaning lab equipment and spills

MEDIA

Reference: Media for Isolation - Cultivation - Identification Maintenance of Medical Bacteriology, McFadden

For media used in the laboratory the resident shall:

1.0 List the main ingredients and describe their functions

2.0 State the classification of the media

3.0 Quality control the medium by

- 3.1 Checking sterility
- 3.2 Checking pH and appearance
- 3.3 Checking biochemical and growth of control organisms
- 3.4 Checking media storage is at proper temperature and length of time

GRAM STAIN

For the Gram stain, the resident shall:

1.0 State principle of Gram's method

2.0 Perform a Gram stain

3.0 Recognize and state problems in poor or incorrectly stained slides

4.0 Recognize and report on Gram stained slides of specimens and cultures

5.0 Quantitatively report on direct smears

MICROSCOPY

The resident shall:

1.0 State the principles of operation of light, phase contrast and UV microscope

2.0 Demonstrate proper use and care of the microscopes

3.0 Be able to perform Kohler illumination

SAFETY / WHIMIS

The resident shall:

1.0 State the safety rules for a clinical laboratory

2.0 State procedure to be used for cleanup of various spills:

- 2.1 Chemical
- 2.2 Radioactive
- 2.3 Blood
- 2.4 Specimens

3.0 Demonstrate proper safety techniques for handling specimens

4.0 State the principles of WHMIS

5.0 Demonstrate the proper handling of chemicals while using MSDS

6.0 Perform all procedures using chemicals using safety techniques as stated in the MSDS

DIAGNOSTIC OBJECTIVES (urine, genital, enteric, respiratory, wound and fluid benches).

Reference: Cumitechs: 4A. Laboratory Diagnosis of Gonorrhea
14A. Laboratory Diagnosis of Central Nervous System Infections
12A. Laboratory Diagnosis of Bacterial Diarrhea
12. Laboratory Diagnosis of Female Genital Tract Infections

Clinical Microbiology Procedures Handbook. Editor: Isenberg

For the following sites:
a) respiratory tract (upper and lower)
b) intestinal tract
c) genitourinary tract
d) skin
e) urine
f) blood
g) CSF
h) sterile fluids
i) tissues

The resident shall:
1.0 State which specimens to be taken for the clinical picture of the patient.

2.0 State the specimens to be taken for proper lab work up.

3.0 State the proper collection methods including appropriate time for specimens to be taken, the amount and how to be transported to the laboratory.

4.0 State the organisms that commonly occur as pathogens, plus state what organisms may occur as commensal flora.

5.0 Recognize and differentiate pathogens from commensal flora in the specimens.

For all the organisms listed below:

Aerococcus urinae
Staphylococcus aureus
Coagulase negative Staphylococcus
Staphylococcus saprophyticus
Streptococcus pyogenes
Streptococcus agalactiae
Streptococcus pnuemoniae
Viridans Streptococcus
Enterococcus spp.
Streptococcus anginosus group
Neisseria gonorrhoeae
Neisseria meningitidis
Moraxella catarrhalis
Pseudomonas aeruginosa
Burkholderia cepacia
Acinetobacter species
Pasteurella multocida
Shigella species
Salmonella typhi
Salmonella spp.
Escherichia coli
Yersinia enterocolitica
Vibrio spp.
and all *Enterobacteriaceae*

Cryptococcus neoformans
Haemophilus influenzae
Haemophilus species
Campylobacter jejuni/coli
Campylobacter fetus
Listeria monocytogenes
Corynebacterium pseudodiptheriae
Corynebacterium diphtheriae
Corynebacterium jeikium
Actinomyces israelii
Nocardia asteroides
Clostridium difficile
Clostridium perfringens
Bacteroides fragilis group
Prevotella melaninogenicus
Fusobacterium species
Propionibacterium species
Mycobacterium species
Mycobacterium tuberculosis
Candida albicans
Candida species

The resident shall:

1.0 Identify and record colonial appearance

2.0 Select optimal temperature, gaseous requirement and suitable media for isolation. Describe any special media that may be required, their main ingredients and functions.

3.0 Describe as applicable the typical cellular morphology, Gram stain reaction or wet mount appearance in direct smears and in culture.

4.0 Select and perform differential tests for identification.

5.0 Interpret all tests performed and identify the organisms.

6.0 State the principles, reagents and controls involved in all the differential tests for identification.

7.0 State the optimal method of susceptibility testing and the typical susceptibility pattern.

8.0 State the epidemiology, clinical significance and infection control implications.

9.0 State the problems of laboratory detection, identification and susceptibility testing.

BLOOD CULTURES

Reference: Cumitech 1A. Blood cultures II

The resident shall:

1.0 State in what clinical situations blood cultures are to be taken.

2.0 State the proper collection methods.

3.0 For the blood cultures bottles

- 3.1 list the major ingredients, and understand the purpose of each.
- 3.2 state the recommended incubation time and temperature for all types of blood cultures.
- 3.3 explain how and why blood cultures are subcultured.

4.0 For the Blood Culture machine in the laboratory

- 4.1 state the principle of operation
- 4.2 demonstrate proficiency in operating the machine
- 4.3 perform regular routine maintenance
- 4.4 recognize and solve minor problems

5.0 Demonstrate the proper procedures for working up positive blood cultures.

6.0 Describe the operation of the following blood culture system

- 6.1 conventional

6.2 lysis centrifugation
6.3 infrared
6.4 radiometric
6.5 colorimetric detection (Virtuo)

ANAEROBES

Reference: Cumitech 5A. Practical Anaerobic Bacteriology

The resident shall:

1.0 Describe the principles of the anaerobic jar and anaerobic chamber, PRAS and rolled tube systems.

2.0 Operate the anaerobic jar utilizing proper techniques for achieving and monitoring anaerobiosis.

3.0 Differentiate organisms by their oxygen requirements.

4.0 State which specimens are suitable for anaerobic culture.

5.0 Describe the proper collection technique and transport for anaerobic specimens.

6.0 Discuss the type of media to be used for anaerobes.

GENITALS

The resident shall:

1.0 Define bacterial vaginosis.

2.0 Differentiate between vaginosis, vaginitis or normal vaginal flora by Gram stain and wet preparations.

3.0 For *Trichomonas vaginalis* the resident shall:

- 3.1 describe how to examine a wet mount.
- 3.2 state it's main differentiating characteristics
- 3.3 explain clinical significance

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Reference: Cumitechs 25. Current Concepts and Approaches to Antimicrobial Agent Susceptibility Testing

6A. New Developments in Antimicrobial Agent Susceptibility Testing: A Practical Guide

CLSI Guidelines

The resident shall:

1.0 Discuss all the antimicrobial agnets used in the laboratory by:

- 1.1 describing their antimicrobial spectrum
- 1.2 stating the kind of activity they have (bactericidal, bacteriostatic)
- 1.3 identifying the agents by their class

2.0 For each of the following antimicrobial susceptibility testing methods:

Kirby Bauer
MIC / MBC broth dilution
MIC agar dilution
MIC micro broth dilution

- 2.1 state the principle
- 2.2 perform and report results of the methods used in the laboratory
- 2.3 select proper quality control procedures and indiate what troubleshooting would be done if the QC did not work
- 2.4 explain limitations of each method

3.0 Describe the Beta Lactamase Test by:

- 3.1 explaining the principle of the nitrocefin, acidometric, and iodometric methods
- 3.2 performing and reporting the results
- 3.3 stating the clinical significance of β-lactamase producation

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The resident shall:

4.0 Identify Methicillin Resistant *Staphylococcus aureus* (MRSA) by:

- 4.1 explaining how MRSA's are detected
- 4.2 stating the clinical significance of such an isolate
- 4.3 discussing the problems in detecting MRSA

5.0 Identify Penicillin resistant Pneumococcus by:

- 5.1 explaining the procedure used for screening and confirmation
- 5.2 stating the criteria used to define resistance, relative resistance, and susceptible to Penicillin
- 5.3 describing the clinical significance

6.0 Discuss the emerging new trends for susceptibility testing of *Enterococcus* by:

- 6.1 explaining what synergy is and shy it's important for *Enterococcus*
- 6.2 determining synergy between Ampicillin and Gentamicin by the different methods available
- 6.3 performing high level aminoglycoside testing and interpreting the results
- 6.4 VRE

7.0 ESBLs and CRES

MYCOLOGY

The student shall:

1.0 Identify properly collected and transported specimens for culture.

2.0 Perform and report on microscopic preparations form direct specimens by identifying fungal elements.

3.0 Select suitable primary media and atmospheric conditions for each specimen.

Describe cultural requirements and differentiate the following microscopically and culturally:

Microsporum
Trichophyton
Epidermophyton
Aspergillus species
Blastomyces dermatitidis
Coccidioides immitis
Histoplasma capsulatum
Sporothrix schenkii
Penicillium species
Yeast (eg. Candida, Crypto)

5.0 Describe the appearance of *Malassezia furfur* in a direct mount of skin scales.

SEROLOGY

Reference: Manual of Clinical Laboratory Immunology, Fourth Edition.

The resident shall:

1.0 Define antibody and antigen

2.0 State the principle, perform, read, report and interpret the results for the following tests:

Observe the following:

Architect i4000 reactive for serology tests

Latex Testing: CMSE – CMV Antibody Capture Test
Monospot
Cryptococcal Antigen

EIA Tests: Galactomannen

Vidas machine for VZ

DFA: PCP

VIROLOGY

PCR Tests:	Chlamydia / NG (Roche 4800) West Nile – NAT & Donor NAT testing (MPX) Enterovirus/EBV/HSV/CMV/Parvovirus/VZ/ Adenovirus//HPV Influenza A, B & RSV

Reference:

1. Schmidt N., Emmons R. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. 1989: American Public Health Association, New York. Sixth Edition.

2. Wiedbrauk D., Johnston S. Manual of Clinical Virology. 1993: Raven Press, New York.

3. Lennette E. (Ed.) Laboratory Diagnosis of Viral Infections. 1992: Marcel Dekker, New York. Second Edition.

The resident shall:

1.0 Describe how a virus differs from bacteria.

2.0 For the following virus families:

a) Herpesviridae
b) Orthomyxoviridae
c) Paramyxoviridae
d) Parvoviridae
e) Retroviridae
f) Togaviridae
g) Picornaviridae
h) Reoviridae

2.1 Classify according to:
- a) RNA or DNA
- b) Single or double stranded
- c) Nucleocapsid symmetry
- d) Positive or negative sense
- e) Prescence / absence of envelope
- f) Size range

2.2 State the important human pathogens within these families.

3.0 Explain what is meant by the following terms and give 2 examples for each:

a) Primary cell line
b) Diploid cell line
c) Continuous cell line

4.0 List the major components of:

a) Viral Transport Media
b) Growth Media
c) Maintenance Media

5.0 For the following culture methods:

a) Shell vial technique
b) Tube culture technique

5.1 Briefly describe the method
5.2 List advantages and disadvantages for each method.

6.0. For the following viral illnesses:
a) Herpes zoster
b) Viral meningitis
c) Systemic CMV disease
d) Viral respiratory illness
6.1 List the appropriate specimens to be collected.
6.2 Describe appropriate specimen collection, transport and storage techniques.
6.3 Describe suitable inoculation and incubation techniques including selection of appropriate cell lines, culture methods and incubation.
6.4 State the virus(es) which may be expected to be detected.
6.5 Describe any rapid tests which may be used to aid in diagnosis.

7.0 For the viruses listed below:

a) Herpes simplex type 1 and 2
b) Cytomegalovirus
c) Varicella Zoster
d) Influenza A and B
e) Parainfluenza 1, 2, and 3
f) Respiratory syncytial virus
g) Adenovirus

h) Enterovirus
i) Coxsackievirus
j) Poliovirus

7.1 State the optimal cell line(s) for isolation.
7.2 State the virus' effect on that cell line including a description of any cytopathic effect produced.
7.3 Describe the test(s) used for definitive identification once isolated, including any controls needed. (e.g. IFA stains)

8.0 Describe one method of anti-viral susceptibility testing.

CMV QUANTITATIVE PCR

The resident shall:

1.0 Explain the significance of determining the presence of CMV antigen in the blood.

2.0 Show proficiency in CMV PCR testing by:

2.1 Discussing proper specimen collection.
2.2 Stating the principle of the procedure.
2.3 Performing the procedure with quality control.
2.4 Reporting results including quantitation of positive samples.

Mycology - Training Manual for Residents

Teaching and Supervising Technologist: Mycology Technologist

Reference:

1. Davise H. Larone: Medically Important Fungi, A guide to identification, 3rd. Edition, ASM Press, 1995
2. Guy St – Germain, Richard Summerbell; Identifying Filamentous Fungi, A Clinical Laboratory Handbook, Star Publisher, 1996
3. Martha E Kern: Medical Mycology; Self Instructional Text, F A Davis, 1985 (1997)
4. Selected Reference Articles

The Trainee at all levels of training shall know:

1.0 ***Processing of Specimens***

1.1 Know about proper mycology specimen collection, transportation and planting and the use of selective and/ or non-selective media

1.2 State the appropriate incubation temperature and length of incubation for recovery of various pathogens

2.0 ***Direct Microscopy***

2.1 Demonstrate the proper of use of microscopy in the detection of fungal elements in clinical specimens using: Fungi-Fluor (FS), 10% KOH and Calcofluor White (CW)

2.2 Demonstrate and interpret fungal elements examined microscopically such as yeast, pseudo hyphae, septate or non-septate hyphae, narrow or broad base attachment etc. and/ or suspect or identify certain pathogens presumptively based on structure

2.3 Explain the different uses, advantages and disadvantages of the FS, CW and 10% KOH

3.0 ***Procedure for identification of Yeast***

3.1 Demonstrate the ability to identify various yeasts using Germ Tube, Oxgall, Cornmeal, Urea, EBM, Rapid Yeast ID System, API 20C procedures

3.2 Explain the principles, uses, advantages and disadvantages of the above methods

3.3 Ability to recognize organisms looking like yeast such as Prototheca based on structure

4.0 ***Protocol for reporting results as per manual policy***

4.1 Reporting positive FS results in SOFT and phoning critical results to ward

4.2 Reporting positive culture results in SOFT and phoning ward where applicable

5.0 ***Procedures for identification of moulds***

5.1 Demonstrate the following methods used: Macroscopic Examination of Cultures, Microscopic Examination of Cultures, Scotch Tape Preparation, Tease Mount Preparation, Slide Culture, Sub-Culture, Phase Conversion, Permanent Mounts and Stock Preparation

5.2 Explain the purpose of each of the above

6.0 ***Identification of the following fungi to the genus and/ or species level where indicated:***

<u>Rapid Grower:</u>

Aspergillus fumigatus, niger, flavus and *terreus*
Penicillium species and Paecilomyces species
Fusarium species and *Acremonium species*
Scopulariopsis species and *Trichoderma species*
Scedosporium species (apiospermum and prolificans) and *P. boydii*

<u>Zygomycetes:</u>

Rhizopus species
Mucor species

Absidia species

Dematiaceous Moulds:

Cladosporium species
Curvularia species and *Bipolaris species*
Alternaria species and *Exophiala species*

Superficial Mycotic Agents:

Dermatophytes and Scytalidium species
Trichosporon beigelii
Malassezia furfur (Pityriasis versicolor – microscopic only, no culture necessary)

Dimorphic Fungi:

Histoplasma capsulatum and Blastomyces dermatitidis
Sporothrix schenckii and *Penicillium marneffei*
(*C. immitis* – Demonstration only)

7.0 ***Identification of Actinomycetes and other Filamentous Organisms***

Nocardia species
Streptomyces species
(Compare to Rapid Grower *Mycobacteria*)

General Microbiology Review

1. Review of common organisms should occur in the first week. This review will include:

   #1 *Staphylococcus aureus*

   #2 CNST

   #3 *Enterococcus faecalis*

   #4 *Escherichia coli*

   #5 *Pseudomonas aeruginosa*

   #6 Group B Streptococcus

   (See Appendix I)

2. Kohler illumination (See Technical Section in Lab Manual)

APPENDIX I - Day One

1. On Day 1, subculture *S. aureus*, CNST, *Enterococcus* spp., *Group B Streptococcus*, *E. coli* and *Pseudomonas aeruginosa* onto MacConkey and Blood agar plates for isolated colonies. (See Appendix II).

2. On the second day gram stain each organism from any one plate. (See Appendix III). Also describe colony morphology for each organism on each of the plates.

3. On the second day also do the following tests and record results: (See Appendix IV).

   Catalase - For either *Staph*. spp and *Enterococcus* spp.

   Coagulase - For *S. aureus* and CNST

   Bile esculin - For *Enterococcus* spp. and *Group B Streptococcus*

   MUG - For *E. coli*

   Oxidase - For *Pseudomonas aeruginosa* and *E. coli*

APPENDIX II- Media

1. MacConkey Agar
   - Is a selective and differential medium to isolate Gram negative bacilli
   - Lactose fermentors are pink
   - Non-lactose fermenters are pale
   - Peptone base with lactose and neutral red as indicator
2. Blood Agar
   - Typticase soy agar, Brucella agar or beef heart infusion base and 5% sheep blood
   - General purpose medium
   - Used to cultivate fastidious microorganisms
   - To determine haemolytic reactions
3. Chocolate Agar
   - Peptone base enriched with 2% haemoglobin
   - To cultivate *Haemophilus* and *Neisseria* species
   - Also a general purpose medium
4. Haemophilus Agar
   - Selective and differential medium
   - Chocolate base containing bacitracin to inhibit respiratory flora
   - To isolate *Haemophilus*
5. *Burkholderia cepacia* Agar
   - Selective agar for *B. cepacia*
6. CNA Agar
   - Columbia colistin nalidixic acid agar with 5% sheep blood
   - Selective and differential medium
   - Used to isolate gram positive organism from mixed cultures
7. Brucella Agar
   - With hemin and vitamin K
   - For the isolation and cultivation of anaerobic bacteria
8. LKV Agar
   - Kanamycin-vancomycin laked sheep blood

- Enriched selective and differential medium for the isolation and cultivation of anaerobic bacteria e.g. *Bacteroides* spp. and *Prevotella* spp.

9. Fastidious Anaerobic Broth
   - Broth culture for anaerobes

10. Hektoen Enteric Agar
    - Selective and differential medium
    - Bile salts and indicator dyes select and inhibit gram positive organisms
    - Lactose, sucrose and salicin present to differentiate between fermenters and non-fermenters
    - Sodium thiosulfate and ferric ammoniumcitrate detect H_2S production
    - *E. coli* are yellow-pink in colour.
    - *Shigella* are green or transparent Salmonella green or transparent with black centers.

11. SMAC
    - MacConkey agar – with sorbitol
    - Selective and differential media used to isolate sorbitol – negative *E. coli*
    - Non-sorbitol fermenters appear colorless

12. Selenite Broth
    - Enrichment broth for isolation of *Salmonella* and *Shigella*
    - Inhibits enterococci and coliforms

13. CIN Medium
    - Cefsulodin-irgasan-novobiocin medium
    - Selective and differential for isolation of *Yersina enterocolitica*

14. Campylobacter Blood Agar
    - Enriched selective blood agar
    - Trimethoprim, Vancomycin, Amphotericin B, Polymyxin B and Cephalothin in medium

15. Martin-Lewis Agar
    - Enriched and selective medium for *N. gonorrhoeae*
    - Vancomycin, anisomycin, trimethoprim and colistin in medium

16. Strep B Carrot Broth
    - Selective enrichment and identification of beta-haemolytic Group B Streptococci

17. Denim Blue Oxoid Chromogenic Agar for MRSA

18. Brilliance VRE Chromogenic Agar

APPENDIX III - Gram Stain Reactions

Gram positive cocci	-	*Staphylococcus aureus*
	-	Coagulase negative *Staphylococcus*
	-	*Enterococcus faecalis*
	-	Viridans streptococcus
	-	Beta-haemolytic group B streptococcus
	-	Peptostreptococcus species
Gram positive bacilli	-	*Clostridium novyi*
	-	*Bacillus* species
	-	*Corynebacterium* species
Gram negative coccus	-	*Moraxella cattarhalis*
	-	*Neisseria* species
	-	*Veillonella* species
Gram negative bacillus	-	*Escherichia coli*
	-	*Proteus mirabilis*
	-	*Pseudomonas aeruginosa*
	-	*Bacteroides* species

APPENDIX IV - Tests

1. Catalase
   - bacteria that contain cytochrome enzymes are catalase positive and those that don't are catalase negative
   - *Staphylococcus* +
   - *Streptococcus* and *Enterococcus* -
   - flood bacteria with 3% hydrogen peroxide and observe for bubbles
     catalase
   a) H_2O_2 (3%) $\rightarrow$ Catalase peroxide + H_2O
   b) Catalase peroxide + H_2O_2 $\rightarrow$ Catalase H_2O + O_2

2. Coagulase
   - the ability to clot plasma
   - two different coagulase tests can be performed, a tube test for free and bound coagulase and a slide test for bound coagulase, or clumping factor
   - *Staphylococcus aureus* +
   - Coagulase negative *Staphylococcus* -

3. Bile esculin
   - to detect beta glucoside which breaks down esculin to form a black precipitate due to the presence of ferric ions
   - *Enterococcus faecalis* +
   - Beta-haemolytic group B streptococcus -

   Esculin (β glucoside) $\rightarrow$ Esculetin + ferric ions (ferric citrate in medium)
   $\downarrow$
   black precipitate

4. MUG
- this test is mainly done on the urine bench
- to detect beta-glucuronidase
- ortho-nitrophenyl-beta-glucuroride substrate is broken down by enzyme glucuroniclase to produce ortho-nitrophenyl which produces a yellow color.
- Indole production

a) tryptophanase (produced by bacteria)

Trytophan → ammonia + pyruvic acid + indole

↓

reacts with p-dimethylamino benzaldehyde (Kovac's reagent)

↓

quinoidal red violet

b) Indole spot test uses Erlich's reagent 1% dimethylaminocinnamalaldehyde

↓

blue colour

Escherichia coli is MUG + and indole +

5. Oxidase
- to test for the production of oxidase
- spot inoculate organism on to a filter paper soaked with 1% tetramethylphenylene diamine dihydrochloride - positive is purple, negative is yellow
- *Pseudomonas aeruginosa* +
- *Escherichia coli* -

Oxidizing reaction

Reagent 1% Dimethyl or Tetramethyl para-phenylenediamine

↓ on colonies

↓

Indopheneloxidase (produced by bacteria)

↓

↓

Indophenol = black colonies (with dimethyl)

= magneta colonies (with tetramethyl)

6. Urease
- Detects urease production
- Peptones in media utilized producing an alkalinity. Phenol red indicator

Urea →→ammonia

7.	TSI	-	Triple sugar iron agar. 0.1% glucose, 1% sucrose and lactose.
		-	If the glucose is fermented, only a small amount of acid will be produced, which will be neutralized by alkali from peptone metabolism along surface of slant.
		-	Oxidation of peptone cannot take place in the anaerobic conditions in the depth of the medium.
		-	Therefore when glucose only is fermented, the butt of the medium becomes yellow and the slant remains red.
		-	If lactose or sucrose is fermented, the amount of acid produced is large enough to offset alkali production and a yellow slant is produced.
		-	Production of hydrogen sulphide is shown by formation of iron sulphide from the ferrous sulphate.
8.	ONPG	-	To detect enzyme β-D-galactosidase in lactose fermenting organisms. O-nitrophenol-B-D-galactopyranoside→→ O-nitrophenol (yellow)
9.	PPA	-	To detect Phenylalanine deaminase production. Phenylalanine→→Phenylpyruvic acid + $FeCl_3$ (ferric ions) = blue green
10.	PYR	-	To detect pyrrolidonyl peptidase
		-	L-pyrolidonyl-β-naphthylamide→β-naphythylamine →add cinnamaldahyde reagent = red colour

**More extensive details for all tests performed in lab can be found in the Technical Manual.

General Overview of Weekly Bench Rotations

Specimen Management and Media

Learn types of media we use in lab and their purpose

Culture the following specimens:

- Urines
- Stools
- Cervical swabs
- Vaginal swabs
- Throat swabs
- Sputa (including screening)
- Bronchial washings
- Miscellaneous swabs
- Tissues
- Fluids, including CSF

Stain and read Gram smears.

Stain and read Kinyoun, Modified Kinyoun and Eosinophil smears..

Be familiar with operation of autoclave.

Urine Bench

Read plates for colony counts and interpret significant colony counts.
Bacteria to have seen and identified:

- *Proteus mirabilis*
- *Escherichia coli*
- *Klebsiella* species
- *Staphylococcus saprophyticus*
- *Enterococcus*
- Yeast
- *Pseudomonas* aeruginosa

Stool Bench

Know biochemical reactions and specific media for:

Salmonella spp.
Shigella spp.
Campylobacter spp.
Yersinia enterocolitca
Escherichia coli 0157:H7
Vibrio spp.

Genital Bench

Read vaginal smears and wet preps. Should see lots of normal smears and vaginosis smears plus see yeast and *Trichomonas*.

Bacteria to have seen and identified:

Neisseria gonorrhoeae
Streptococcus agalactiae (Group B strep)

Respiratory Bench

Read sputum gram stains and know how to screen smears for oropharyngeal contamination.

Bacteria to have seen and identified:

Streptococcus pyogenes (Group A strep)
Pseudomonas aeruginosa
Burkholderia cepacia
Haemophilus influenzae
Haemophilus parainfluenzae
Streptococcus pneumoniae
Moraxella catarrhalis
Candida albicans
Cryptococcus neoformans

Miscellaneous & Sterile Fluids

Read gram smears and correlate with cultures.

Bacteria to have seen and identified:

Staphylococcus aureus including MRSA
Serratia marcescens
Enterococcus species
Streptococcus anginosus group
Streptococcus pyogenes
Streptococcus pneumoniae
Bacteroides species
Prevotella species
Clostridium perfringens
Fusobacterium species
Enterobacteriaceae
Pseudomonas species
Corynebacterium jeikeium

Blood Culture Bench

Operate the BacT Alert machine and assist with processing the specimens.
Assist in working up the positives.

Bacteria to have seen and identified:

Staph. aureus
Neisseria meningitidis
Listeria monocytogenes
Corynebacterium spp.
Viridans streptococcus
Coagulase negative staphylococcus
Streptococcus pneumoniae
Enterobacteriaceae
Enterococcus spp.
Anaerobes
Fastidious Gram negative bacilli

Infection Control Bench

Learn about the different selective media used on the specific benches to identify MRSA, VRE and CPE.
Bacteria to have seen and identified:

MRSA
VRE
ESBL
CPE

Virology/Serology

Observe the following:

Accessioning and processing of specimens
Preparing smears for DFA (PCP)
Processing of FLU/RSV samples
Cryptococcal Latex Agglutination test

Architect Bench: theory , loading specimen, reflex tests, reporting

MPX Bench: Donor NAT testing, Monospot testing,
routine and STAT VZV Ab on Vidas

CMV PCR other PCR tests

Performing Chlamydia/GC/HPV PCR

Mycology

Observe the following procedures:

Fungi Fluor stain for fungus

Isolation of various fungi and yeast, including knowledge of different media used.

Mycology - Training Manual for Students

Teaching and Supervising Technologist: Mycology Technologist

Reference:

1. Davise H. Larone: Medically Important Fungi, A guide to identification, 3rd. Edition, ASM Press, 1995
2. Guy St – Germain, Richard Summerbell; Identifying Filamentous Fungi, A Clinical Laboratory Handbook, Star Publisher, 1996
3. Martha E Kern: Medical Mycology; Self Instructional Text, F A Davis, 1985 (1997)
4. Selected Reference Articles

The student should know the following:

1.0 ***Processing of Specimens***

1.1 Know about proper mycology specimen collection, transportation and planting and the use of selective and/ or non-selective media

1.2 State the appropriate incubation temperature and length of incubation for recovery of various pathogens

2.0 ***Direct Microscopy***

2.1 Demonstrate the proper of use of microscopy in the detection of fungal elements in clinical specimens using: Fungi-Fluor (FS), 10% KOH and Calcofluor White (CW)

2.2 Explain the different uses, advantages and disadvantages of the FS, CW and 10% KOH

3.0 ***Procedure for identification of Yeast***

3.1 Demonstrate the ability to identify various yeasts using Germ Tube, Oxgall, Cornmeal, Urea, EBM, Rapid Yeast ID System, API 20C procedures

3.2 Explain the principles, uses, advantages and disadvantages of the above methods

4.0 ***Protocol for reporting results as per manual policy***

4.1 Reporting positive FS results in SOFT and phoning critical results to ward

4.2 Reporting positive culture results in SOFT and phoning ward where applicable

5.0 ***Procedures for identification of moulds***

Demonstrate the following methods used: Macroscopic Examination of Cultures, Microscopic Examination of Cultures, Scotch Tape Preparation, Tease Mount Preparation, Slide Culture and Sub-Culture. State the purpose of each step as listed above

TRAINEE'S NAME: _____ TRAINOR'S NAME:_____

Evaluation for Bench : URINE☐ ENTERIC/GYNAE☐ RESP☐ MISC☐
BLOODS☐ INF. CTL☐ QC☐ SPECIMEN PROCESSING☐

Dates of Rotation: _____-_____

Length of Rotation adequate_____ inadequate _____

Were your objectives covered?

Were you allowed to actively participate on the bench?

Were questions welcomed and answered?

Things you would suggest to other students to prepare for doing unknowns from this bench:

General Comments:

Trainee Evaluation Form for Technologist - Mycology Section

Name of the Trainee:

Dates of training:

Unacceptable ——————————— **Superior**
1 2 3 4 5

1.	**Skill and competence in performance:**		
	a.	Manual dexterity	1 2 3 4 5
	b.	Accuracy	1 2 3 4 5
2.	**Knowledge base:**		
	a.	Demonstrates relationship between principles & procedures	1 2 3 4 5
	b.	Interpretation of test results	1 2 3 4 5
	c.	Recognition of errors	1 2 3 4 5
3.	**Care and concern for order and clarity:**		
	a.	Attention to detail	1 2 3 4 5
	b.	Neatness in maintenance of work area	1 2 3 4 5
	c.	Accuracy and legibility in reporting results	1 2 3 4 5
4.	**Efficiency and organization:**		
	a.	Ability to sequence task (priority setting)	1 2 3 4 5
	b.	Adherence to established protocol	1 2 3 4 5
5.	**Dependability and commitment:**		
	a.	Carries out duties promptly with enthusiasm	1 2 3 4 5
	b.	Able to work independently	1 2 3 4 5
	c.	Shows initiative (ideas for improvement)	1 2 3 4 5
	d.	Responsible	1 2 3 4 5
	e.	Demonstrates emotional stability in stressful situation	1 2 3 4 5

6. Interpersonal relation:

a.	Co-operation with others	1 2 3 4 5
b.	Ability to accept constructive criticism	1 2 3 4 5
c.	Tactful in dealing with colleagues and personnel	1 2 3 4 5

7. Communication:

a.	Ability to give clear and precise instructions and/ or explanations	1 2 3 4 5
b.	Good communication	1 2 3 4 5
c.	Ability to ask for clarification in case of doubt	1 2 3 4 5

Comments:

Supervising Technologist's signature

Bench Training Evaluation Form for Trainee - Mycology Section

To be completed by the trainee at the end of training

Name of the trainee: _____

Dates of training: _____

Supervising Technologist:

Disagree		Agree		Strongly Agree
1	2	3	4	5

(Circle one)

1.	The objectives specified in the bench manual as applicable to your specific needs were covered during bench training. **If disagree please explain:**	1 2 3 4 5
2.	The supervising technologist was patient and supportive (Approachable)	1 2 3 4 5
3.	Frequent opportunities for questions and discussions were offered	1 2 3 4 5
4.	The supervising technologist was able to give:	
	a. Clear and precise instructions	1 2 3 4 5
	b. Explanations	1 2 3 4 5
5.	Feed back about your performance was given throughout the rotation	1 2 3 4 5
6.	There were adequate hands-on participation on the bench	1 2 3 4 5

4. The supervising technologist's teaching abilities are:

☐ Poor ☐ Fair ☐ Average ☐ Above Average ☐ Outstanding

Comments:

Trainee's signature:

Trainee Evaluation Form For Virology/Serology Section

Name of the Trainee:

Dates of training:

	Observed
1. Virology Accessioning Bench: a. Accessioning and processing of specimens b. Preparing smears for DFA (PCP) c. Run FLU/RSV samples d. Perform Cryptococcal Latex Agglutination test	☐
2. Architect Bench: a. Theory of Architect testing b. Loading of specimens c. Reflex tests. (Patient vs. Donors) d. Printing of results and Reporting	☐
3. MPX Bench: a. Donor NAT testing b. Perform Monospot testing c. Perform routine and STAT VZV Ab on Vidas	☐
4. PCR Benches : a. CMV PCR b. other PCR tests	☐
5. Viral Load Bench: a. Performing Chlamydia/GC/HPV PCR	☐

Books in Microbiology Laboratory

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28. Laboratory Safety CSLT Guidelines. 4th Edition. Gene Shematek. Wayne Wood. CSLT 1996.

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Record of Edited Revisions

Manual Section Name: Education Manual

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	May 26, 2004	Dr. T. Mazzulli
Annual Review	May 12, 2005	Dr. T. Mazzulli
Annual Review	July 5, 2006	Dr. T. Mazzulli
Annual Review	August 13, 2007	Dr. T. Mazzulli
Annual Review	August 12, 2008	Dr. T. Mazzulli
Annual Review	August 12, 2009	Dr. T. Mazzulli
Annual Review – Micro Rotation, CSMLS, Append.1, Books in Micro.	September 13, 2010	Dr. T. Mazzulli
Annual Review	March 30, 2011	Dr. T. Mazzulli
Pg.3 – Training - deleted "Mycology"	June 22, 2012	Dr. T. Mazzulli
Pg.4 – Revised Performance Assessments	June 22, 2012	Dr. T. Mazzulli
Pg.9 – Training – deleted "Mycology"	June 22, 2012	Dr. T. Mazzulli
Pg.10 – Revised Performance Assessments	June 22, 2012	Dr. T. Mazzulli
Pg.21 – Antimicrobial Sus. Testing – added CRES (7.0)	June 22, 2012	Dr. T. Mazzulli
Pg.21 – Serology changed AXSYM to Architect i2000	June 22, 2012	Dr. T. Mazzulli
Pg. 23 – Virology – Revised Observation	June 22, 2012	Dr. T. Mazzulli
Pg. 29 – General Micro Review – Added #6 – GBS	June 22, 2012	Dr. T. Mazzulli
Pg.33 – Appendix II(Media) - Revisions	June 22, 2012	Dr. T. Mazzulli
Annual Review	June 22, 2012	Dr. T. Mazzulli
Annual Review	August 19, 2013	Dr. T. Mazzulli
Pg. 3 & 9 – Remove Dr. Low & MacDonald	June 6, 2014	Dr. T. Mazzulli
Annual Review	June 6, 2014	Dr. T. Mazzulli
Update Headers Update Serology/Virology Tests & Competencies	June 12, 2014	Dr. T. Mazzulli
p.2 – Added perform AST to end of Welcome message	April 30, 2015	Dr. T. Mazzulli
p.5 – caveat: classical methods due to use of vitek MS - correlate grams read with culture results.	April 30, 2015	Dr. T. Mazzulli
p. 17 Residents: added to organism to know list: *Corynebacterium pseudodiptheriae* *Aerococcus urinae*, *Vibrio* spp.	April 30, 2015	Dr. T. Mazzulli
p. 21 – changed to architrect i4000	April 30, 2015	Dr. T. Mazzulli

p.30 - change ocbl to B.cepacia agar – selective medium	April 30, 2015	Dr. T. Mazzulli
p.37 – remove prepare a batch of medium	April 30, 2015	Dr. T. Mazzulli
p. 38/38 – add vibrio to stool, p.aer and B. cep to resp, GAS and pneumo to misc. pneumo to BC	April 30, 2015	Dr. T. Mazzulli
Removed RPR VD in virology sections	April 30, 2015	Dr. T. Mazzulli
Updated bench evaluations forms added	April 30, 2015	Dr. T. Mazzulli
Updates to: -Evaluation Form for Specimen Processing -Bench Evaluation for Student -Bench Evaluation Forms for Technologist	January 14, 2016	Dr. T. Mazzulli
Annual Review Updated MSH logo in header	April 30, 2016	Dr. T. Mazzulli
Update Simplexa to “Influenza” testing	September 7, 2016	Dr. T. Mazzulli
Updated - Trainee Evaluation Form For Virology/Serology Section (p.51)	November 2, 2016	Dr. T. Mazzulli
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