Microbiology / Mycology / Parasitology / Virology - Multi-institutional Rotation

The rotation in the Microbiology/Virology Laboratories is designed to provide the residents with a core program in microbiology and virology, with concentrations on general microbiology, antibiotic susceptibility testing, molecular diagnostic approaches in this field, mycology, parasitology, virology, and mycobacteriology. In addition to first-hand experience with methods and techniques, specimen processing, and a general understanding of workflow, there is opportunity for assessing how to introduce new technologies or substitution of new assays for older assays, as well as for appropriate utilization of the laboratory.

Coordinating Teaching Faculty Members
  Patricia Ferrieri, MD
  Sophie Arbefeville, MD

Training Site:
  University of Minnesota Medical Center, East Bank
  Hennepin County Medical Center

Duration of Rotation:
  2 months (1 month at UMMC, 1 month at HCMC)

Post Graduate Level of Residents Involved
  PGY 1-4

Goals and Objectives

General Aerobic and Anaerobic Microbiology

Following this course, a trainee who has satisfactorily completed the rotation will

1. be able to perform a good Gram stain and will be able to distinguish between
   a. gram-positive and gram-negative organisms, and
   b. rods, cocci, and yeasts
2. be able to read direct Gram stains on clinical specimens, recording the approximate numbers and types of organisms and cells seen
3. be able to streak plates and obtain isolated colonies
4. be able to distinguish colonies of streptococci, staphylococci, and gram-negative rods on sheep blood agar plates. Be able to recognize colonies which are not streptococci, staphylococci, or gram-negative rods, such as diphtheroids, *Bacillus, Neisseria*, yeast, etc. Perform Gram stains of colonies when unable to distinguish them macroscopically
5. be able to distinguish the various species of gram-negative bacilli on the commonly used differential media on the basis of their colonial morphology and fermentation reactions
6. be familiar with the media described in Laboratory Procedures in Diagnostic Microbiology. When questioned, will
   a. give at least the use for each medium
   b. explain why the medium is used, and
   c. how the medium works
7. when given a mixture of gram-positive cocci and gram-negative rods be able to separate them in one day by using proper media and techniques
8. when given a species of a clinically important bacterium be able to identify it using the reference material at hand (the laboratory manual or other recommended references)
9. when given any clinical specimen select the appropriate media necessary for the isolation of clinically important bacteria from that specimen and
   a. be able to recognize and identify the normal flora that might be found in any given clinical specimen and will be able to list the important pathogens to be found in any specimen
   b. be able to state the reason for using each type of medium used for a given clinical specimen
10. be able to understand and discuss modern blood culture and blood fungus detection systems and other automated equipment for microbial identification
11. be able to state the clinical significance for each organism when given actual laboratory reports of respiratory, urine and other miscellaneous culture
12. be able to relate the principles upon which antimicrobial dilution, disc diffusion, and E-strip susceptibility testing is based
13. be able to outline the methodology for two different antimicrobial assay procedures and describe the appropriate temporal collection of specimens and, in addition, be able to perform an assay by one of the procedures outlined for defining the antibiotic level in serum
14. be able to outline the methodology and value of the serum bactericidal assay and be able to perform this procedure
15. be able to describe the methods of appropriately obtaining culture specimens from all body areas and be able to outline proper methods of specimen transport to a laboratory facility
16. be able to describe the necessary components of an effective and efficient quality control program for a microbiology laboratory
17. be able to relate the necessary features of a laboratory safety program
18. understand the principles of cDNA probes and PCR-based assays for pathogens, e.g., \textit{N. gonorrhoeae} and \textit{Chlamydia trachomatis}
19. understand enzyme immunoassay principles for detection of microbial products and toxins, e.g., \textit{C. difficile} toxin A
20. understand rapid antigen detection systems, e.g., direct specimen tests CSF, urine and direct bacterial colony tests (examples: latex agglutination for Hib and group B strep, ELISA-based assays)
21. understand general principles of anaerobic bacteriology, including collection and processing of specimens
22. understand epidemiology of mycobacterial infections and general concepts of identification of major species of Mycobacteria, including molecular approaches
23. have an understanding of principles of general virology, indications for virus cultures, rapid antigen detection, and antibody assays for various viruses, including HIV.

Antimicrobial Testing

Following this course, a trainee who has satisfactorily completed the rotation will become familiar with the following methods performed in the antimicrobial testing section:

A. Susceptibility testing methods
   1. disc diffusion
   2. micro broth dilution
   3. macro broth dilution
   4. agar dilution
   5. semi-automated
   6. E-strip
B. Methods for testing fastidious organisms
   1. micro broth dilution
   2. agar dilution
C. Specialized test methods
   1. MBC
   2. serum bactericidal titer (SBT)
   3. synergy
D. Resistance screening methods
   1. staphylococci
   2. *Streptococcus pneumoniae*
   3. Beta lactamase
   4. *Neisseria gonorrhoeae*
   5. high-level aminoglycoside resistance
E. Antimicrobial resistance
   1. resistance mechanisms
   2. antimicrobial biopatterns (statistics)
F. Antifungal susceptibility testing
   1. yeasts
   2. filamentous fungi
G. Antimicrobial levels in body fluids by bioassay
H. Serological tests for group A *Streptococcus*
   1. ASO screen
   2. ASO titer
   3. Anti-DNase B titer

Mycobacteriology
I. Introduction
   A. Clinical manifestations of mycobacterial infection
   B. Pathogenesis of TB and leprosy
   C. Histology of mycobacterial infection
   D. Epidemiology of TB
   E. TB infection control
   F. Treatment

II. Isolation and Identification of Mycobacteria
   A. Sources of isolates
   B. Specimen collection and transportation
   C. Specimen processing
   D. Microscopy
   E. Culture examination and identification
      1. Runyon's grouping
      2. Radiometric techniques
      3. Differentiation of NTB from mycobacteria other than TB
         a. Niacin
         b. NAP test
         c. Nucleic acid probes
         d. Gas liquid chromatography
         e. Susceptibility testing
            1. solid media
            2. radiometric
         f. Molecular amplification techniques and direct identification of mycobacteria

Mycology

Following this course, a trainee who has satisfactorily completed the rotation will

A. understand what media and incubation temperatures best facilitate the isolation of fungi from various clinical specimens
B. become familiar with direct preparations and tests used in Mycology
   1. perform/interpret KOH prep
   2. review use of India Ink prep
   3. Gram stain as it pertains to Mycology
   4. modified acid-fast stain
   5. tease preps
   6. cryptococcal antigen
C. understand the difference between yeasts and molds
D. learn methods for identifying yeast
   1. colony morphology
   2. microscopic morphology
   3. cream of rice media
4. sugar fermentations
5. ascospore agar
6. caffeic acid disc
7. Vitek YBC and other sugar assimilation systems

E. differentiate various species of yeast (through observation of patient cultures and use of reference books) including:
   - Candida albicans, tropicalis, glabrata, krusei, lusitaniae
   - Cryptococcus neoformans, albidus, laurentii
   - Rhodotorula
   - Saccharomyces cerevisiae
   - Torulopsis
   - Trichosporon beigeli
   - Blastoschizomyces
   - Prototheca

F. learn methods for identifying molds
   0. colony morphology
   1. microscopic morphology
   2. slide culture technique
   3. subculture media used to enhance sporulation, exhibit resistance to cycloheximide, and demonstrate thermotolerance
   4. specialized media for dermatophyte ID such as urea, cornmeal agar with dextrose, trichophyton agars, hair test, rice grains
   5. specialize media for identification of aerobic actinomycetes such as casein, xanthine, tyrosine, lysozyme broth/basal broth, urea agar

G. differentiate various species of molds (through observation of patient cultures, reference slides/reference books) including:
   - DIMORPHIC MOLDS
     - Blastomyces dermatitidis
     - Histoplasma capsulatum
     - Coccidioides immitis
     - Sporothrix schenki
   - BRANCHING FILAMENTOUS ORGANISMS (aerobic Actinomycetes)
     - Nocardial/Streptomyces
   - DERMATOPHYTES
     - Microsporum
     - Trichophyton
     - Epidermophyton
   - ASPERGILLUS Sp.
     - A. fumigatus, flavus, niger, terreus
   - MISCELLANEOUS HYALINE MOLDS INCLUDING
     - Penicillium
     - Paecilomyces
     - Scopulariopsis
- Acremonium
- Fusarium
- Chrysosporium
- Pseudallescheria boydii

- **ZYGOMYCETES**
  - Rhizopus
  - Mucor
  - Cunninghamella
  - Absidia

- **DEMATIACEOUS MOLDS**
  - Alternaria
  - Curvularia
  - Drechslera
  - Cladosporium
  - Aureobasidium pullulans
  - Phoma/Chaetomium
  - Phialophora verrucosa
  - Exophiala jeanselmei
  - Fonsecaea pedrosoi

H. become familiar with the hazards involved in this area and use proper safety measures
I. become acquainted with helpful Mycology references

**Parasitology**

Following this course, a trainee who has satisfactorily completed the rotation will

A. become familiar with appropriate techniques for identification of intestinal parasites (primarily stool specimens)
   1. criteria for evaluating satisfactory specimens
   2. use of preservatives
   3. methodology
      - concentration techniques
      - permanent smears
      - swube collection kit for pinworm detection
      - Entero Capsule string test
   4. differentiation of intestinal parasites (larvae, cysts, trophozoites) through studies of positive samples and reference books including:
      - **PROTOZOA/FLAGELLATES**
        - *Entamoeba histolytica and coli*
        - Endolimax nana
        - Iodamoeba buetschlii
        - Dientamoeba fragilis
        - Chilomastix mesnili
- Giardia lamblia
- Trichomonas hominis

**TREMATOIDES**
- Fasciolopsis buski/Heterophyes/Metagonimus

**CESTODES**
- Taenia
- Hymenolepis nana and diminuta
- Diphyllobothrium latum
- Schistosoma haematobium, mansoni, and japonicum

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**NEMATODES**
- Ascaris lumbrichoides
- Trichuris trichiura
- Enterobius vermicularis
- Hookworm

**COCCIDIA**
- Cryptosporidium and Cyclospora cayetanensis
- Isospora and Sarcocystis
- Blastocystis hominis

B. become familiar with appropriate techniques for identification of blood and tissue parasites

1. requirements for blood smears, Knott's concentration, skin snips for microfilariae, Leishmania culture, Acanthamoeba/Naegleria - FA smear and culture and smear for Toxoplasma gondii

2. methodology
- Giemsa stain
- Knott's concentrate
- skin snips for microfilariae
- Amoeba culture/FA smear
- CSF for T. gondii

3. differentiation of blood and tissue parasites through studies of positive samples and reference books including:

**SPOROZOA**
- Plasmodium vivax, ovale, malariae, falciparum
- Babesia spp.
- Pneumocystis carinii

**FLAGELLATES**
- Leishmania braziliensis, donovani
- Trypanosoma cruzi, gambiense/rhodesiense
- Trichomonas vaginalis

**AMOEBAE**
- Naegleria/Acanthamoeba

**COCCIDIA**
- Toxoplasma gondii

**FILARIAL WORMS**
- *Wuchereria bancrofti*
- *Brugia malayi*
- *Loa loa*
- *Onchocerca volvulus*
- *Mansonella perstans*

**TREMATODES**
- liver/lung - *Clonorchis sinensis*

**C.** become familiar with techniques for identification of
  - ticks
  - mites
  - lice

**D.** become acquainted with the life cycles of the more common parasites of medical importance through study of the Atlas of Medical Helminthology and Protozoology

**E.** become acquainted with helpful parasitology references

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**Virology**

Residents taking the Virology Laboratory rotation will become familiar with cell culture techniques, specimen processing, viral isolation and identification of viruses in cell culture, techniques to detect viral antigens and antibodies, and molecular diagnostic techniques, especially PCR.

At the end of the Virology rotation, the resident should have knowledge in the following areas:

- **CELL CULTURE**
  Understand sterile technique; understand the source, type and usage for various cell lines in a clinical virology laboratory; understand splitting procedures for cell lines.

- **PROCESSING AND RECEIVING**
  Understand specimen collection, transportation of primary specimen types, test requesting and processing procedures for primary specimen types.

- **ISOLATION**
  Be familiar with the common human viruses and know the cell lines in which each produces cytopathic effect; identify specific viruses by cytopathic effect (presumptive ID); distinguish between HSV and VZV by use of monoclonal antibody typing - direct immunofluorescence and ELISA; understand the hemadsorption test for respiratory viruses and complete identification by monoclonal antibody typing - indirect immunofluorescence.

- **ANTIGEN/ANTIBODY TESTING**
  Understand several of the most common immunological procedures routinely used in a clinical virology laboratory (i.e., ELISA, latex agglutination, indirect immunofluorescence, and culture enhanced immunofluorescence); know principles of all assays performed, the
advantages and limitations of each procedure and the interpretation and the clinical significance of test results.

• **MOLECULAR DIAGNOSTICS**
  Observe molecular methods for the detection of viruses and virus mutations; understand the principle of methods performed and the interpretation and the clinical significance of test results.

**Guidelines for Patient Care and Specimen Handling**

Specimen handling in the Microbiology and Virology Laboratories is the direct responsibility of the laboratory technologists and medical laboratory technicians. The residents under supervision of these individuals have an opportunity to perform specimen processing of a limited number of specimens at the beginning of the rotation at the University Hospital Laboratory. Supervision of the residents in their decision-making in the laboratory is under the supervision of the medical staff of the laboratory.

**Overview of Daily and Responsibilities**

The residents rotate in different sections of the laboratory and start with an introduction and audiovisual tutorial, demonstration of media and specimen processing. They carry out various tests under supervision of technical leads in the lab or the laboratory supervisor. The residents do not generate interpretative reports in Microbiology and Virology, but are familiarized with the interpretations generated by the medical director. Daily laboratory rounds are carried out by the Medical Director, Dr. Ferrieri, and these rounds are attended by Infectious Diseases faculty, fellows in training, residents and medical students. "Significant" laboratory results are presented, and there are frequently demonstrations. The residents are given the opportunity to present exhibits on lab rounds, which would include showing growth on specialized media, microscopic features, and biochemical reactions of particular organisms they are seeing. The residents are involved in evaluating patients' clinical records on the patient care units, in order for us to assess the needs for some of the requested tests. A typical rotation for the residents is attached to this description.

**Resident Opportunities to Function as a Consultant to Other Physicians**

Frequently, the residents obtain relevant patient information from the charts or from a requesting physician. The medical directors refer some inquiries to the residents, commensurate with their level of training, and then they pursue investigation, which may also include literature searches with very unusual or difficult patient problems. The interactions may include any physician on the attending staff at the hospitals and infectious diseases specialists in the departments of internal medicine, pediatrics, and surgery. We interact with other laboratories in our complex, including the Molecular Diagnostic Laboratory, Special Hematology, as well as the above-cited Surgical Pathology Laboratory.

**Description of On-call Duties**
At the present time, the resident is "on-call" in Microbiology during regular working hours. The resident interacts with the faculty member supervising the laboratories. There is very close interaction at all times between the faculty and the residents during daytime, and every opportunity is used to teach. For example, if there are emergency blood smears for malaria (which are processed in the Microbiology Laboratory), the residents and faculty member would collaborate on these and discuss the findings.

**Structured Formal Education in Management of the Microbiology/Virology Laboratories:**

The resident is requested to attend the weekly Microbiology staff meetings. The residents now participate in inspecting the laboratories for our internal laboratory accreditation for CAP. In addition, financial decision-making choice of laboratory instruments and assays and staffing issues are discussed by the Directors with the residents.

**Required Conferences/Seminars**

- Laboratory Medicine and Pathology Grand Rounds
- Infectious Diseases Conference (Departments of Pediatrics and Medicine). This conference permits the discussion of unusual infectious diseases problems and the role of the laboratory.
- The Clinical Pathology Conference

**Optional Conferences**

- Residents Conference
- Morbidity, Mortality, and Management Conference, Department of Pediatrics. Frequently, there are infectious diseases patients presented and an opportunity for the resident to present what the Microbiology Laboratory has done on behalf of that patient.
- Laboratory Medicine and Pathology Research Forum

**Scholarly Activities/Research Activities During the Rotation**

When the rotation was longer than two months, the residents were expected to carry out a few weeks of research; for example, doing molecular characterization of bacteria involved in nosocomial infections or hospital epidemics. The two-month rotation does not permit that activity. However, the residents are expected to carry out literature searches on unusual cases or microbiologic issues. In addition, the Medical Director provides many original articles relevant to each section of the laboratory rotation, and these are discussed then with the residents on rotation.

There has not been a fellow in Microbiology for several years, but such fellows have had more graded responsibility in the laboratory and devoted 50% or more of their time to research activities.

**Basis, Method and Criteria for Resident Evaluation**
Residents are evaluated on the assessment of their performance of their daily activities at the bench by the Medical Directors directly observing their performance, as well as soliciting information from the laboratory supervisors and laboratory technical leads. Residents are also evaluated on the presentation of their clinical pathology conferences, focused on microbiology or virology. In addition, they are evaluated on the performance of their proficiency (unknown) specimens, and on their presentations at daily laboratory rounds. The residents are given feedback on their performance midway during the rotation, and then again a person-to-person discussion of the written evaluation at the end of the rotation.

**Educational Resources**

The Microbiology Laboratories have teaching files of slides of interesting microorganisms and a tutorial that is used at the beginning of the rotation as well as throughout the rotation. Other key references include all of the procedure books that are at each of the specialty areas of the laboratory. In addition, there are copies of the Manual of Clinical Microbiology by Murray, P.R., et al, of which each resident has a copy, with the latest edition available in the Quality Control/Research and Development section of the laboratory. The other references include monthly copies of the Journal of Clinical Microbiology and Antimicrobial Agents and Chemotherapy, available in the laboratory. Original articles are provided as well by the Medical Directors, and the residents are given a packet of articles, both at the Minnesota Department of Health, where they carry out their mycobacterial experience, and at the Veterans Administration Hospital. There are too many articles to list them separately, but these are constantly updated.

**Computer Information Systems Available**

Patient data is currently available on two systems: the Abaton system for University of Minnesota Medical Center laboratories; the Lab Care system for the other entities in the Fairview Health System.

Literature searches can be performed on-line via the University of Minnesota Bio-Medical Library Databases and Information Sources, and this is utilized frequently by all the residents.