General Medical/Clinical Microbiology

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Objectives

1. To provide an overview of a typical clinical microbiology laboratory
2. To review how to operate a clinical microbiology laboratory using a Quality Management System
Typical Clinical Microbiology Laboratory
Who we are

• Shared microbiology service between UHN and MSH
• Serve nine Ontario hospitals (~5000 beds) and five non-hospital clients
• Approximately 35,000 specimens processed per month
Who we are

• Site:
  – 14th Floor Mount Sinai Hospital
• Website:
  – www.microbiology.mtsinai.on.ca
What we do

• Clinical Service
  – Routine Diagnostics
  – Infection Control
  – Reference Testing

• Research

• Education
Clinical Service
A. Routine Diagnostics

- Bacteriology
- Mycology
- Virology
- Serology
- Parasitology
- Mycobacteriology
Process

- Specimen collection
- Specimen transport
- Specimen receipt
- Specimen processing
- Testing
- Interpretation
- Reporting
Process

- Specimen collection
- Specimen transport
- Specimen receipt
- Specimen processing
- Testing
- Interpretation
- Reporting

Pre-analytical

Analytical

Post-analytical
Specimen Collection → Receipt

- Transport media
  - Stool cultures (Cary-Blair)
  - Viral/Mycoplasma/Chlamydia (transport media)
- Transport temperature
  - Sterile Site Specimens (room temp/incubate)
  - Nonsterile Site Specimens (room temp/4°C)
  - Virology/Serology/NAAT (4°C)
Tests Overview

- Direct detection
  - Stained smears, EM, LA, DFA, EIA
- NAAT
- Culture
  - Media, Cell lines
- Serology
  - EIA, IFA, Immunoblots
- Susceptibility Testing
Direct Detection
The Gram Stain

Steps in Staining | State of Bacteria
--- | ---
Step 1: Crystal violet (primary stain) | Cells stain purple.
Step 2: Iodine (mordant) | Cells remain purple.
Step 3: Alcohol (decolorizer) | Gram-positive cells remain purple; Gram-negative cells become colorless.
Step 4: Safranin (counterstain) | Gram-positive cells remain purple; Gram-negative cells appear red.
Calcofluor White
Electron Microscopy

Norovirus by EM
Latex Agglutination

- Cryptococcal Antigen (CRAG)
DFA/IFA
Membrane EIAs
Nucleic Acid Target or Signal Amplification Testing
NAAT - Target

- PCR most common
- Real-time instruments
PCR
NAAT - Signal

bDNA Hybridization Assay

AP label probe → bDNA → label extender

preamplifier → capture probe

capture extender
Blood Agar
Media

• Enrichment media
  – Special nutrients required for growth of particular pathogens
  – e.g. BCYE – L-cysteine for *Legionella*

• Supportive media
  – Support growth of most nonfastidious organisms
  – e.g. sheep blood agar
• Selective media
  – One or more agents that are inhibitory
  – e.g. MacConkey with crystal violet

• Differential media
  – Factor(s) that allow colonies of one type to be distinguished from others
  – e.g. MacConkey lactose with pH indicator
MAC CONKEY’S AGAR

Lactose Negative

MAC CONKEY’S AGAR

Lactose Positive
THERMONUCLEASE TEST
Microbiology

CATALASE TEST

Micro Slide

Inoculating Loop

Hydrogen Peroxide

CATALASE TEST

Negative

Positive

Mount Sinai Hospital

University Health Network

Microbiology
UREA AGAR

Uninoculated or Negative  Negative  Positive
Automated Identification
Culture – Cell Lines
Cell Lines

• Primary cell lines (primary)
  – Passes only once or twice since harvesting
    • E.g. PMK (primary monkey kidney)

• Low passage cell lines (diploid)
  – Remains virus sensitive through 20-50 passages
    • E.g. HDF (human diploid fibroblast cells)

• Continuous cell lines (heteroploid)
  – Can be passes and remain sensitive to virus infections indefinitely
    • E.g. Hep-2 (Human epidermoid carcinoma cells)
Tube Culture
Vero Cells – SARS-CoV
Shell vials

• Rapid modification of conventional cell culture
• Mixed cell lines (e.g. R-mix)
Shell Vial
Serologic Tests

• Enzyme Immunoassay (EIA)
• Immunofluorescent Assays (IFA)
• Complement Fixation (CF)
• Hemagglutination Inhibition Assays (HAI)
• Western Blot
• Neutralization Tests
EIA

**Step 1**
Specific antigen is attached to a solid-phase surface

**Step 2**
Test specimen is added, which may or may not contain the antibody

**Step 3**
An enzyme-labeled antibody specific to the test antibody is added (conjugate)

**Step 4**
Chromogenic substrate is added, which in the presence of the enzyme, changes color.

- e
- color
Step 1
Microbial antigen is dried on a glass slide and treated with a chemical fixative

Step 2
Dilutions of patient serum are incubated with the antigen on the slide, and then rinsed

Step 3
A fluorescein-labeled antibody (conjugate) is added
**Complement Fixation Test**

1. **Serum with antibodies**
   - Serum with antibodies

2. **Antigen binds with antibodies**
   - Antigen binds with antibodies

3. **Complement binds with Ag/Ab complex**
   - Complement binds with Ag/Ab complex

4. **Unbound Antigen**
   - Unbound Antigen

5. **Unbound complement**
   - Unbound complement

6. **Hemolysin**
   - Sensitized red blood cells serve as an indicator

7. **Hemolysin**
   - Sensitized RBCs serve as an indicator

8. **RBCs settle into a pellet**
   - RBCs settle into a pellet

9. **no lysis**
   - no lysis

10. **Reactives**
    - Reactive

11. **Nonreactive**
    - Nonreactive
W. Blot

SDS-polyacrylamide gel

Incubate with Ab$_1$ (Y) and then wash excess Ab$_1$

Porous membrane sheet

Incubate with enzyme-linked Ab$_2$ (Y) and then wash excess Ab$_2$ and then activate color reaction

DEVELOPMENT

Add substrate

(c)
Hemagglutination Inhibition (HAI)

Red blood cells + Virus (Orthomyxovirus, Paramyxovirus) → Hemagglutination

Red blood cells + Anti-viral antibodies from serum + Virus → Viruses neutralized and hemagglutination inhibited
Neutralization Tests

• Neutralization of a virus is defined as the loss of infectivity through reaction of the virus with specific antibody.

• Virus and serum are mixed under appropriate condition and then inoculated into cell culture, eggs or animals.
Titres

• Dilute specimen to determine how concentrated antibody titre is
• Expressed as 1:8, 1:16, 1:32, 1:64 etc.
• Positive
  – +IgM test
  – >set cutoff (specific to each agent)
  – >=4 fold rise between acute and convalescent specimens
Definitions

• MIC (Minimum Inhibitory Concentration)
• MBC (Minimum Bactericidal Concentration)
• Tolerance
  – MBC/MIC ≥ 32
  – Clinical relevance not established
  – Mostly related to beta-lactam drugs
Definitions

**Combination Testing**

- MCBT (multiple combination bactericidal testing)
- Synergy Testing (synergy, indifference, antagonism)
  - Checkerboard Titration
  - Time Kill Curves
MIC

• Interpretive Standards
  – NCCLS (changed to CLSI in Jan 2005)
  – Susceptible (S), Intermediate (I), Resistant (R)

• MIC breakpoints based on studies assessing:
  – PK/PD based on systemic antibiotic delivery
  – Clinical efficacy studies
    » Clinical resistance vs. biologic resistance
Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement
Susceptibility Testing

• Bacterial
  – Agar dilution, broth macrodilution, broth microdilution
  – Automated broth microdilution
  – Disk diffusion
  – E test
  – Screening Plates
  – Molecular (latex agglutination, NAAT)

• Fungal
  – Macrodilution, microdilution

• Mycobacteriology
  – Macrodilution
Susceptibility Testing

- **Bacterial**
  - Agar dilution, broth macrodilution, broth microdilution
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- **Fungal**
  - Macrodilution, microdilution

- **Mycobacteriology**
  - Macrodilution
Agar Dilution

Penicillin 1 mg/L
Penicillin 2 mg/L
Penicillin 4 mg/L
**Agar Dilution**

- **Penicillin 1 mg/L**
- **Penicillin 2 mg/L**
- **Penicillin 4 mg/L**
Agar Dilution

Penicillin
1 mg/L

Penicillin
2 mg/L

Penicillin
4 mg/L

Pen MIC = 4 mg/L
Broth Macrodilution Testing

Penicillin (mg/L)

1 2 4 8 16 32 64 128 256 512
Broth Macrodilution Testing

Penicillin (mg/L)
Broth Microdilution Testing
# Broth Microdilution Testing

<table>
<thead>
<tr>
<th>ISOLATE #</th>
<th>1</th>
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<tbody>
<tr>
<td>A MHB CONT.</td>
<td>Vanco</td>
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<td>Dapto 0.12</td>
<td>Dapto 0.25</td>
<td>Dapto 0.5</td>
<td>Dapto 1</td>
<td>Dapto 2</td>
<td>BMS 756</td>
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<td>Genta 0.5</td>
<td>Gemi 4</td>
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<td>H Cefoxitin 0.5</td>
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<td>Cefoxitin 32</td>
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<td>Cefoxitin 2</td>
<td>NG</td>
<td>CONT.</td>
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**Note:** Tetra = Tetra, Genta = Gentamicin, NG = Not growth.
## Broth Microdilution Testing

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Broth Microdilution Testing
**Broth Microdilution Testing**

Mupirocin (mg/L)

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Mupirocin MIC = 128 mg/L
Automated Broth Microdilution
Disk Diffusion Testing
Disk Diffusion Testing
E test
E test
Screening Plates
(DIRECT FROM ISOLATE)
Screening Plates
(DIRECT FROM SPECIMEN)
Latex Agglutination
NAAT
Limitations of Susceptibility Tests

• Interpretative guidelines
• Cost (NAAT)
• New resistance determinants
  – MRSA
  – VRE
  – ESBL, KPC
  – VRSA, VISA
• Turn-around-times
B. Infection Control

• Epidemiology of Infectious Disease
  – Reportable diseases
  – Tracking rates of select pathogens
    • e.g. C. difficile, AROs

• Epidemiology of Antimicrobial Resistance
  – Annual antibiogram
  – Antibiotic Stewardship Program
    • Formulary, guidelines

• Outbreak investigation
  – Epidemiology typing, treatment options
PFGE
eg.
C. Reference Work

- Susceptibility testing
- Identifying resistance determinants
- Epidemiologic typing (esp. AROs)
- NAAT
Research

- Collaborative studies
- Surveillance studies
- Mechanisms of resistance
Education
Education

• Undergraduate lectures
• Postgraduate lectures
• Plate rounds
  – ID team with pharmacists
• Medical/Clinical Microbiology Residency
• Internships
  – Students, pharmaceutical industry reps
Quality Management System
Quality

• Right thing
  – Test algorithm
• Right way
  – Quality control
• Right time
  – Benchmarks
Definitions

• Quality Management System
  – System that encompasses all processes relating to assuring quality service

• Quality Assurance
  – Activities to ensure quality outcome of lab

• Quality Control
  – Techniques to ensure diagnostic test accuracy
Quality Management Systems

• Models
  – QMP-LS
  – CLSI (NCCLS) GP-26
Quality Management System

- Twelve Quality System Essentials (QSEs):
  1. Organization
  2. Personnel
  3. Equipment
  4. Purchasing/Inventory
  5. Process Control
  6. Documents/Records
Quality Management System

• Twelve QSEs (cont’d):
  7. Information Management
  8. Occurrence Management
  9. Internal Assessment
  10. Process Improvement
  11. Service/Satisfaction
  12. Facilities and Safety
Quality Management System

- Twelve QSEs (cont’d):
  7. Information Management
  8. Occurrence Management
  9. Internal Assessment
  10. Process Improvement
  11. Service/Satisfaction
  12. Facilities and Safety
Elements of a QMS

• Quality Policy
• Quality Manager
• Quality Manual
Quality Manual

• Quality Manual = Management’s
  Procedure Manual = Department Manual
  – For each QSE, document:
    • Policy (What to do) – one for each QSE
    • Process (How it happens) – flowchart
    • Procedure (How to do it) – classic SOP
  – Taking into account pre-analytical, analytical, post-analytical workflow
1. Organization

- Organizational Chart
- Define level of authority, responsibility
- Allocation of resources
2. Personnel

- Job descriptions, qualifications
- Orientation, training
- Competency assessment
- Continuing education
- Performance appraisals
- Collaboration with human resources
3. Equipment

• Appropriate use
• Documentation for each piece of equipment
  – Select
  – Acquire
  – Install
  – Calibrate
  – Maintenance
  – Service
  – Verification before use
4. Purchasing/Inventory

• Identify product/service
  – Desired qualities
  – Vendor evaluation and selection

• Inventory of material supplies

• Material tracking
  – Expired inventory
5. Process Control

• **Validation** of processes
  – Internal quality control
  – External quality assessment (EQA)
  – **Corrections of problems**
6. Documents/Records

• Create, review, approve, revise regularly
  – Annual review of quality manual by management
• Distribution, storage, and retrieval of records
• Retention policies
7. Information Management

- Privacy/confidentiality issues regarding patient information
- Information access/security
- Data integrity
  - e.g. check final reports
- Billing practices
8. Occurrence Management

- Standardized reporting mechanism (incident reports)
- Receive, review, code, analyze
- Correction of problems
* 9. Internal Assessment *

= Quality Assurance

- Quality indicators
  - compare against benchmark where available
    - Lab own experience, other labs, guidelines, trend
- Internal audits
- Correction of problems
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QA Program encompasses following all QSEs through Quality Indicators
Quality Indicators

• Each lab to define their own indicators
• Examples are bolded text in each QSE
• Important to cover
  – each QSE
  – pre-analytical, analytical, and post-analytical
  – problem areas
• Follow critical indicators – given limited resources, can’t follow all always
* 10. Process Improvement *
= Continuous Quality Improvement

PLAN
ACT
DO
CHECK
* 10. Process Improvement *

- Identify opportunities for improvement
- Correction of problems
11. Service/Satisfaction

• Internal satisfaction
  – Physicians, staff
  – surveys

• External satisfaction
  – Physicians, patients, public health
  – surveys
12. Facilities and Safety

• Facilities
  – Lab design, access
  – Air handling
  – Biological safety cabinets
  – Pest control
12. Facilities and Safety

• Safety
  – Safety training
  – Chemical safety
    • WHMIS (workplace hazardous material information system)
    • Spill kits, eyewash stations, showers
  – Biosafety
    • Routine practices
    • Immunizations, TB skin test
  – Transportation of Dangerous Goods

• Audits
What Haven’t We Covered?

• Financial management
  – budgets
• Workload units
  – Capturing lab efficiency
• LEANing the lab
  – Maximizing lab efficiency
Objectives

1. To provide an overview of a typical clinical microbiology laboratory
2. To review how to operate a clinical microbiology laboratory using a Quality Management System
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