An Introduction to Clinical Microbiology

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Microbiologist & Infectious Disease Consultant
Objectives

1. To provide an introduction to a typical microbiology laboratory
2. To address specific microbiology laboratory test issues as they apply to public health
Who we are

- Shared microbiology service between TML (UHN & MDS) 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
What we do

• Clinical Service
  – Routine Diagnostics
  – Infection Control
  – Reference Testing
• Research
• Education
Clinical Service
A. Routine Diagnostics

- Bacteriology
- Mycology
- Virology
- Serology
- Parasitology
- Mycobacteriology
Terminology

• Bacteriology
  – Prokaryotic, single cell organisms
  – Divided into aerobic and anaerobic
  – Divided into gram-positive and gram-negative

• Mycology
  – Eukaryotic, multi-cellular organisms
  – Divided into yeast and filamentous fungi
Terminology

- Virology
  - Acellular infectious particles consisting of core of RNA or DNA surrounded by a protein coat unable to replicate without a host cell

- Serology
  - Detection of antibodies against infectious agents
Terminology

• Parasitology
  – Eukaryotic organisms
  – Divided into protozoa (e.g. *Plasmodium* spp., *Giardia lamblia*) and nematodes (i.e. worms)

• Mycobacteriology
  – Prokaryotic, single cell organisms
  – Acid-fast bacteria
Process

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

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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

<table>
<thead>
<tr>
<th>Steps in Staining</th>
<th>State of Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1: Crystal violet</td>
<td>Cells stain purple.</td>
</tr>
<tr>
<td>(primary stain)</td>
<td></td>
</tr>
<tr>
<td>Step 2: Iodine</td>
<td>Cells remain purple.</td>
</tr>
<tr>
<td>(mordant)</td>
<td></td>
</tr>
<tr>
<td>Step 3: Alcohol</td>
<td>Gram-positive cells remain purple; Gram-negative cells</td>
</tr>
<tr>
<td>(decolorizer)</td>
<td>become colorless.</td>
</tr>
<tr>
<td>Step 4: Safranin</td>
<td>Gram-positive cells remain purple; Gram-negative cells</td>
</tr>
<tr>
<td>(counterstain)</td>
<td>appear red.</td>
</tr>
</tbody>
</table>

The Gram stain is a procedure used to differentiate bacteria into two major groups: Gram-positive and Gram-negative. The process involves staining the bacteria with various dyes and decolorizers. The result is used to identify and classify bacteria based on their cell wall structure.
**Bacterial Classification**

**Gram Stain**
- **Gram Positive**
  - Cocci
    - Anaerobic: e.g. peptostreptococcus
    - Aerobic: e.g. Staphylococcus
  - Bacilli
    - Anaerobic: e.g. Clostridia, Actinomyces
    - Aerobic: e.g. Listeria, Corynebacterium
- **Gram Negative**
  - Cocci: e.g. Haemophilus, Moraxella, Neisseria
  - Bacilli
    - Aerobic: e.g. Lactose fermenter: e.g. Klebsiella, E. coli, Enterobacter
    - Oxidase Positive: e.g. Pseudomonas
    - Oxidase Negative: e.g. Serratia, Proteus, Acinetobacter, Sternotrophomonas
  - Non-lactose fermenter

**Bacterial Classification**

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Calcofluor White
Electron Microscopy

Norovirus by EM
Latex Agglutination

- Cryptococcal Antigen (CRAG)
Membrane EIAs

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NAAT

- PCR most common
- Real-time instruments
Culture – Media
Blood Agar
THERMONUCLEASE TEST
COAGULASE TEST

Rabbit Plasma

Nutrient Broth

Pipette

COAGULASE TEST

Positive

Fibrin Clot

COAGULASE TEST

Negative

No Fibrin Clot

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CATALASE TEST

Inoculating Loop

Micro Slide

Hydrogen Peroxide

Positive

Negative
Automated Identification
Culture – Cell Lines
Tube Culture
Vero Cells – SARS-CoV
Shell Vial
Serology
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.
IFA

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

- Serum with antibodies
- Serum without antibodies
- Antigen binds with antibodies
- Unbound Antigen
- Complement binds with Ag/Ab complex
- Unbound complement
- Hemolysin
- Sensitized red blood cells serve as an indicator
- Hemolysin
- Sensitized RBCs serve as an indicator
- RBCs settle into a pellet
- no lysis
- Reactive

- RBCs lysed by unbound complement
- lysis
- Nonreactive
Incubate with Ab1 (Y) and then wash excess Ab1

Incubate with enzyme-linked Ab2 (Y) and then wash excess Ab2, and then activate color reaction

(c) DEVELOPMENT

Add substrate
HAI

Red blood cells + Virus
Orthomyxovirus, Paramyxovirus → Hemagglutination

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

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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
January 2005

Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth 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
  – Automated broth microdilution
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  – Molecular (latex agglutination, NAAT)

• 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

Pen MIC = 4 mg/L

Penicillin 1 mg/L
Penicillin 2 mg/L
Penicillin 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)

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

<table>
<thead>
<tr>
<th>ISOLATE #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
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<tbody>
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<td>A</td>
<td>MHB CONT</td>
<td>Vanco 2</td>
<td>Vanco 4</td>
<td>Vanco 8</td>
<td>Oxacillin 0.25</td>
<td>Oxacillin 0.5</td>
<td>Oxacillin 1</td>
<td>Oxacillin 2</td>
<td>Oxacillin 4</td>
<td>Oxacillin 8</td>
<td>Oxacillin 16</td>
<td>Oxacillin 32</td>
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<tr>
<td>B</td>
<td>MHB 2%NaCl Ery 0.5</td>
<td>Ery 1</td>
<td>Ery 2</td>
<td>Ery 4</td>
<td>Ery 8</td>
<td>Clinda 2</td>
<td>Clinda 4</td>
<td>Clind/ery 4/0.25</td>
<td>Tetra 4</td>
<td>Tetra 8</td>
<td>Tetra 16</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Doxy 4</td>
<td>Doxy 8</td>
<td>Doxy 16</td>
<td>Mup 1</td>
<td>Mup 2</td>
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<td>Mup 8</td>
<td>Mup 16</td>
<td>Mup 32</td>
<td>Mup 64</td>
<td>Mup 128</td>
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<td>D</td>
<td>Mino 4</td>
<td>Mino 8</td>
<td>Mino 16</td>
<td>Rif 1</td>
<td>Rif 2</td>
<td>Rif 4</td>
<td>Rif 8</td>
<td>TMP/SMX 2</td>
<td>TMP/SMX 4</td>
<td>TMP/SMX 8</td>
<td>Genta 4</td>
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<tr>
<td>E</td>
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<td>Cipro 4</td>
<td>Cipro 8</td>
<td>Fucidic 0.5</td>
<td>Fucidic 1</td>
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<td>G</td>
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<td>Dapto 0.12</td>
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<td>Dapto 0.5</td>
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<td>Dapto 2</td>
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<td>Genta 500</td>
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<td>BMS 756 4</td>
<td>BMS 756 2</td>
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### Broth Microdilution Testing

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<th>ISOLATE #</th>
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<th>D</th>
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<th>F</th>
<th>G</th>
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<td>Ery</td>
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<td>Ery</td>
<td>Clinda</td>
<td>Clinda</td>
<td>Clind/ery</td>
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<tr>
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<td>Mup</td>
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<td>Rif</td>
<td>Rif</td>
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<td>15</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>16</td>
<td>32</td>
<td>64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Broth Microdilution Testing
Broth Microdilution Testing

Mupirocin (mg/L)
1  2  4  8  16  32  64  128  256

Mupirocin MIC = 128 mg/L

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Automated Broth Microdilution
Disk Diffusion Testing
Disk Diffusion Testing
Disk Diffusion Testing
E test
E test
Screening Plates
(DIRECT FROM ISOLATE)
Screening Plates
(DIRECT FROM SPECIMEN)
Latex Agglutination
NAAT

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Limitations of Susceptibility Tests

- Interpretative guidelines
- Cost (NAAT)
- New resistance determinants
  - MRSA
  - VRE
  - ESBL
  - VRSA, VISA
- Turn-around-times
Turn-around-times (TATs)
TATs

• Direct detection
  – STAT or within 24 hours

• Culture
  – Varies

• Serology
  – Usually within 24 hours (excluding weekends)

• Susceptibility Testing
  – Varies (typically requires positive culture)
Culture TATs

- **Bacteriology**
  - Routine: 24-48 hours
  - BC: 5 days (21 days if endocarditis)
- **Mycology**
  - 2-6 weeks
- **Virology**
  - 1-2 weeks
- **Mycobacteriology**
  - 6 weeks
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 Subcommittee
    - Formulary, guidelines

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

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

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Research

- Collaborative studies
- Surveillance studies
- Mechanisms of resistance
- Animal model
  - PK/PD
  - MPC
Education
Education

• Undergraduate lectures
• Postgraduate lectures
• Plate rounds
  – ID team with pharmacists
• Internships
  – Students
  – Pharmaceutical industry representatives
Resources

The Testing Guideline provides an overview of the laboratory testing available through the Ontario Public Health Laboratories (OPHL).

The guideline is listed in alphabetical order by disease, syndrome and/or causal agent.

Information includes:

- Laboratory tests available
- Laboratory test code
- Appropriate specimens
- Collection kit numbers
- Section / location where test is performed
- Turn-around-times for negative and for positive or confirmatory results
- Additional information as required

Please note that the turn-around-times are based on Monday to Friday business working days.

For further assistance, please use the OPHL HELPLINE at 1-800-540-7221 and your call will be appropriately directed.

Document download

This Publication requires knowledgeable interpretation and is intended primarily for professional health care practitioners, health care organizations and public health units.

Specimen Collection Guide - August 2006
Testing Guidelines
74 pages | 306 Kb | PDF format
Specimen Collection Guide
- Testing Guidelines

Public Health Laboratories
Ministry of Health and Long-Term Care

August, 2006

http://www.health.gov.on.ca/english/providers/pub/labs/specimen.html
<table>
<thead>
<tr>
<th>Disease / Syndrome / Causal Agent / Test</th>
<th>Test Code</th>
<th>Specimens</th>
<th>Collection Kit</th>
<th>Test Available</th>
<th>Section</th>
<th>TAT Negative Results Reported</th>
<th>TAT Positive or Confirmatory Results Reported</th>
<th>Notes *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascariasis</td>
<td>P04</td>
<td>Faeces in SAF preservative</td>
<td>Para</td>
<td>Microscopy</td>
<td>Parasitology</td>
<td>3 days</td>
<td>Within 3 days</td>
<td></td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>P02</td>
<td>Adult worm (passed in vomit or faeces)</td>
<td>Sterile container with normal saline</td>
<td>Microscopy</td>
<td>Parasitology</td>
<td>2 days</td>
<td>Within 2 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S06</td>
<td>Blood, clotted or serum</td>
<td>BL-S</td>
<td>EIA</td>
<td>Immunodiagnostics</td>
<td>7 days</td>
<td>Within 7 days</td>
<td></td>
</tr>
<tr>
<td>Aspergillosis (Invasive)</td>
<td>S05</td>
<td>Blood, clotted or serum</td>
<td>BL-S</td>
<td>Immuno-diffusion</td>
<td>Immunodiagnostics</td>
<td>7 days</td>
<td>Within 7 days</td>
<td></td>
</tr>
<tr>
<td>Astrovirus Infections</td>
<td>V06</td>
<td>Faeces</td>
<td>Sterile container</td>
<td>Electron microscopy</td>
<td>Virus Detection</td>
<td>3 days</td>
<td>Within 3 days</td>
<td>If transportation is delayed, refrigerate at 4°C</td>
</tr>
<tr>
<td>Astrovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avian Flu</td>
<td>V23</td>
<td>Nasopharyngeal aspirate/swab</td>
<td>Virus-Resp</td>
<td>Virus isolation</td>
<td>Virus Detection</td>
<td>Preliminary 7 days</td>
<td>Within 10 days</td>
<td>Contact the local Health Unit and Head, Virus Detection at 416-235-5730 prior to submitting specimen.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 day</td>
<td>1 day</td>
<td>Submit travel history and clinical information.</td>
</tr>
</tbody>
</table>
OPHL

• “For further assistance, please use the OPHL HELPLINE at 1-800-640-7221 and your call will be appropriately directed.”
Susan M. Poutanen, MD, MPH, FRCPC
spoutanen@mtsini.ai.on.ca
(416) 586-3139