The Role of Microscopy in the Diagnosis of Infectious Disease

MHD I 2018-19

Objectives:
1. Describe how to prepare a specimen and perform Gram stain.
2. Explain the clinical utility of a Gram stain.
3. Summarize the steps of an acid fast stain.
4. List organisms that stain positive in acid-fast stains.
5. List 4 types of fungal stains
6. List 4 types of parasitology stains
7. Identify following bacteria from a Gram stain image:
   Staphylococcus aureus, Neisseria meningitides, Streptococcus pneumoniae, E. coli, Actinomyces species, Campylobacter species, Nocardia species

Patient Perspective

• What do patients (and their families) want to know when they are diagnosed with an “infection”?
  • What is it?
  • How did I get it?
  • Why am I so sick?
  • What is the treatment?
  • When will I feel/get better?
  • Can I get this again?
  • What can I do to not get it again?
  • Can I get it? (family, friends)
Diagnosis of Infectious Disease

From History and Physical Develop a Differential Diagnosis

- What are likely diagnoses?
- If an “infectious disease” – what are the most likely etiologic microbes?

What we need to know about the microbes?

- Family
  - Important Species
- Habitat
  - Where do they live
- What diseases do they cause?
  - In whom?
    - Host predisposing factors
  - Habitats
- How do they do what they do?
  - What are their virulence factors?
    - Do they Adhere, Invade, Evade, Destroy
    - Disease Pathogenesis
- How do we detect them?
  - Laboratory identification
- Approach to treatment
  - Antibiotic sensitivity, resistance patterns
Examples of important host and microbe factors: Age of Patient

Acute Meningitis

Present as fever, headache, meningismus, altered mental status

Most common etiologic bacteria vary depending on age of patient

Newborn (0-6 months):
- Group B Streptococci
- E. coli
- Listeria

Children (6 months - 6 years):
- Neisseria meningitidis
- Haemophilus influenzae type B

6-60 years:
- S. pneumoniae
- N. meningitidis (#1 in teens)

60+ years:
- S. pneumoniae
- Gram negative rods
- Listeria

Acute Meningitis

Presents as fever, headache, meningismus, altered mental status

Most common etiologic bacteria vary depending on age of patient

Examples of important host and microbe factors: Travel History

From History and Physical Develop a Differential Diagnosis

• What are likely diagnoses?
• If an “infectious disease” – what are the most likely etiologic microbes?

• What do I have to do to identify the pathogen?
  • What specimen?
  • What is the appropriate diagnostic testing?
Serum/blood

Diagnostic Method | Time to Pathogen Identification
---|---
Culture and Phenotypic Biochemistry in/on artificial media (bacterial, fungal, mycobacteria, viral) | Days to Weeks
Microscopy | Morphology in minutes
Gram Stain | General category in minutes
In vitro antimicrobial sensitivity | Days to weeks
Acute and convalescent antibody | Weeks
Monoclonal antibodies | Hours
Antigen Detection | Minutes to hours
Real chain-polymerase chain reaction for microorganisms and drug-resistance genes | One to several hours


A microbial culture is a method of multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions.
Bacterial Culture

- Use different "agars" to cultivate bacteria
- Depends on specimen type and/or clinical disease
- Use additional methods to identify what is growing
  = Organism Identification

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Stains Used To Aid in Microbial Diagnosis

- **Bright field (light) microscopy**
  - Bacteria, fungi, parasites
  - Gram
  - Acid Fast
  - Silver
  - GMS
  - PAS
  - Mucin
  - Lectinphenoil Cotton Blue
  - Wright-Giemsa
  - Trichrome
  - Iodine
Gram Stain

• Invented by Hans Christian Gram over 100 years ago
• Classify organisms into two groups
  • Gram positive = retain dye complex
  • Gram negative = loses purple color after decolorization
• Based on the composition of the cell wall
• Observe presence of inflammatory cells

Gram Stain Procedure

1. Cells on the slide
2. Primary stain (Crystal Violet)
3. Mordant (Gram's iodine)
4. Decolorizer (Alcohol and/or acetone)
5. Counterstain (Safranin)

Gram Positive = Purple
Gram Negative = Red (pink)
Clinical Utility of Gram Stain

• Direct Examination of clinical material to make an initial diagnosis
• Determine the quality of the specimen
• Suggest an unusual organism or provide early presumptive bacterial identification
• Verify the clinical relevance of the culture

Gram Stain
Pneumococci (Streptococcus pneumoniae) in Sputum

[Image of Gram-positive diplococci]
Gram Stain
Gonococci (*Neisseria gonococcus*) in Urethral Smear


Gram Stain
*Neisseria meningitidis* in cerebrospinal fluid (CSF)

P. Schreckenberger – Loyola University Chicago

Clinical Utility of a Gram Stain

• Direct Examination of clinical material to make an initial diagnosis
• Determine the quality of the specimen
• Suggest an unusual organism or provide early presumptive bacterial identification
• Verify the clinical relevance of the culture
A poor quality respiratory specimen with large numbers of squamous cells

A good quality respiratory specimen

Clinical Utility of a Gram Stain

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Actinomyces - Gram Stain

Gram Positive Filamentous bacteria

Campylobacter jejuni Gram Stain

Gram negative Bacilli “Seagulls”

Nocardia Gram Stain

Gram Positive Filamentous Bacteria with “Beading”
**Clostridium tetani**

- Small positive rod with round terminal spores giving a drumstick appearance. Often isolated from deep wounds (e.g., nail puncture)

**Gram negative rods** from a blood culture

**Clinical Utility of a Gram Stain**

- Direct Examination of clinical material to make an initial diagnosis
- Determine the quality of the specimen
- Suggest an unusual organism or provide early presumptive bacterial identification
- Verify the clinical relevance of the culture
Acid Fast Stains

- Designed to stain bacteria with cell walls containing long chain fatty acids
- Use heat or detergent to drive stain into cell, once in the cell resists decolorization = acid fast bacilli
- Stains mycobacteria, nocardia, Cryptosporidia, Cyclospora
- Methods: Ziehl-Neelsen or Kinyoun

Acid Fast Procedure
Acid fast stain for Mycobacterium

Modified Acid Fast Stain
Nocardia Brain Abscess

Silver Stains

- Warthin-Starry or Steiner
  - Silver impregnated stain for formalin fixed tissue
  - Demonstrate
    - Spirochetes (syphilis)
    - Bartonella henselae (cat scratch disease)
Warthin-Starry Stain
Spirochetes
Treponema pallidum

Special Stains for FUNGI

• Gomori methenamine silver (GMS)
  • Yeast cells and hyphae
  • Pneumocystis jiroveci
• Periodic acid-Schiff (PAS)
  • Yeast cells and hyphae
• Mucicarmine (mucin)
  • Cryptococcus capsule
• Lactophenol cotton blue

GMS (Gomori methenamine silver) Stain
Aspergillus

Hyphae with 45 degree angle branching, septated
PAS (Periodic acid-Schiff) Stain
Aspergillus in lung Bx

GMS and PAS Stain
Blastomyces dermatitidis
Yeast with broad-based budding

Mucin Stain
Cryptococcus in lung Bx
Mucin stains capsule
Stains for Parasites

- **Wright Giemsa**
  - Used to examine peripheral blood smear
  - Detect Leishmania, Trypanosomes, Malaria

- **Trichrome**
- **Modified acid fast**
- **Iodine**

- **Wet Mounts**

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**Wright Giemsa Stain**
Malaria in Blood Smear
Trichrome Stain - stool
Giardia

Wet Mount
Iodine Stain

Other microscopes
Fluorescence Microscope

- Fluorophores or fluorochromes absorb energy from nonvisible UV and short visible wavelengths, become excited and emit the energy in the form of longer visible wavelengths.
- Color of fluorescence depends on dye and filters on the special microscope.

Fluorescent Microscopy Stains

- Auramine-rhodamine stain
  - Mycobacteria
- Calcofluor white stain
  - Fungi
- Fluorescein conjugated antibodies
  - Viruses, Bordetella, Legionella, Pneumocystis

Immunofluorescence Stain
CMV Antigen
Darkfield Microscopy

special condenser and objective with a diaphragm that scatters light from the object observed, with the result that the object appears bright on a dark background.

Spirochete

Learning Objectives:

Explain the clinical utility of a Gram stain.
- Direct Examination of clinical material to make an initial diagnosis; Determine the quality of the specimen; Suggest an unusual organism or provide early presumptive bacterial identification; Verify the clinical relevance of the culture

List organisms that stain positive with acid-fast stains
- Mycobacteria, Nocardia, parasites: Cryptosporidium, Cyclospora

List 4 types of fungal stains
- GMS, PAS, Mucicarmine, Lactophenol Blue
- Calcofluor White (fluorescence microscope)

List 4 types of parasitology stains
- Wright Giemsa, Trichrome, Modified Acid Fast, Iodine

Identify following bacteria from a Gram stain image:
- Staphylococcus aureus, Neisseria meningitides, Streptococcus pneumoniae, E. coli, Actinomyces species, Campylobacter species, Nocardia species

Sample Question

Which one of the following stains is most useful for demonstrating Aspergillus in lung tissue?

a. Modified Acid-Fast
b. Trichrome
c. Wright’s Giemsa
d. Gomori Methenamine Silver
e. Gram Stain
Sample Question

Which bacteria would be suggested by the Gram stain image shown here.

a. E. coli  
b. Streptococcus pyogenes  
c. Staphylococcus aureus  
d. Clostridium tetani

Sample Question

75-year-old man presents to ED with fever, shortness of breath, chest pain and severe, extremely productive cough. A chest x-ray revealed a right lower lobe infiltrate. Sputum, urine and blood culture were collected. The direct Gram stain of the specimen is show below:

Based on the colony morphology seen in this Gram stain, what organism is suspected as cause of pneumonia?

a. Staphylococcus aureus  
b. Streptococcus pneumoniae  
c. Haemophilus influenzae  
d. Klebsiella pneumoniae  
e. Legionella pneumoniae