

Advanced Specimen Collection and Culture Work-up

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Objectives

- Review collection and transport procedures for wound, stool, respiratory, and genitourinary specimens submitted for microbiological culture.
- Summarize appropriate algorithms for culture workup of wound, stool, respiratory, and genitourinary specimens submitted for microbiology culture.
- Discuss the importance of the clinician laboratory interface

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Routine Stool Culture

- Q-Probe Study of 601 institutions
 - Majority of laboratories (99.3%) included *Salmonella* and *Shigella* in the routine stool workup
 - 96% routinely included *Campylobacter*
 - 30-60% of laboratories surveyed also included other organisms such as *Aeromonas*, *Plesiomonas*, *Yersinia*, *Escherichia coli* O157, & *Vibrio*

Valenstein, P. M. Pfaller, and M. Yungbluth. 1996. The use and abuse of routine stool microbiology. *Arch Pathol Lab Med.* 120:206-211.

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Role of Rapid Nonculture Screening Tests

- Fecal Leukocyte Test
- Fecal Lactoferrin Assay
- Gram stain

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Fecal Leukocyte Test

Pathogen	No. of patients	Mean % positive (range)
<i>Campylobacter</i> sp.	194	58 (25-80)
<i>E. coli</i> O157:H7	112	54 (42-65)
<i>Salmonella</i> sp.	140	52 (11-82)
<i>Shigella</i> sp.	252	73 (49-100)
<i>Yersinia</i> sp.	27	48
<i>C. difficile</i>	160	42 (24-63)

Hines, J and I. Nachamkin, 1996. Effective use of the clinical microbiology laboratory for diagnosing diarrheal diseases. Clin Infect Dis. 23:1292-1301. 5

Fecal Lactoferrin Assay

- To detect *Salmonella*, *Shigella* and *Campylobacter* spp.
 - The sensitivity ranged from 83 to 93%, and specificity ranged from 61% to 100% ¹
 - 85% sensitive and 79% specific when compared to culture ²
- As a marker for inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) ³
 - Significantly higher lactoferrin levels in patients with active and inactive IBD (85.9% sensitivity and 100% specific)
 - Elevated fecal lactoferrin levels – 100% specific in ruling out IBS

1. Choi et. al. 1996. To culture or not to culture: fecal lactoferrin screening for inflammatory diarrhea. J. Clin. Microbiol. 34:928-932.
 2. Silletti, R. P. et. al. 1996. Role of stool screening tests in diagnosis of inflammatory bacterial enteritis in selection of specimens likely to yield invasive enteric pathogens. J. Clin. Microbiol. 34:1161-1165.
 3. Kane, S. V., et. al. 2003. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. Am. J. Gastroenterol. 98:1309-1314. 6

Gram Stain

- The only role of Gram stain is in the diagnosis of *Campylobacter* sp.
- The sensitivity ranges from 66 to 94% with high specificity
- No value for detecting other enteric pathogens

Hines, J and I. Nachamkin. 1996. Effective use of the clinical microbiology laboratory for diagnosing diarrheal diseases. *Clin Infect Dis.* 23:1292-1301.

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Number of Specimens Required to Identify a Pathogen

Number of Specimens Collected per patient	Cumulative Percent of Infected Patients Detected	
	Bacteria/Fungi (n = 3349)	Parasites (n = 1159)
1	96.9	91.9
2	99.0	97.6
3	99.3	99.8
4	99.4	99.9

Valenstein, P, M. Pfaller, and M. Yungbluth. 1996. The use and abuse of routine stool microbiology. *Arch Pathol Lab Med.* 120:206-211.

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Bacteriology Results on Inpatients by Day of Hospital Stay

Result	Hospital Day				
	1	2	3	4	>4
Total number of specimens collected	7924	6886	3369	2022	10009
Total number of 1st positives, with or without previous specimens	374	155	35	19	49
Percentage of total specimens that were patient's 1st positive specimen	4.7	2.3	1.0	0.9	0.5
Number of 1st positive specimens from patients for whom previous negative specimens were collected	6	0	1	0	1

Valenstein, P, M. Pfaller, and M. Yungbluth. 1996. The use and abuse of routine stool microbiology. *Arch Pathol Lab Med.* 120:206-211.

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CAP CHECKLIST

- Question MIC.22440 PHASE: I
 - Does the laboratory have guidelines (developed with clinicians) for the number and/or timing of collection of stool specimens submitted for routine bacterial testing?
- Question MIC.22336 PHASE: I
 - Does the final report for routine bacterial stool cultures list the organisms for which the specimen was cultured (e.g., Salmonella, Shigella, Vibrio, etc.)?

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Acceptable Specimens

- Fecal sample
 - Fresh
 - received within 1 to 2 h of passage
 - Preserved
 - Buffered Glycerol Saline
 - recommended for *Salmonella* & *Shigella* but not for *Campylobacter* or *Vibrio* sp., unless enriched with CaCl₂
 - Modified Carey-Blair
 - good overall transport media
 - inappropriate for *C. difficile*

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Acceptable Specimens

- Rectal swabs
- Duodenal, colostomy or ileostomy contents
 - stool transport vials
- Rectal biopsy samples
 - sterile container with a small amount of sterile water

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Unacceptable Specimens

- Unpreserved stool samples > 2h old
- Dry rectal swabs or biopsy specimens
- Multiple specimens received on the same day
- Specimens received from inpatients after the third hospital day, without prior consultation

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Workup Guidelines for *Salmonella*, *Shigella*, *Aeromonas* & *Plesiomonas* species

- Media
 - **Non-selective**
 - BAP
 - *Aeromonas* sp., *Plesiomonas shigelloides*, Yeasts, *Staphylococcus aureus*, *Pseudomonas aeruginosa*
 - **Differential enteric agar**
 - MacConkey agar, Eosin Methylene Blue (EMB)
 - differentiates lactose-fermenting from non-lactose fermenting colonies
 - **Moderately selective**
 - Hektoen Agar, Xylose Lysine Deoxycholate Agar (XLD), or Salmonella Shigella Agar
 - allows growth of *Salmonella* & *Shigella* sp. while suppressing the growth of most members of family *Enterobacteriaceae*
 - **Enrichment broth**
 - GN Broth, Selenite F
 - increases chances of detecting low numbers of pathogens

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Workup Guidelines for *Salmonella*, *Shigella*, *Aeromonas* & *Plesiomonas* species

- Enteric Screening Procedure
 - Conventional
 - TSI or KIA, LIA, and Urea
 - Commercial kits
 - latex agglutination
- Full ID of suggestive screening results
- Serological identification of *Salmonella* and *Shigella* sp.

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Campylobacter sp.

- **Most frequently isolated**
- **Selective media**
 - Campy-Thio (enrichment broth)
 - Campy-BAP
 - Skirrow medium
 - Campylobacter-cefoperazone-vancomycin-amphotericin (CVA)
- **Identification**
 - growth at 42°C, oxidase and catalase positive, Hippurate positive
 - Nalidixic Acid susceptible, Cephalothin resistant
 - Latex agglutination test

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Campylobacter sp.

- ProSpecT® *Campylobacter* Microplate Assay
- detects *Campylobacter* specific antigens in stool (fresh or in transport medium)
- utilizes polyclonal anti- *Campylobacter* specific antigens capture antibody
- can be read visually or spectrophotometrically
- Evaluated in three studies
 - Sensitivities of 80, 89 and 96%
 - Specificities of 99%
- Flexible, easy to use
- reduces cost, reduce turnaround time
- Cross-reactivity with *C. upsaliensis*, *C. hyointestinalis*, or *C. helveticus* unknown

1. Endtz, H. P., et. al. 2000. Evaluation of a New Commercial Immunoassay for Rapid Detection of *Campylobacter jejuni* in Stool Samples. *Eur. J. Clin. Microbiol. Infect. Dis.* 19:794-797.
 2. Hindiyyeh, M. et. al. 2000. Rapid Detection of *Campylobacter jejuni* in Stool Specimens by an Enzyme Immunoassay and Surveillance for *Campylobacter upsaliensis* in the Greater Salt Lake City Area. *J. Clin. Microbiol.* 38: 3076-3079.
 3. Tolén, R. et. al. 2000. Evaluation of the Alexon-Trend ProSpecT *Campylobacter* Microplate Assay. *J. Clin. Microbiol.* 38: 3853-3855.

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Genus *Vibrio*

- **Laboratory Diagnosis**
 - Media
 - TCBS (thiosulfate citrate bile salts sucrose) agar
 - sucrose-fermenting (Yellow colonies)
 - *V. cholerae*, *V. alginolyticus*, & *V. fluvialis*
 - non-sucrose-fermenting (green colonies)
 - *V. parahaemolyticus*, *V. vulnificus* (Lactose Fermentor)
 - Susceptible to 150 µg of vibriostatic agent (O/129)

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Escherichia coli

- Three paradigms by which diarrhea is produced:
 - enterotoxin production
 - Enterotoxigenic *E. coli* (ETEC)
 - Enteroaggregative *E. coli* (EAEC)
 - invasion
 - Enteroinvasive *E. coli* (EIEC)
 - intimate adherence with membrane signaling
 - Enteropathogenic *E. coli* (EPEC)
 - Enterohemorrhagic *E. coli* (EHEC/ STEC)

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Enterohemorrhagic *E. coli*

- Aka Shiga toxin-producing *E. coli* (STEC)
- There are at least 100 serotypes of STEC
- Only one serotype, namely *E. coli* O157:H7 can be detected in clinical laboratories.
 - Selective media: sorbitol-MacConkey agar
 - confirm by latex agglutination
- Varied geographic distribution - evaluate prevalence for the need of routine workup
- Availability of EIA for detection of STEC

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Yersinia enterocolitica

- Detection based on conventional methods
- Selective media - CIN agar
 - dark red "bull's eye" with a transparent border

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Clostridium difficile Testing

- Acceptable specimen
 - Unformed stool specimen unless ileus due to *C. difficile* is suspected.
- Rejection criteria
 - Specimens that are not liquid or soft
 - Specimens from infants under 1 year old should be discouraged
 - Specimen more than 24 hours old.
 - Rectal swab specimens
 - "Test for cure" or testing from asymptomatic individuals

Gerding, D.N. et. al. 1995. Clostridium difficile-associated diarrhea and colitis. Infect. Control. Hosp. Epidemiol. 16:459-477.
Johnson, S. and D. N. Gerding. 1998. Clostridium difficile associated diarrhea: a review. Clin. Infect. Dis. 26:1027-1034. 22

Clostridium difficile Testing

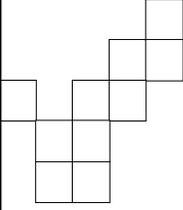
- Culture – most sensitive
 - Selective medium - Cycloserine-cefoxitin-fructose agar
 - Characteristic horse-dung smell
 - typical yellow-green fluorescence under UV light
 - Limitations
 - does not distinguish between toxigenic and non-toxigenic strains
 - delayed turn-around time
- Use of latex agglutination test that detects glutamate dehydrogenase is discouraged

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Clostridium difficile Testing

- Cell Culture Cytotoxicity Assay – most specific
 - detects Toxin B
 - Limitations
 - Requires 24 to 48 hours
 - Tedious
 - Non-commercial versions are not standardized
- EIAs for toxin A or toxins A and B
 - Rapid
 - Less sensitive than cell cytotoxicity assay
 - Tests that detect only toxin A may miss isolates that are toxin A/B*

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Wounds

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Wound Cultures: Controversies

- Is sampling a wound for culture relevant?
- When and how should wounds be sampled?
- How should samples be transported?
- What analysis should be requested?
 - Gram stain only? Culture only? Susceptibility testing?
 - Quantitative cultures?

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Wounds: Classification

<p>Acute</p> <ul style="list-style-type: none"> ■ Caused by external damage to intact skin ■ Types <ul style="list-style-type: none"> <input type="checkbox"/> Surgical <input type="checkbox"/> Bites <input type="checkbox"/> Burns <input type="checkbox"/> Minor cuts <input type="checkbox"/> Abrasions <input type="checkbox"/> Severe traumatic 	<p>Chronic</p> <ul style="list-style-type: none"> ■ Precipitated by predisposing conditions that lead to compromise of dermal/epidermal tissue ■ Types <ul style="list-style-type: none"> <input type="checkbox"/> Impaired venous drainage <input type="checkbox"/> Impaired arterial supply <input type="checkbox"/> Metabolic diseases eg. diabetes
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Bowler PG, et. al. 2001. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 14: 244.

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Wound Infections: Etiology

- Surgical wounds
 - Aerobes: *S. aureus*, coagulase negative staphylococci, *Enterococcus* spp., *E. coli*, *P. aeruginosa*, *Enterobacter* spp.
 - Anaerobes: *Bacteroides* spp., *Peptostreptococcus*, *Clostridium* spp.
- Acute soft tissue infections
 - *Staph aureus* only organism in 30%
 - 30-50% mixed aerobes/anaerobes
 - 20-30% other eg. Group A streptococci, *Clostridium* spp.
- Bite wounds
 - Special pathogens: *Pasteurella multocida*, *Capnocytophaga canimorsus*, *Bartonella henselae*, *Eikenella corrodens*
 - Other mixed aerobes and anaerobes

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Wound Infections: Etiology

- Burn wounds
 - Primarily aerobic organisms: *P. aeruginosa*, *Staphylococcus aureus*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp. and *Candida* spp.
- Diabetic foot ulcers
 - Aerobes: *Staph aureus*, *Streptococcus* spp., *P. aeruginosa*, *Enterococcus* spp., enterics
 - Anaerobes: *Peptostreptococcus*, *Bacteroides* spp., *Prevotella* spp.
- Decubitus ulcers
 - Mixed aerobic and anaerobic bacteria

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Wound Cultures

- For open wounds
 - Clean the wound margins with surgical soap or 70% ethyl or isopropyl alcohol
 - Aspirate from the depth of the wound using a sterile syringe and needle
 - Aspirated fluid should be sent to the laboratory in an appropriate transport system
 - Alternatively, a curette may be used to obtain tissue from base of the wound
 - Swabs are strongly discouraged

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Wound Cultures

- For closed wounds
 - Prepare site as described for obtaining blood culture
 - Aspirate as much purulent material as possible
 - Transport in aerobic/anaerobic transport system

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Wound Cultures: Gram stain

<p>Pros</p> <ul style="list-style-type: none"> ■ Useful in estimating organism load from tissue biopsies ■ Presence of microorganisms on smear from swabs correlates with $\geq 10^6$ organisms (burns) ■ Facilitates identification of etiologic agent of wound infection following clean surgery 	<p>Cons</p> <ul style="list-style-type: none"> ■ Poor correlation seen between Gram stain and culture results from biopsy of diabetic foot infections ■ In mixed infections, little value although presence of leukocytes indicates infection
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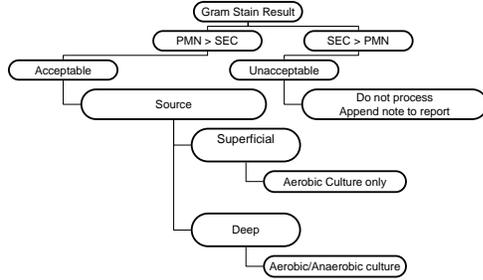
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Wound Specimens: Algorithms

- Three approaches
 - PMN predominance
 - Q Score
 - Q 2 3 4system

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Wound Specimens: Algorithms



Modified from Sharp SE. *Clin Micro Newsletter* 21:14, 1999

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Wound Cultures

- Culture for aerobic and anaerobic bacteria if appropriately collected
 - Gram stain results suggest adequate collection or presence of inflammation
 - Tissues or aspirates vs. swabs
 - Primary plating media: 5% SBA, Choc agar, MacConkey agar; anaerobic plates and thio if appropriately collected
- Identify anaerobes to Genus level only
- Perform susceptibility testing of predominant organisms only

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Wound Cultures: Extent of Workup

Possible approaches

- Use Gram stain result
 - Work up organisms seen on stain only
 - List others
- Work up any potential pathogens to maximum of three, list others present by morphology
- Work up any quantity *S. aureus*, *P. aeruginosa*, beta hemolytic streptococci, enterics and gram-negative anaerobes

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Wound Cultures: Examples

Gram stain results: (Acceptable)
 Many neutrophils, no epithelial cells
 Many gram positive cocci in clusters
 Many gram negative bacilli
 Few morphotypes resembling skin flora
 Work up (identify and perform susceptibility testing): Gram positive cocci in clusters and gram neg bacilli
 Culture report: Many *S. aureus*, many *Klebsiella pneumoniae*, light aerobic bacteria resembling skin flora

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Wound Cultures: Examples

Gram stain: many neutrophils, few epithelial cells,
 Gram positive cocci in clusters, Gram positive cocci in chains,
 Culture grows: many *S. aureus*, many Group A streptococci, few enteric bacilli
 Work up: *S. aureus*, Group A streptococcus: limited ID and no susceptibility on enteric bacilli; susceptibility testing on Group A strep not required

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Wound Cultures: Examples

- Gram stain: Many neutrophils, few epithelial cells, multiple morphotypes
- Culture grows: more than 3 potential pathogens
- Consider source
 - Tissue or aspirate ?
 - Contamination likely ?
 - Type of patient
 - May need to consult with clinician or Infectious Diseases service

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Q-Score

Q-Score = # of potential pathogens (PP) to work up

		Squamous Epithelial Cells				
		No Cells	1-9/lpf	10-24/lpf	≥25/lpf	
Neutrophils	No Cells	0	3	0	0	0
	1-9/lpf	+1	3	0	0	0
	10-24/lpf	+2	3	1	0	0
	≥25/lpf	+3	3	2	1	0

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Workup of Wound Cultures

■ Q-Score System

- Good quality specimen (Q3)
 - Up to 3 organisms can be considered as potential pathogens and worked up (ID/AST)
- Lower quality specimen (Q2, Q1)
 - More SEC
 - Fewer organisms are worked up

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Workup of Wound Cultures

■ Q-Score System

- If the Q score is greater than or equals the PP in culture
 - Workup all potential pathogens
- If Q score is less than the PP in culture
 - Look at the Gram stain
 - Workup all PP that are seen on GS
 - Morphologically ID others
 - If all PP present on GS then only Morph ID all

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Workup of Wound Cultures

- Q/2 3 4 System
 - Culture workup is based on the # of PP present
 - 2PP – ID/AST
 - 3PP
 - Look at the Gram stain
 - Workup two PP if they are seen on GS
 - If all 3 present on GS then Morph ID
 - 4PP
 - Morph ID only

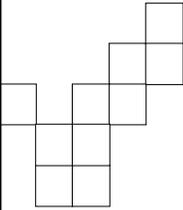
Wound Cultures: Example

Gram stain: many neutrophils, few epithelial cells, Gram positive cocci in clusters, Gram positive cocci in chains,
 Culture grows: many *S. aureus*, many Group A streptococci, few enteric bacilli

Q score = 2 [PMN (+3), few epi (-1)]
 Q/2-3-4 = 3 PP

- look at gram stain

Work up: *S. aureus*, Group A streptococcus, Morph ID and no susceptibility on enteric bacilli

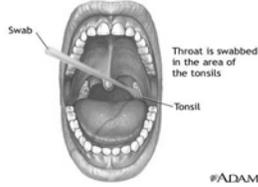


Respiratory Specimens

Respiratory Specimens

- Upper respiratory tract specimens

- Throat
 - detection of streptococcal pharyngitis

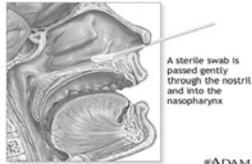


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Respiratory Specimens

- Upper respiratory tract specimens

- Nose
 - detection of MRSA carriers
- Nasopharyngeal swabs
 - diagnosis of *Bordetella pertussis*
- Nasopharyngeal swabs and washings
 - diagnosis of viral disease



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Lower Respiratory Tract Infections

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“ The culture of lower respiratory specimens may result in more unnecessary microbiologic effort than any other type of specimen.”
Raymond C Bartlett

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Lower Respiratory Tract Infections Epidemiology

- Pneumonia is the sixth leading cause of death in US
- Increasing numbers of patients at risk
 - Aging population
 - Increase in patients with immunocompromising conditions
- Overtreatment has lead to resistance
 - Multidrug resistant *Streptococcus pneumoniae*
 - Resistance among hospital acquired pathogens such as *Acinetobacter*, *Pseudomonas aeruginosa* and others

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Cumitech 7B:2003 Lower Respiratory Tract Infections

- 6 contributing authors
- Major sections
 - Clinical aspects of diseases of LRT
 - Specimen collection
 - Specimen processing
 - Interpretation of bacterial cultures
 - Most common pathogens
 - Methods for implementing change
 - Guidelines for frequency of testing
 - Public health issues
 - Reimbursement codes

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Categories of Lower Respiratory Tract Infections

- Acute bronchitis
- Community acquired pneumonia
- Hospital acquired pneumonia
- Pneumonia in the immunocompromised host

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Community Acquired Pneumonia Etiologic Agents

Pathogen	Frequency (%)
<i>Streptococcus pneumoniae</i>	66
<i>Haemophilus influenzae</i>	1-12
<i>M. catarrhalis</i>	10
<i>Legionella</i> species	2-15
<i>Mycoplasma pneumoniae</i>	2-14
<i>Klebsiella</i> species	3-14
Enteric gram negative bacilli	6-9
<i>Staphylococcus aureus</i>	3-14
<i>Chlamydia</i> species	5-15
Influenza viruses	5-12
Other viruses	<1-12
Unknown	23-49

Carroll KC. 2002. *J Clin Microbiol* 40:3115-3120. Sharp SE, et al. *Cumitech* 2003

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Community Acquired Pneumonia Diagnosis

Available Test Methodologies

- Sputum Gram stain and culture
- Blood cultures
- Serologic studies
- Antigen detection tests
- Nucleic acid amplification tests

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Sputum Gram Stain and Culture

Proponents

- Demonstration of predominant morphotype on Gram stain guides therapy
- Accuracy is good when strict criteria are used
- Cheap, so why not?

Antagonists

- Poor specimen collection
- Intralaboratory variability (Gram stain interpretation)
- Low sensitivity and specificity
- Empiric treatment guidelines
- Not cost effective

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Sputum Collection

- Proper patient instruction
 - Food should not have been ingested for 1-2 h prior to expectoration
 - The mouth should be rinsed with saline or water
 - Patient should breathe and cough deeply
 - Patient should expectorate into a sterile container
- Transport container immediately to lab
- Perform Gram stain and plant specimen as soon as possible

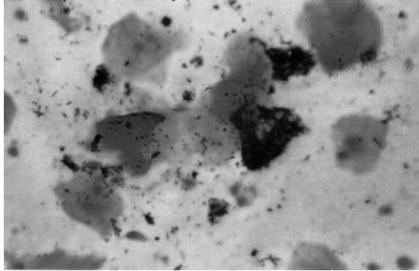
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Sputum Gram Stain

- Screen for acceptability
 - Examine specimen under low power (x 10 objective)
 - Examine 10 representative fields
 - Specimens that show few squamous epithelial cells (< 10/lpf) and many PMNs (> 25/lpf) are acceptable
 - Notify physician of unacceptable samples

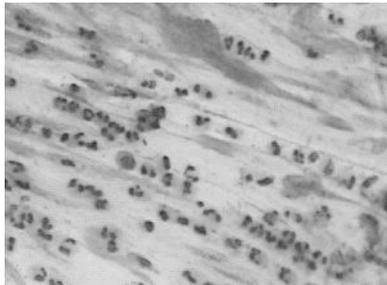
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Sputum Gram Stain
Unacceptable



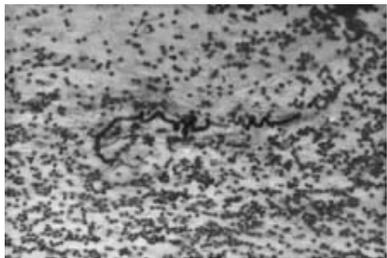
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Sputum Gram Stain
Good Quality



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Sputum Gram Stain
Good Quality



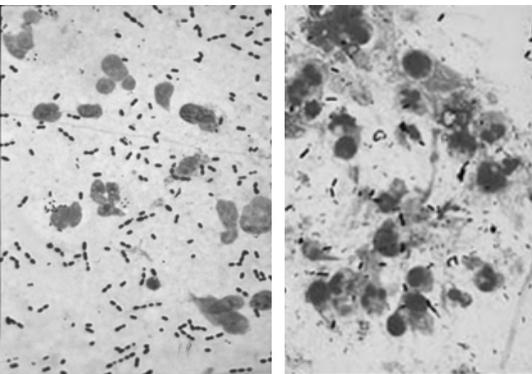
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Sputum Gram Stain

Good quality specimens

- Quantify number and types of inflammatory cells
- Note presence of bronchial epithelial cells
- Concentrate on areas with WBCs when looking for organisms
- Determine if there is a predominant organism (> 10 per oil immersion field)
 - Semiquantitate and report organism with descriptive
 - If no predominant organism is present, report "mixed gram positive and gram negative flora"

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Utility of the Gram Stain in Diagnosis of Pneumonia

Roson, B, et. al. 2000. *Clin Infect Dis* 31:869-74.

- Prospective study
- Non immunocompromised patients hospitalized with CAP
- 1,000 bed hospital in Spain
- ER physicians instructed on sputum collection for Gram stain and culture
- Sputum collected under supervision of nurse or resident
 - Samples were processed immediately
 - Screened for epithelial cells
 - Screened for predominant morphotype (> 75% of the organisms seen)
 - Sputum planted to blood agar, chocolate agar and MacConkey agar
- Strictly defined clinical and diagnostic parameters

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Utility of the Gram Stain in Diagnosis of Pneumonia

Roson, B, et. al. 2000. *Clin Infect Dis* 31:869-74

Results

- 190/533 (35.6%) patients had no sputum sample submitted (these patients were included in the calculations)
- 133/533 (25%) patients had a poor quality specimen
- 210/533 (39.4%) patients had a good quality specimen
- Overall sensitivity and specificity for pneumococcal pneumonia: 57% and 97%
- Overall sensitivity and specificity for *H. influenzae* pneumonia: 82% and 99%
- Gram stain gave presumptive diagnosis in 80% of patients who had a good specimen submitted
- > 95% of patients in whom a predominant morphotype was seen on Gram stain received monotherapy

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Gram Stain Reports

- Be as descriptive as possible
 - Moderate neutrophils
 - Moderate Gram positive diplococci suggestive of *Streptococcus pneumoniae*
 - Few bacteria suggestive of oral flora
- Keep report short—avoid line listing of all morphotypes present

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Sputum and Endotracheal Suction Culture Evaluation

- Identify and perform susceptibility testing on 2-3 potential pathogens seen as predominant on Gram stain
- Alpha strep—rule out *S. pneumoniae*
- Yeast—rule out *Cryptococcus neoformans* only
- *S. aureus*, Gram negative bacilli
 - < normal flora, quantify and limit ID; no susceptibility
 - Add comment that organism not predominant on stain
- ID mould, Mycobacteria or *Nocardia spp.*

Modified from Sharp SE, et. Al. 2003. *Cumitech* 7B. ASM Press. ⁶⁶

IDSA Practice Guidelines
Diagnostic Tests for CAP

- Outpatients
 - Empiric therapy with a macrolide, doxycycline, or a fluoroquinolone
- Hospitalized patients with CAP
 - Gram stain and culture of sputum
 - 2 pretreatment blood cultures
 - Studies for Mtb, Legionella in select patients
- Rationale
 - To improve patient care
 - Advance knowledge of epidemiologically important organisms
 - Prevent antibiotic abuse
 - Reduce antibiotic expense

Bartlett JG. 2000. *Clin Infect Dis* 31:347-82.

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ATS Guidelines
Diagnostic Tests for CAP

- Empiric therapy for outpatients
 - Macrolide or tetracycline
- Hospitalized patients with CAP
 - 2 sets of pre-treatment blood cultures
 - Pleural fluid Gram stain/culture when appropriate
 - Studies for Legionella, Mtb, fungi in select patients
 - Sputum Gram stain/culture only if resistant or unusual pathogen is suspected
 - Avoid extensive testing

ATS. 2001. *Am J Respir Crit Care Med* 163: 1730-1754.

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Hospital Acquired Pneumonia

- Most frequent nosocomial infection (30-33% of cases) among combined medical surgical intensive care units
- 83% are ventilator associated
- Etiologic agents

	Frequency (%)
□ Gram positive cocci	
■ <i>S. aureus</i>	17
■ <i>S. pneumoniae</i>	2-20
□ Aerobic gram-neg bacilli	60
■ <i>Pseudomonas aeruginosa</i>	
■ <i>Enterobacter sp.</i>	
■ <i>Klebsiella pneumoniae</i>	
■ <i>Acinetobacter</i>	
■ <i>Legionella</i>	
□ Anaerobes	10-20
□ Fungi	0-10

Modified from: Carroll KC. 2002. *J Clin Microbiol* 40: 3115-3120.

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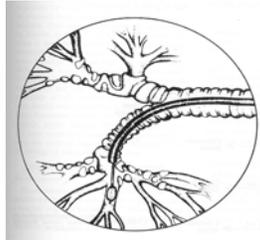
Hospital Acquired Pneumonia Diagnosis

- American College of Chest Physicians: Clinical findings are not sufficient for definitive diagnosis
- Qualitative culture of endotracheal sputum has poor predictive value
- Bronchoscopy is recommended by many pulmonologists
 - Bronchial brushings
 - Bronchial washes
 - Protected specimen brushing
 - Bronchoalveolar lavage specimens (BAL)
 - Transbronchial biopsy

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Respiratory Specimens

- Protected Brush Specimen
 - To procure uncontaminated lower airway secretions
 - Brush within 2 catheters



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Respiratory Specimens

- Bronchoalveolar Lavage (BAL)
 - Samples large area of the lung
 - Performed using a bronchoscope
 - 100 to 250 ml of saline injected
 - Injected saline along with secretions is collected by aspiration
- Transthoracic Aspiration
 - Involves percutaneous introduction of a needle directly into the infiltrate

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**Bronchoalveolar Lavage (BAL)
Specimen Acceptability**

- Microscopic examination of Gram-stained smear
 - Acceptable
 - <1% of cells present are squamous epithelial cells
 - Unacceptable
 - >1% of cells present are squamous epithelial cells

Thorpe JE et. al. 1987. Bronchoalveolar lavage for diagnosing acute bacterial pneumonia. *J. Infect. Dis.* 155:855-861 73

**Processing Bronchoscopy
Specimens**

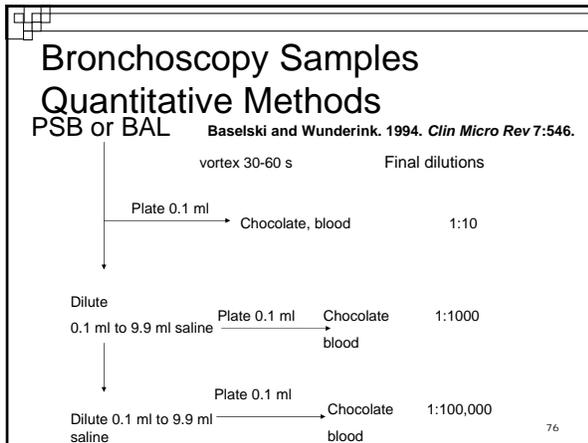
- Bronchoscopy brush protected
 - Aerobic bacterial culture and Gram stain
 - Anaerobic bacterial culture
 - Limited volume
- Bronchoscopy brush, unprotected
 - No anaerobic culture
 - Limited volume
- Bronchial washings
 - Useful only for pneumonia caused by strict pathogens
 - Reasonable requests: *Mtb*, *Fungi*, *Legionella*, *Pneumocystis*
- Bronchoalveolar lavage
 - No anaerobe culture
 - Amenable to extensive testing for all opportunistic pathogens

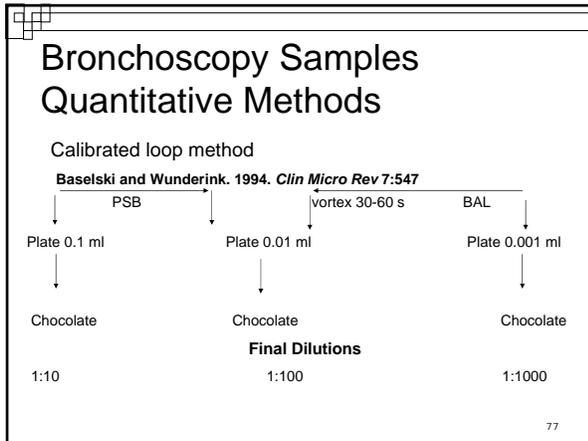
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**Interpretation of Quantitative
PSB/BAL**

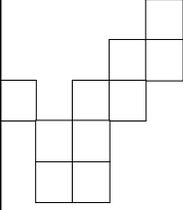
- Dilution Method
 - Quantify each morphotype present and express as CFU/ml
- Calibrated Loop Method
 - Quantify each morphotype present and express as log₁₀ colony count ranges
- Thresholds for significance
 - PSB > 10³ CFU/ml
 - BAL > 10⁴ CFU/ml

Baselski and Wunderink. 1994. *Clin Micro Rev* 7:547 75





- ### Immunocompromised Patients Suggested BAL Protocol
- Aerobic Gram stain quantitative bacterial culture
 - Fungal stain and culture
 - Mycobacterial stain and culture
 - Viral culture/Respiratory DFA
 - Pneumocystis DFA
 - Legionella culture
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Genital Specimens

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GENITAL TRACT SPECIMENS

- **Patients in high risk situations:**
 - Patients known to have gonorrhea
 - Male patients with NGU, PGU, epididymitis, and Reiter's Syndrome
 - Females with mucopurulent cervicitis, urethral syndrome, endometriosis, and salpingitis
 - Neonates born to infected mothers
- Infertility investigations

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GENITAL TRACT SPECIMENS

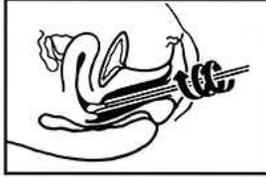
- **Sexually active asymptomatic females who:**
 - Are age 25 years or younger
 - Are pregnant
 - Have evidence of purulent or mucopurulent cervical discharge
 - Exhibit endocervical bleeding, induced by swabbing on examination
 - Have had a new sex partner in the preceding 2 months
 - Use no contraceptives or a non-barrier method for contraception

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GENITAL TRACT SPECIMENS

■ For Females

- Cervical specimens should be collected after removing excess mucous from the cervical os and surrounding mucosa
- Use a second swab to collect specimen by rotating the swab for 10 to 30 secs. in the endocervical canal
- Collect vaginal specimens using a speculum without any lubricant

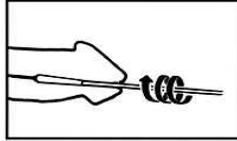


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GENITAL TRACT SPECIMENS

■ For males

- Urethral specimens are collected by inserting a swab 2 to 4 cm. into the urethra and rotating the swab for 2 to 3 seconds



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GENITAL TRACT SPECIMENS

■ For HSV lesions

- Fluid from lesions should be aspirated using a syringe
- Swab can be used to collect vesicle fluid or cellular material from the base of the lesion before crusting and healing have begun

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Genital Specimens

Specimen source	Potential Pathogens	Primary plating media	Special considerations
Cervix	Chlamydia; GC; herpes	SBA, Choc, TM; viral transport media for herpes	NAT testing recommended for GC, CT
Cul-de-sac	Anaerobes, GC, CT, enterics	SBA, choc, TM, Mac, ana, thio	Collect aspirate; Anaerobe transport
Endometrium	Mixed aerobes /anaerobes	SBA, choc, TM, Mac, ana, thio	Surgical biopsy or sheathed catheter
Vagina	Group B strep; Mixed aerobes anaerobes BV	SBA; LIM or other special broth	Culture for Group B strep only; do not culture for BV

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LABORATORY DETECTION OF BV

- Clue Cells: vaginal epithelial cells studded with coccobacilli
 - wet mount
- pH > 4.5
- Whiff test + (10 20% KOH)
- Scored gram stain
- Culture = NO
 - *G. vaginalis* isolated in > 92% women with BV and 70% asymptomatic woman
- Probe: AFFIRM (Becton Dickinson)
 - agrees well with high count *G. vaginalis*

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Trichomonas vaginalis

- Common sexually transmitted disease
- Disease associations and adverse outcomes
 - Vaginitis
 - Urethritis—men and women
 - Outcomes
 - Adverse pregnancy events
 - Associated with increased HIV shedding

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Trichomonas vaginalis
Diagnosis

- Culture “gold standard”
 - Diamond’s media
 - InPouch TV; BioMed Diagnostics, San Jose CA)
 - Barenfanger J, et. al. 2002. *J Clin Microbiol* 40:1387.
- Wet mount—insensitive (~ 50%)
- Rapid tests
 - XenoStrip-Tv (GenzymeDiagnostics, Inc. San Antonio, Tex.)
 - more sensitive than wet prep
 - less sensitive than culture
 - useful as a POC test

Pillay A, et. al. 2004. *J Clin Microbiol* 42:3853.
 Kurth A, et. al. 2004. *J Clin Microbiol* 42:2940.

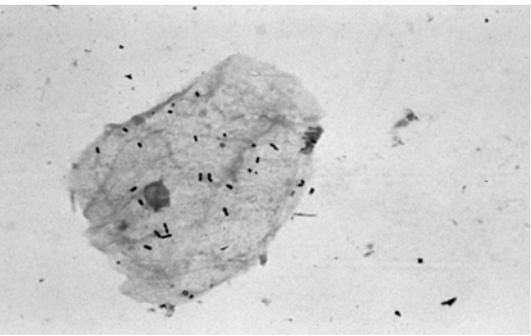
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Lab detection (cont.)

- GLC—no longer used
- Detection of sialidases (neuraminidases that remove sialic acid from sialoglycoconjugates)
 - In BV, associated with *Prevotella* and *Bacteroides* sp.
 - Colorimetric test BVBlue System (Gryphus Diagnostics—91.7% sensitive; 97.8% specific

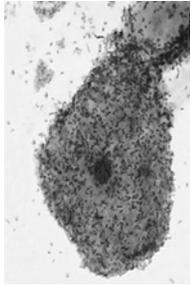
Myziuk L, et. al. 2003. BVBlue test for diagnosis of bacterial vaginosis. *J Clin Microbiol* 41:1925.

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Clue Cell of BV



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BV Scored Gram Stain

(from Nugent RP 1991;29:297)

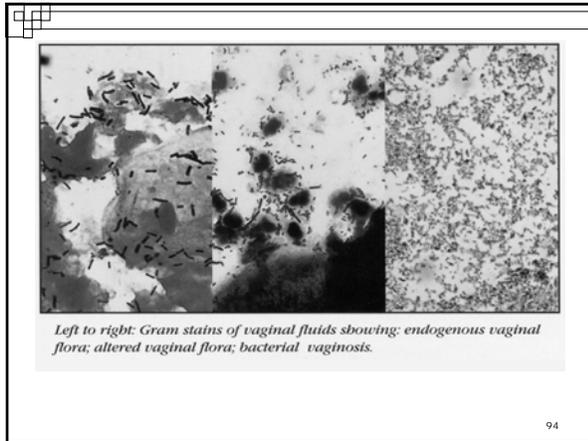
TYPE	Number seen/OIF				
	None	<1	1 5	6 30	>30
Lacto	4	3	2	1	0
Gard/Bact	0	1	2	3	4
Curved GNR	0	1	2	3	4

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Interpretation of Scored Gram Stain

- 0-3 = Normal
- 4-6 = Intermediate
 - may indicate trichomoniasis, GC or CT
 - abnormal gram stain, but not consistent with BV
- 7-10 = Consistent with Bacterial Vaginosis
 - Significance of results unknown in premenarchal girls or postmenopausal women

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- ### Who Should be Screened for BV?
- Women with vaginal symptoms
 - esp. if failed therapy
 - Pregnant women at high risk of preterm birth
 - Pregnant women with genital symptoms
 - rule out trichomoniasis as well
 - Women with gynecologic surgery
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Thank You

Questions ?????

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