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The Clinical Microbiology Laboratory and the Dx of Infectious Diseases

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Objectives

- Understand changing landscape of clinical and laboratory medicine.
- Understand the Path of Workflow in the Clinical Laboratory (including the pre-analytic, analytic and post-analytic phase components) and their importance to patient outcomes.
- Recognition of differences between microbial contamination, colonization and true infection, including naming components of the human microbiome at various body sites.
- Understand the parameters for appropriate laboratory use, including definition of test sensitivity, specificity and appropriate specimen choice.
- Be able to summarize appropriate approaches to collection, processing and interpretation of culture of specimens from various infectious processes (including blood, respiratory tract, wound, normally sterile body sites, urinary tract, and GI tract).

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Changing Landscape – Healthcare Reform

- 2010 Patient Protection & Affordable Care Act (ACA)
  - Goals of healthcare reform:
    - Increasing access to healthcare
    - Improving quality of care
    - Increasing accountability and efficiency
      - Clinical value
      - Patient satisfaction
      - Decreasing costs
  - Value Based Purchasing Model: Reimbursement based on quality metrics; shift from a **fee-for-service** reimbursement model to a **value-based** model
  - Accountable Care Organizations (ACOs)
    - **What does one get for the resources spent?**

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Changing Landscape of Clinical Microbiology

- Changing laboratory infrastructure and dwindling personnel (centralization; pros and cons)
- Maturation of new technologies (automation & nucleic acid amplification tests)
  - Increasing number of test menus and decreasing understanding of test application, interpretation by clinicians in general
  - Algorithms for Dx of infectious diseases & computerized physician order entry (CPOE)
- Increasing antimicrobial resistance
  - Necessitated antimicrobial stewardship
- Healthcare reform
  - Cost expectations; clinical value, outcome studies

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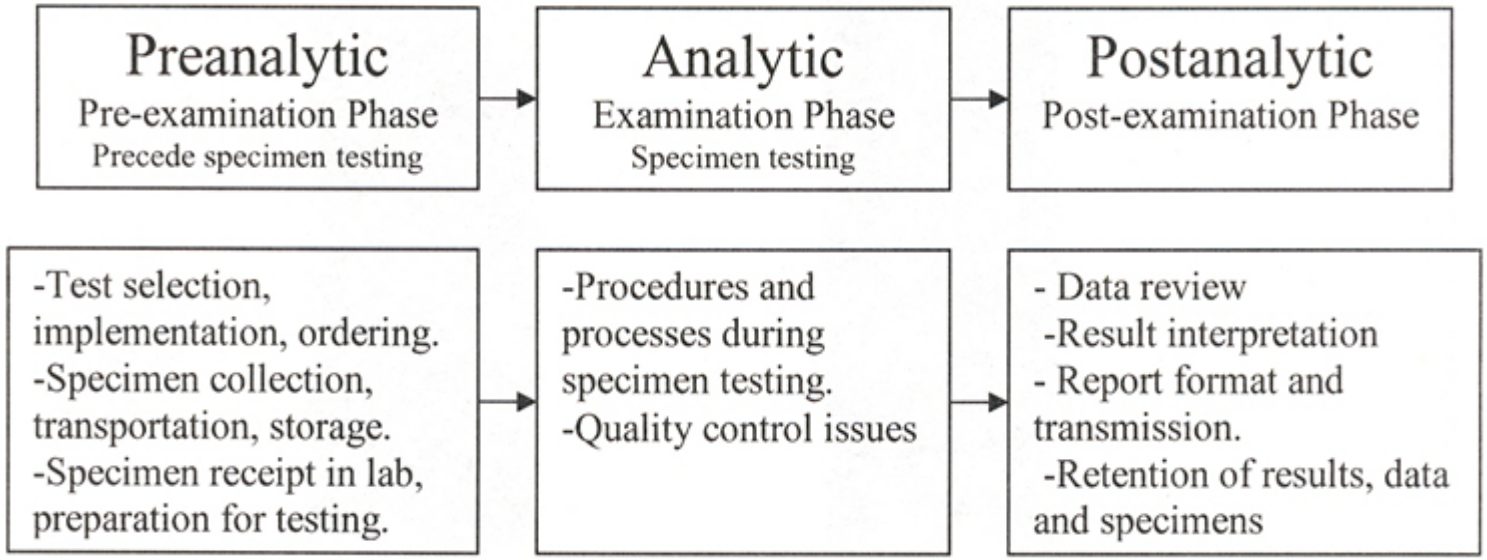
Historically Normal Laboratory Work Flow Patterns: good at looking at mostly in-lab processes :
We determine accuracy, TAT, Costs to lab, testing personnel needed, PT, QC, QA, etc.



From NCCLS (CLSI) Document HS1

Described and defined by Clinical Laboratory Improvement Amendments (CLIA) regulations and CLSI guidelines

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Clinical Relevance vs. Cost of Laboratory Testing and Results

- Screening testing should be limited to clinically relevant situations
- The more testing performed the more false-positives will be picked up
- Issues with testing:
  - **sensitivity/specificity**
  - **false-positives usually expands inappropriate testing**
  - **Cost (actual, set by provider, reimbursed by payers such as Medicare, Medicaid, private insurance, etc)**

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Parameters for Appropriate Laboratory Use

- Understanding infectious process
- Understanding in vitro evaluation process (lab)
- Limitations (sensitivity, specificity, errors)
- Significance of results
- Communication (action taken)
  - Appropriate clinician interpretation of results is crucial to correct utilization.

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Infectious Diseases
Crucial Points in Diagnosis

Pre-analytic

- PE, history, immune status, time of year
- Appropriate choice of testing to be performed
  - (which test(s) to order)
- Choice of correct specimens
  - (bypass normal / colonizing flora; timing must be correct).
- Appropriate specimen collection, handling, transportation crucial.

Differences in Human Microbiome Composition by Body Site

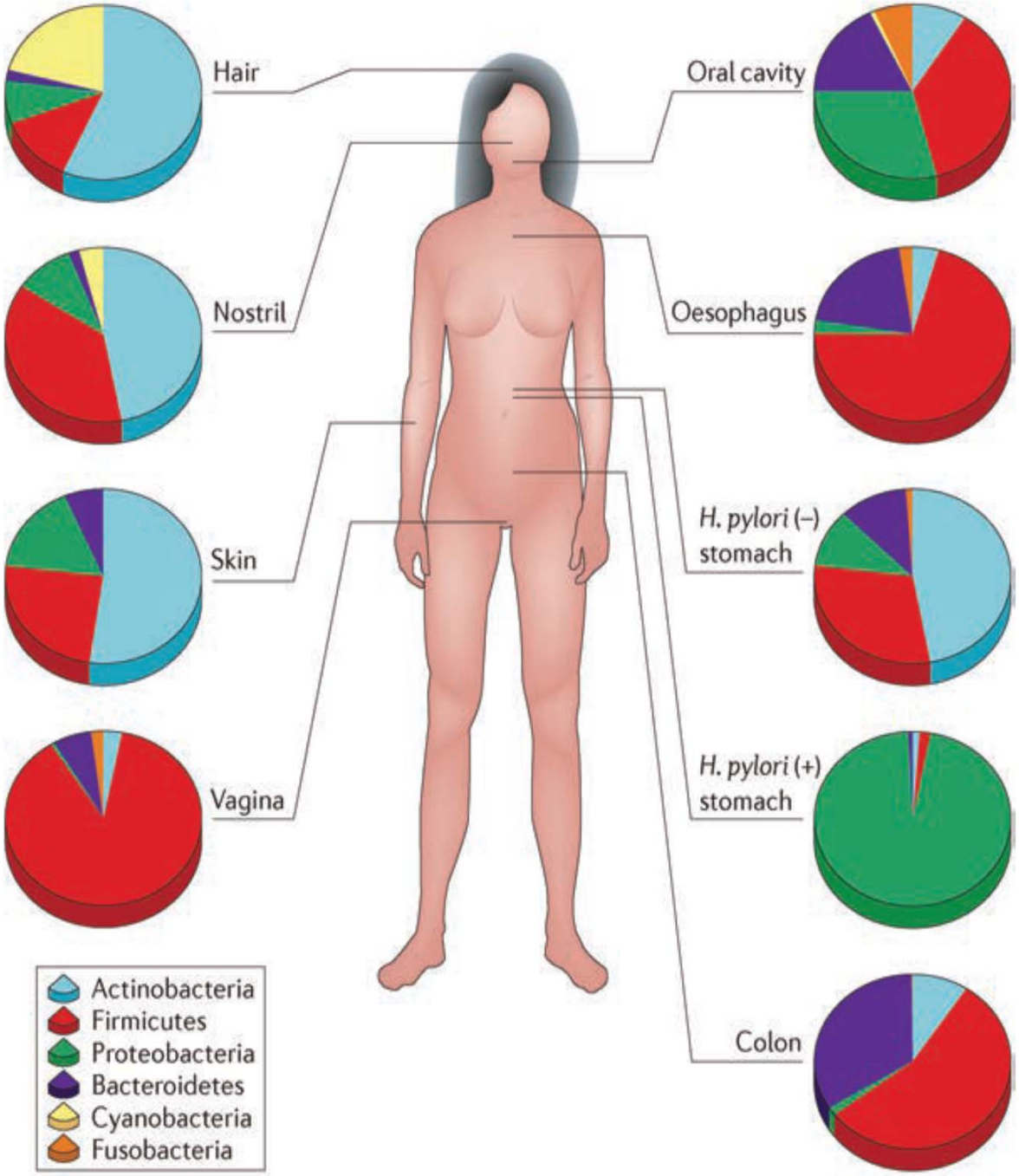

Table 1. Approximate numbers of bacteria inhabiting various regions of the human body, as collated from various printed sources[1–6,8,9,48,87]

Region	Size of the bacterial population
Skin	10^{2}–10^{6} per cm^{2}
Oral cavity	
Saliva	$10^{7–8}$ per mL
Dental plaque	$10^{10–11}$ per gram
Stomach	$10^{4–7}$ per gram
Intestinal fluid	$10^{4–7}$ per mL
Colon contents	$10^{11–12}$ per gram
Genital econiche	
Preputium, perurethral area	$10^{2–6}$ per cm^{2}
Vaginal fluid	$10^{6–8}$ per gram
Bacterial vaginosis	$10^{8–9}$ per gram
Bacterial vaginosis, biofilm	$10^{10–11}$ per gram

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Physicians and Laboratory Tests

Children's Hospital Colorado

"Remember, ordering a diagnostic test is like picking your nose in public: you must first consider what you will do if you find something."

Catherine D. DeAngelis, MD
Arch Pediatr Adolesc Med 1994; 148:1277.

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Clinical Relevance of Laboratory Results

Diagnostic Sensitivity:

- **Ability of test to detect a condition**
  - Frequency of abnormal or positive test results in individuals who have a selected disease

$$\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$= \text{probability of a positive test, given that the patient is ill}$$

Diagnostic Specificity:

- **Ability of test to define a true condition**
  - Frequency with which a normal or negative test results in individuals free of the disease

$$\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

$$= \text{probability of a negative test given that the patient is well}$$

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Clinical Relevance of Laboratory Results

Predictive value of a test is significantly effected by the prevalence of the disease in the selected population

$$PPV = \frac{(\text{sensitivity})(\text{prevalence})}{(\text{sensitivity})(\text{prevalence}) + (1 - \text{specificity})(1 - \text{prevalence})}$$

Prevalence of Disease within Population (%)	Predictive Value of Positive Result (%)
1	16.1
2	27.9
5	50.0
10	67.9
25	86.4
50	95.0

Test Sensitivity 95%, Test Specificity 95%

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Postanalytic : Why evaluate appropriate utilization of tests and their outcomes

One of the great mistakes is to judge policies and programs by their intentions rather than their results

~

Milton Friedman (Nobel Prize 1976, US Economist)

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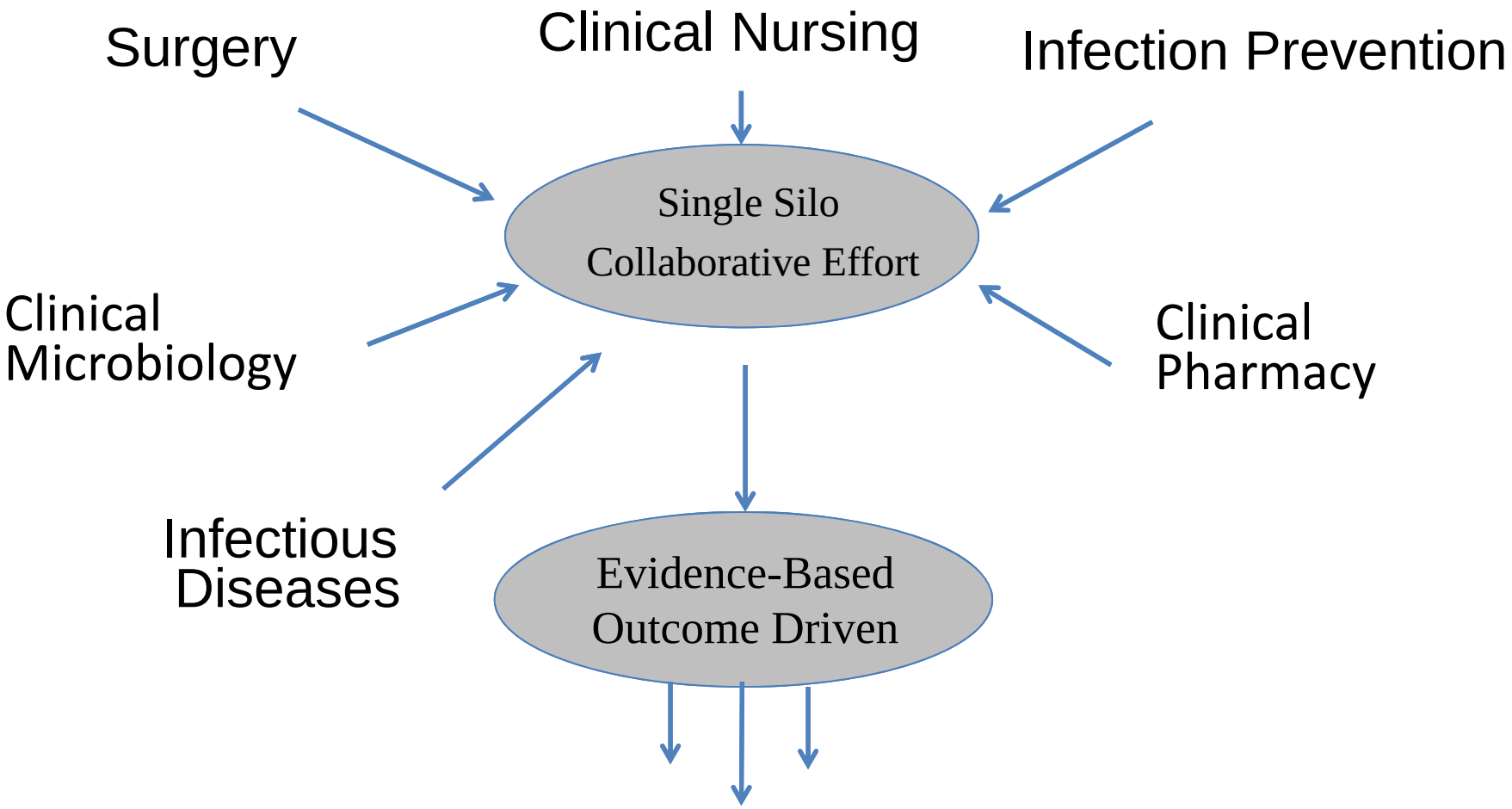
New paradigm in clinical microbiology

- Collaborate with ID and other CCGs as well as pharmacy, etc in new programs – partnering with other clinical entities for laboratory and resource utilization – the lab provides value-added concepts to patient care
- Develop programs (initiation of new procedures) to not only verify and validate tests but also validate their expected effects on clinician compliance and patient outcomes
- Document and verify outcomes – work with administration to measure returns for output

Lancet. 1973;2:349

Evidence-based best practices clinical approach



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Based on: CDC Guidelines
and Website Accessed 2007

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Appropriate Specimen Collection
General Principles

- Consider specimen location and infectious process (contaminated or sterile)
- Choose location in which pathogen might be at (stage of disease process)
- Advancing margins on skin or soft tissue
- Bypass contaminated or colonized sites
- Decontaminate sites that might be colonized or contaminated
- Collect adequate specimen size or volume (no swabs except in special situations)
- Collect before initiation of antimicrobial therapy
- Transport in correct container
- Transport in adequate environment and appropriate time

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Effect of Antibiotic Therapy on Sputum Cultures in Patients with Consolidated Community-Acquired Pneumonia
******Collect specimen BEFORE initiation of therapy**

	Prior Rx (52 Pts)	No Prior Rx (24 Pts)
S. pneumoniae	0	15 (63%)
H. influenzae	0	7 (29%)
S. aureus	1 (2%)	0
Gram-negative bacilli	15 (29%)	0
No pathogen	36 (69%)	2 (8%)

Lancet. 1973;2:349

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Specimens Sent for Microbiological Studies

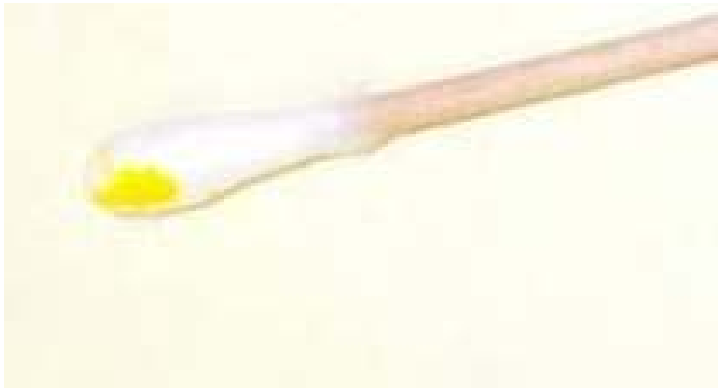
Swab **WORST** type of sample

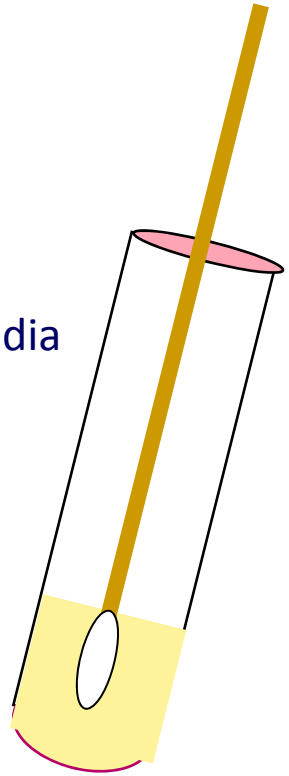
A. Picks up extraneous microbes

B. Holds extremely small volume of specimen

C. Hard to get bacteria or fungi away from fibers and onto media

D. Inoculum not uniform across several different agar plates





Slide from: Ellen-Jo Baron, Stanford

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Microbiologic Evaluation

Traditional

- Direct microscopic evaluation
- Isolation of etiologic agent as needed
  - Under Rx influence
  - Slow growing /Unable to grow
- Susceptibility studies when warranted
  - Phenotypic
  - No standard for many organisms
  - New mechanisms of resistance
    - ESBLs, KPCs, Ps/Acinetobacter, MRSA and vanco

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More Rapid Procedures in Microbiology

- Microscopic (wet mount, Gram/AFB/Calcofluor/Acridine orange, etc)
- Direct or Indirect Antigen detection (direct: EIA, FA, Latex; Indirect: selective broth testing)
- Rapid biochemical (leukocyte esterase, oxidase, beta-lactamase, Vitek bio card, etc)
- Nucleic Acid Testing (NAT; molecular)
- Mass Spectrophotometric Methods (e.g.MALDI-TOF)
- Next Generation Sequencing Studies

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Microbiologic Stains

- Direct set-up (unspun)
  - WBCs, epithelial cells and organisms reported semi quantitatively (1+/scant; 2+/light; 3+/moderate; 4+ = heavy)
- Centrifuged (e.g. spinal fluid)
  - Reported only as "unable to quantitate"
- AFB the only difference – reported quantitatively even though specimen concentrated

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Growth Quantitation

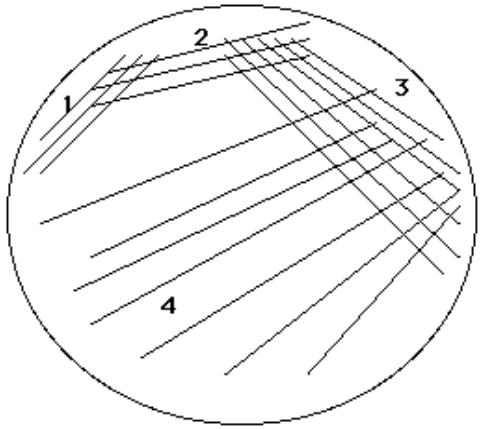
- Depending on the type of inoculation method used, the microbiologist will determine the actual number of colonies present or an estimate of the bacteria present.
  - When plates are inoculated with a measured amount of specimen as in **quantitative** inoculation, the colonies can be counted and the actual numbers of organisms in the specimen reported **(10^3, 10^4, 10^5, etc.).**
  - When the plates are inoculated in a **semi-quantitative** manner, the numbers of organisms growing on the plates are graded to give an estimate of the organisms present in the original specimen

    1+ = scant

    2+ = light

    3+ = moderate

    4+ = heavy



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Blood Cultures

Routine (includes the yeast Candida)

- Number (x 2-3; 2 bottles/culture),
- 20 mls/culture of blood
- Prior to Rx; one after another in sepsis; peripheral venous draw; spaced about 1 or more hrs if abscess suspected
- Contamination
  - skin disinfection (following contamination rates, $2-6 thousand/case)
  - interpretation: isolate types and number positive cultures
  - Do not collect through indwelling catheter (higher contamination rate)

Fungal (Histo, Crypto, Coccy, do not need for other yeasts)

AFB (Rapid growers: MAC, TB, other NTM in Icpts - non-IC should not be cultured for TB)

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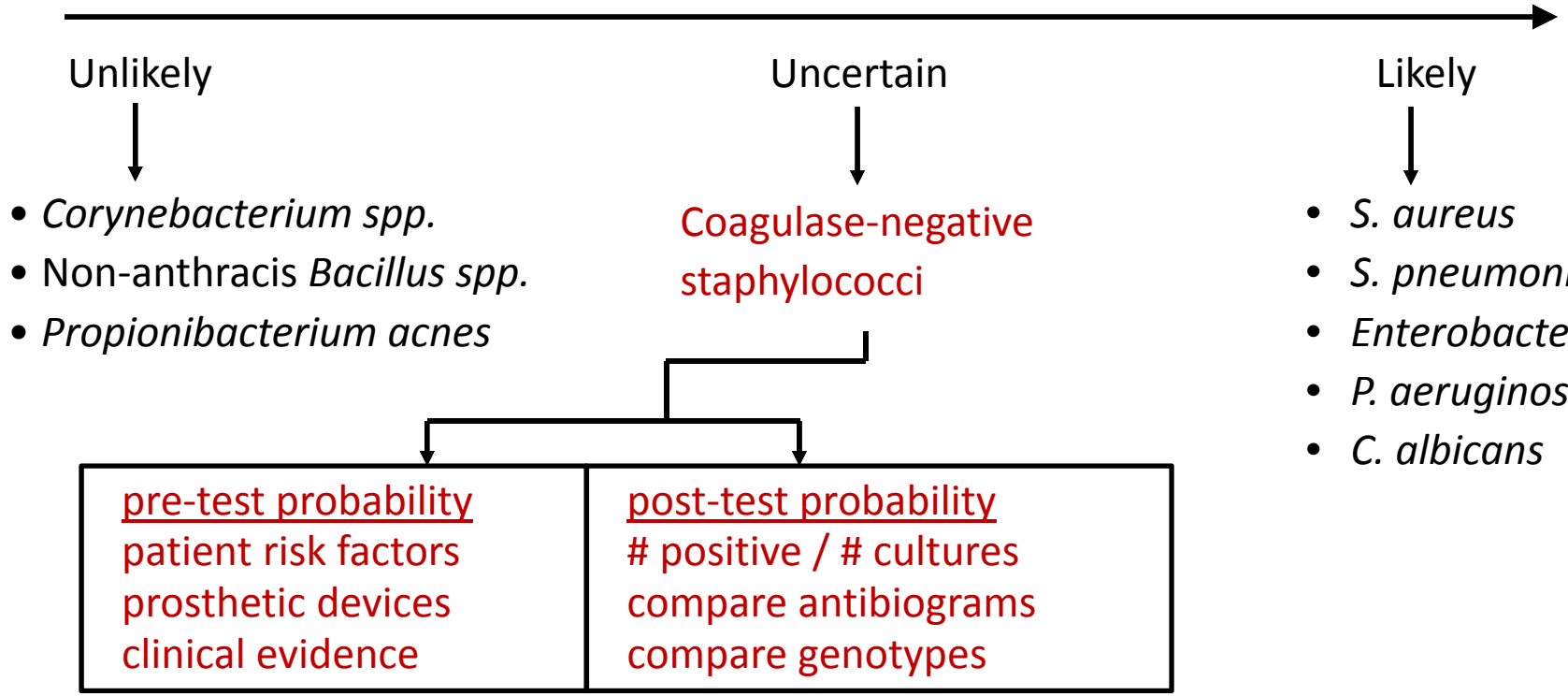
Interpreting a "Positive" Blood Culture

True Bacteremia:



Source: Kim SD, et al: Infect Control Hosp Epidemiol 2000;21:213-7

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Clinical and Microbiological Relevance

- Wound specimens:
  - Normally sterile sites / tissue, pus
  - Topical : ulcers, diabetic foot, mixed colonization (only after debridement) - careful attention to organism types and numbers
  - Pay attention to interpretation of results

Diagnosis of Wound Infections

- CBC, Blood cultures X 2-3
- Culture and Gram stain of any drainage from lesion (PUS is good)
- Needle aspiration
- Punch biopsy
- But do not use swabs (even on pus)
  - DO NOT SWAB infected pressure ulcers or diabetic foot wounds

Stevens, et al. CID 2005;41:1373

Use of Cultures in Wound Infections

- Can be difficult to interpret
- Superficial swab cultures are of limited value
  - *Staph aureus*
- Sinus tract (outside third usually different organism than deeper specimens)
- Tissue biopsy
  - Can be very useful, especially in mixed flora, fungal and with histopathology)
  - Bone biopsy in cases of osteomyelitis

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Obtaining Specimens for Culture

- Culture in cellulitis infrequently positive

  (neg. in 75-80% of cases; including blood cultures, < 5% are positive)

- Gram stain and culture should be obtained from other SSTIs

  (except perhaps diabetic feet and infected pressure ulcers)

- Tissue or pus are superior specimens
  - Tissue biopsy or curettage (scraping with a scalpel blade) are better sources for culture
  - Needle aspiration of pus : (do not use swabs)

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Soft Tissue Specimen Collection

When

- Culturing clinically *uninfected* lesions is unnecessary, unless done as part of an infection-control surveillance protocol (C-III).
- Cultures of infected wounds are valuable for directing antibiotic choices, but may be unnecessary in cases of acute mild infection in an antibiotic-naive patient (B-III).
- Blood cultures should be performed for a patient with a severe infection, especially if the patient is systemically ill (C-III).

How

- Cleanse and debride the lesion before obtaining specimens for culture.
- In cases involving an open wound, obtain tissue specimens from the debrided base (whenever possible) by means of curettage (scraping with a sterile dermal curette or scalpel blade) or biopsy (bedside or operative) (A-I).
- Avoid swabbing undebrided ulcers or wound drainage. If swabbing the debrided wound base is the only available culture option, use a swab designed for culturing aerobic and anaerobic organisms and rapidly transport it to the laboratory (B-I).
- Needle aspiration may be useful for obtaining purulent collections or, perhaps, a specimen from an area of cellulitis.
- Clearly identify samples (specimen type and anatomic location), and promptly send them to the laboratory in an appropriate sterile container or transport media for aerobic and anaerobic culture.

From: Lipsky, et al. Clin Infect Dis 2004; 39:885

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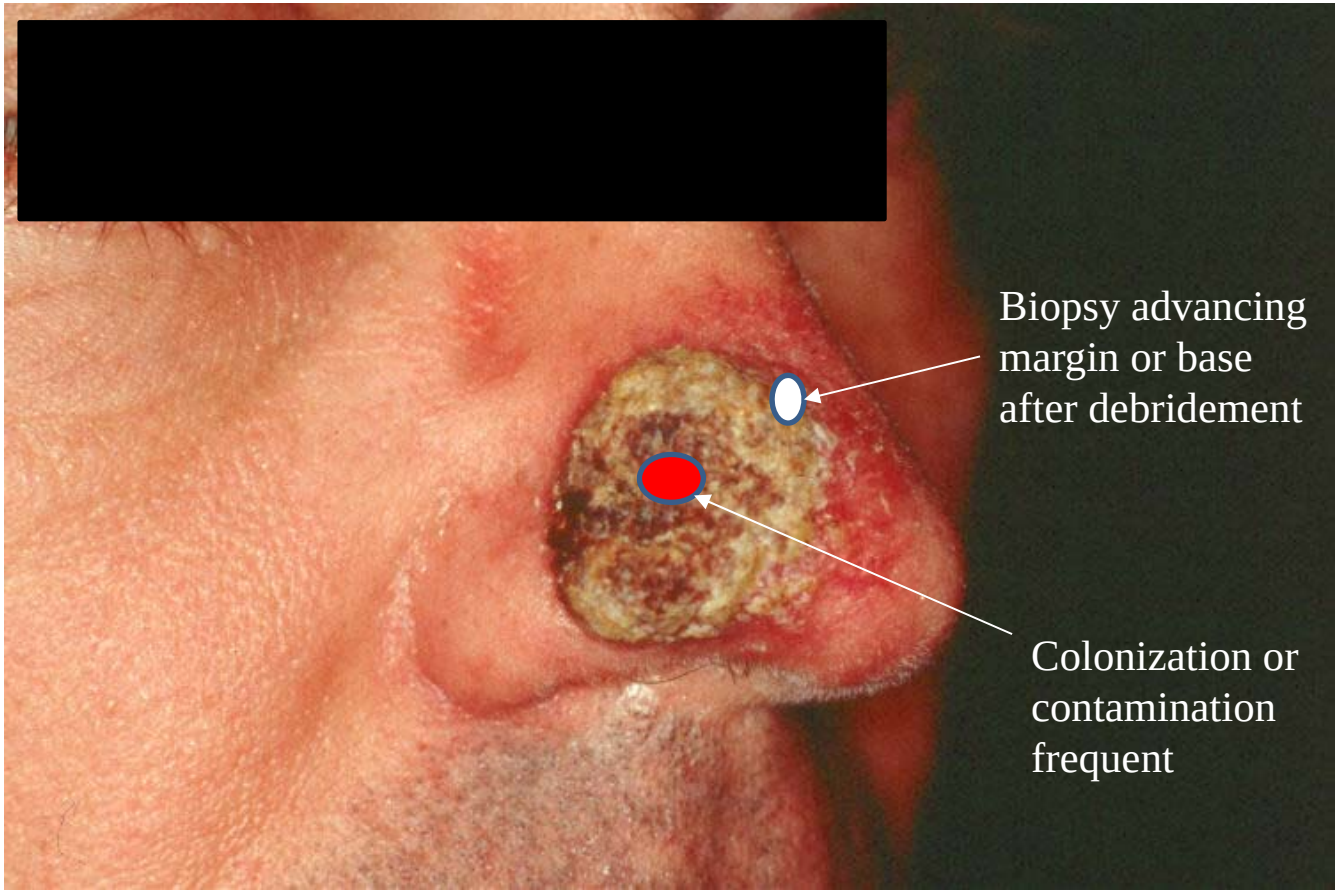
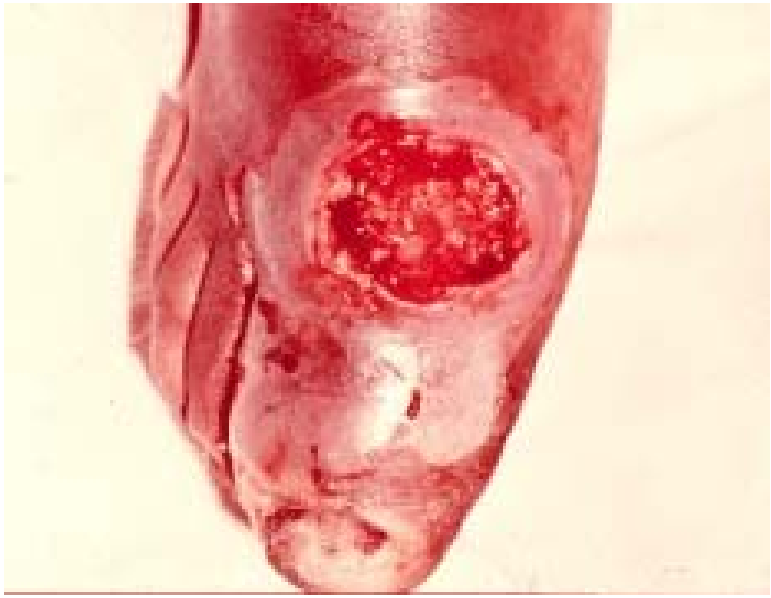
Ulceration due *Coccidioides spp.*



A patient with a diabetic foot ulcer is seen in clinic and the ulcer looks ugly but otherwise has no red streaking outside the borders. It is best to:

1. Not culture the ulcer surface?
2. Culture surface of ulcer with swab?
3. Debride carefully and submit advancing margin biopsy or deep base and bone tissue for culture?
4. Cauterize surface of ulcer prior to collection of specimen for culture?



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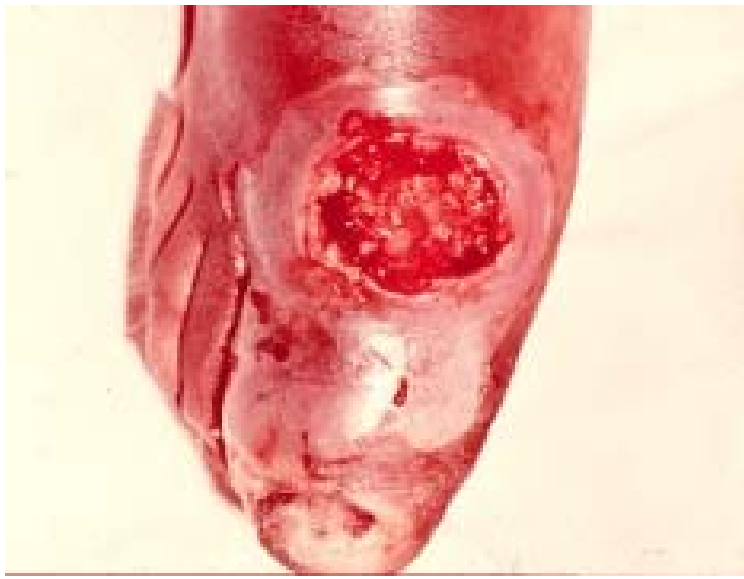
A patient with a diabetic foot ulcer is seen in clinic and the ulcer looks ugly but otherwise has no red streaking outside the borders. It is best to:

1. Not culture the ulcer surface (correct)
2. Culture surface of ulcer with swab
3. Debride carefully and submit advancing margin biopsy or deep bone tissue for culture if osteo suspected (possible choice)
4. Cauterize surface of ulcer prior to collection of specimen for culture



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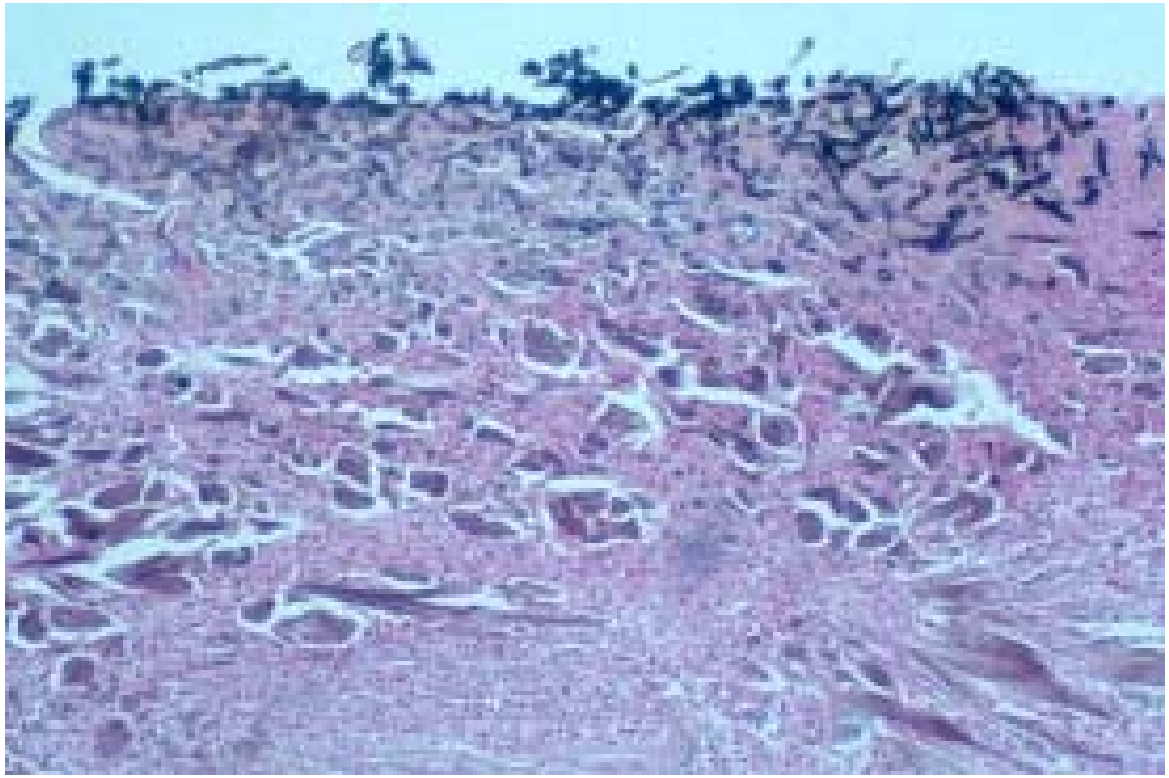
- Culture of swab of surface of ulcer grew *Bipolaris spicifera* (dematiaceous mould)
- Clinician wanted to start Amphotericin B therapy for fungal infection
- Biopsy showed the following:

H & E stain from the diabetic ulcer



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Clinical and Microbiological Relevance

Upper Respiratory tract

- Pharyngitis (throat): Strep grp A only
- Otitis (ear): tympanocentesis
- Sinusitis:
  - Aspirate (not nasal swab or drainage)
  - Endoscopically guided NP swab at meatus

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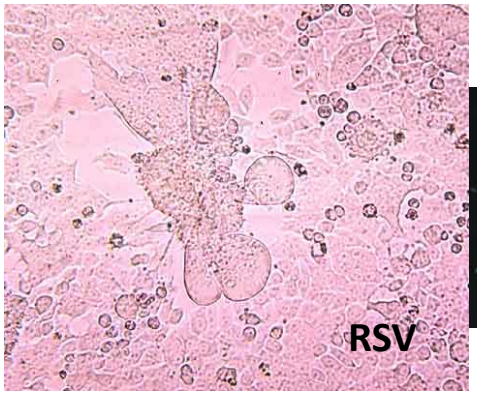
Upper Respiratory Tract Pathogens

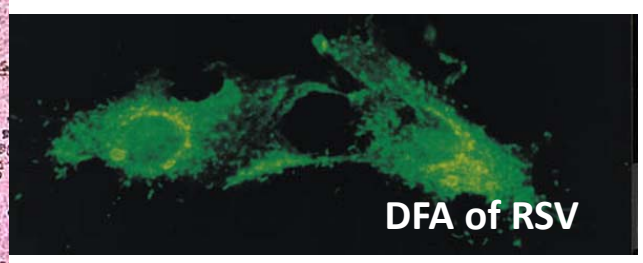
Respiratory Syncytial Virus

- Wheezing, bronchiolitis
- EIA, DFA, culture, (RT)-PCR

Other respiratory viruses

- DFA, culture, PCR





Pertussis

- very active in AZ; most common vaccine-preventable disease in children under 5; increased in older pop.
- Common in adults, carriers
  - DFA, culture on Regan-Lowe, Bordet-Gengou,
  - PCR on older vaccinated children and adults, serology

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Clinical and Microbiological Relevance

Lower Respiratory Tract

- Sputum: routine culture - screen for WBCs/ squamous epithelial cells (expectorated, induced)
- Most groups use <10-12 epis, > 25 WBCs per LPF
  - Work up only organism(s) associated with WBCs as indicated by Gram-stain
- Endotracheal aspirate- often confusing results due to colonization, especially in endotracheal tubes; results in overtreatment of patients
- Protected-Brush Bronchoscopy or Bronchoalveolar lavage: quantitative culture and cytospin-centrifuged Gram-stain (intracellular organisms)

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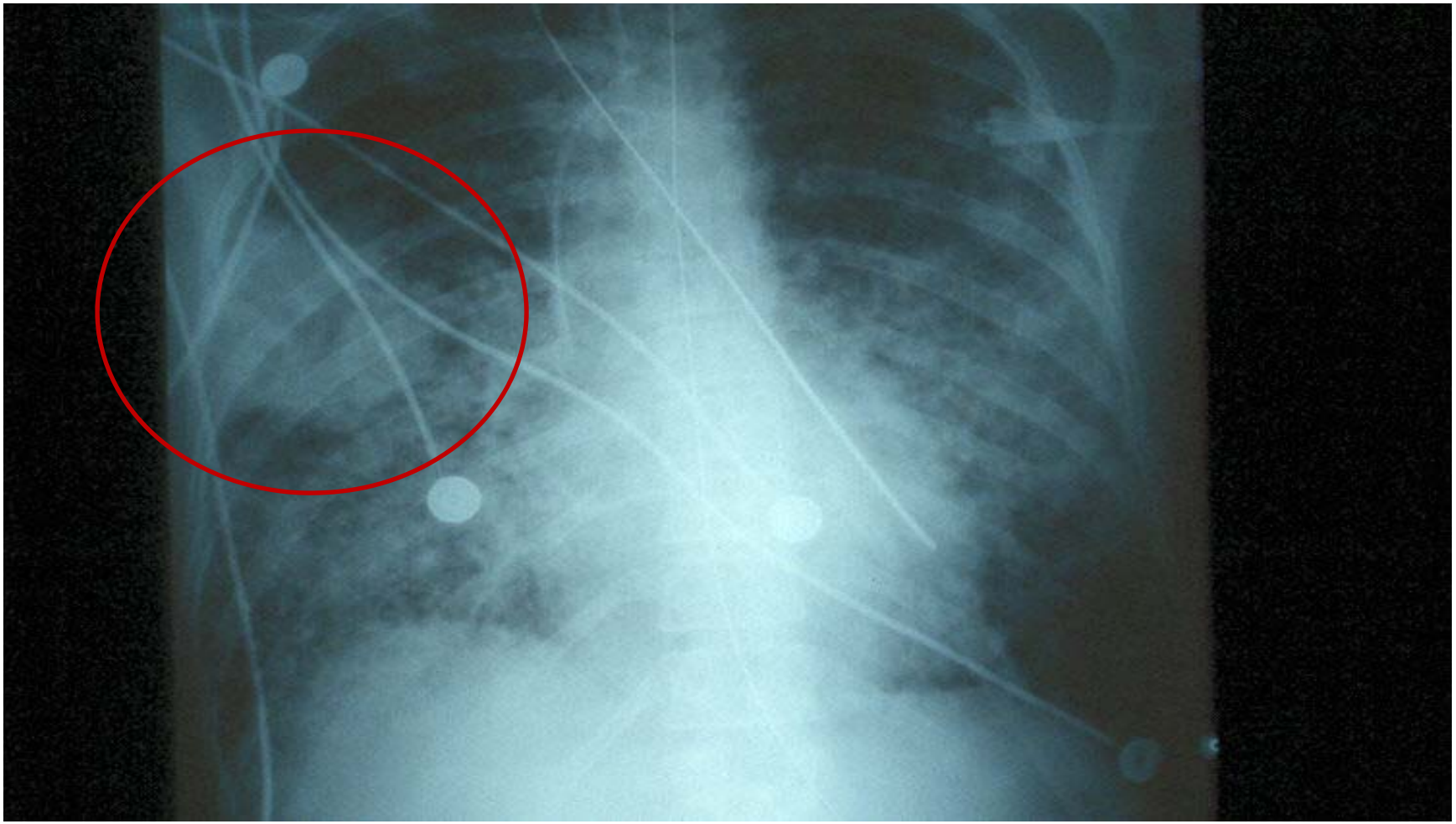
Dx of CAP - X-ray



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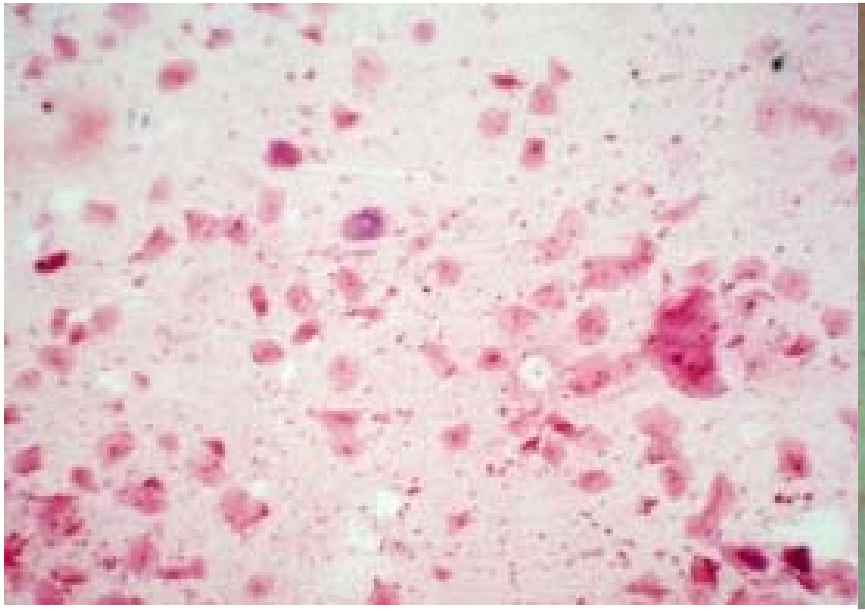
Sputum Screening: Gram stain of sputum contaminated with saliva



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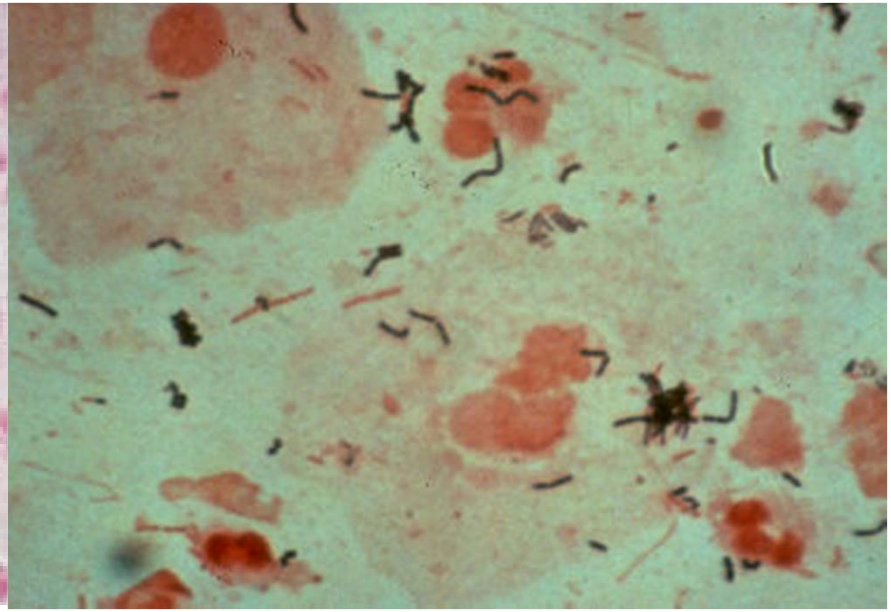

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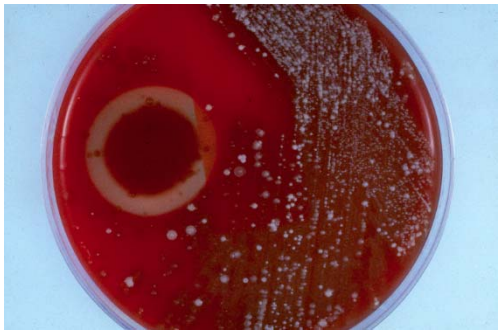

Growth of mixed flora on culture

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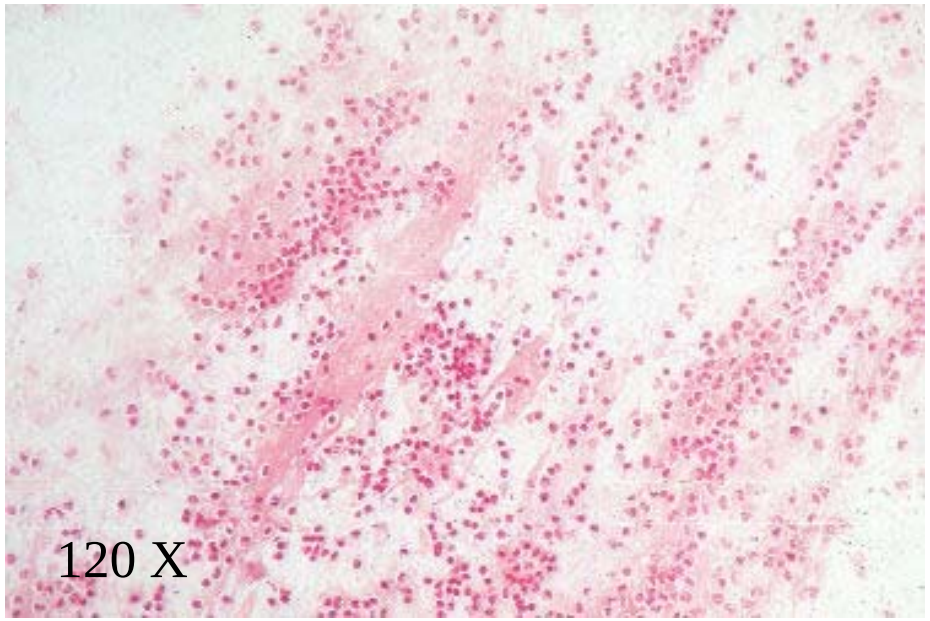
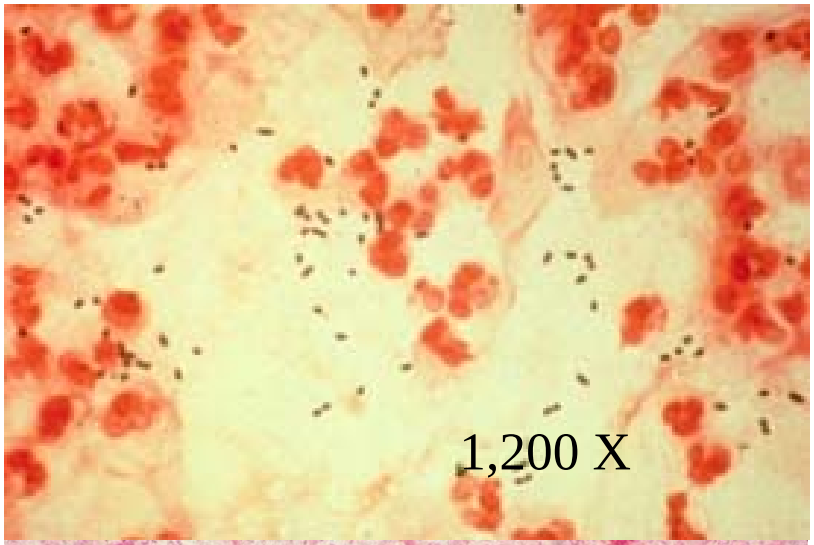
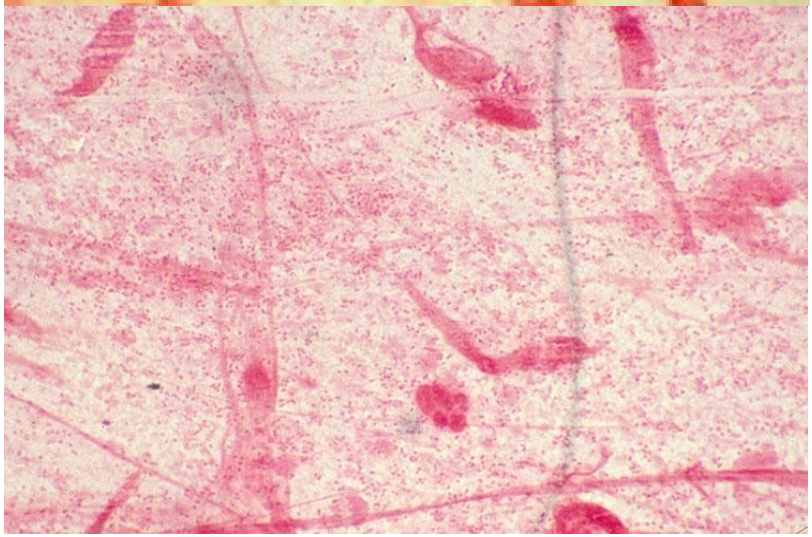
Gram stain of sputum with polymorphonuclear cells (PMNs) 120 X







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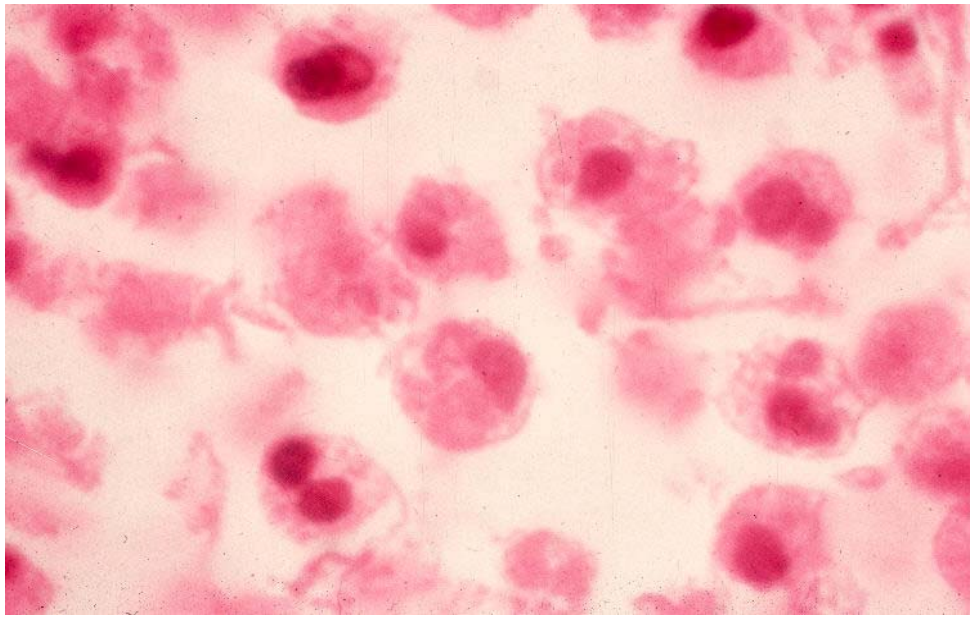
Gram stain of sputum with PMNs but no pathogenic organisms

- Potential reasons:
  - Therapy prior to specimen being collected
  - Stealth bug (TB, Legionella, Coccy, Mycoplasma, Viruses)



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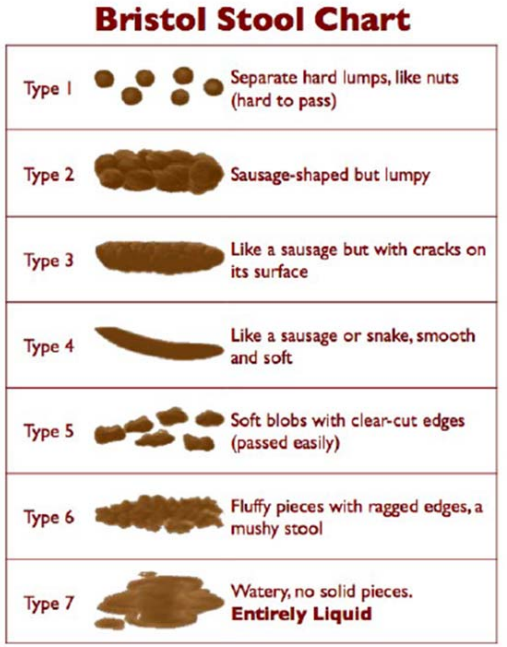
Gastrointestinal Tract

- Use separate appropriate transport systems for Dx of bacterial and parasitic infections
- Test only loose, diarrheal stools
- Acute presentation, <=7 days:
  - Stool examination for routine pathogens:*Salmonella, Shigella, Campylobacter*; in high numbers: *Yersinia, Vibrio, Aeromonas, E. coli* 0157 or Shiga Toxin
    - Giardia Ag or FA (if history indicates camping, travel to endemic areas, day-care center)
    - Norovirus (in right setting and if clinically warranted)



The Brecher Guidelines

If it ain't loose, it's of no use
Put a lab stick in the stool:
If the stick stands, the test is banned; if the stick falls, test them all

And my favorite so far…

"If you can't slurp it with a straw it ain't diarrhea"

An anonymous physician from Tampa General Hospital

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Clinical Microbiology and ID

Gastrointestinal Tract

- Chronic presentation, >7 days, history supports travel or from endemic area and with negative routine studies:
  - DFA/EIA for Giardia
  - Full O&P Exam (X 1 initially; additional 2 spaced over several days if warranted)
- *Clostridium difficile* (GDH + Toxin and/or PCR vs Toxin A &B)
  - If patient has diarrhea after >=3 days in hospital (other studies should not be ordered)
  - Consider if patient is on laxatives (do not order Cdiff unless severe and protracted)

PRACTICE APPROACH:
Recommended Clinical Practice

PRACTICE STATEMENT:
Laboratory evaluation of stools for diagnosing adult and pediatric diarrheal disease will be done when patient symptoms include at least one of the following:

- Severe diarrhea
- Temperature of >38.5 0 C or 101.3 0F
- Passage of bloody stools
- Stools positive for leukocytes, lactoferrin, or hemoccult testing
- Persistent diarrhea which has not been treated with antibacterial agents empirically

Rationale:
Studies in the United States have found that routine laboratory studies on stool specimens are frequently ordered inappropriately, resulting in excessive medical costs and overutilization of decreasing available resources. Laboratory detection of bacterial pathogens in stools remains in the 2-4 percent rate, while detection of parasites has fallen to below 1%. Laboratory evaluation of stools from patients should not be ordered routinely but reserved for the appropriate clinical and epidemiologic setting

CLINICAL APPROACH:
Select laboratory studies that best match the patient condition:

1. **Community-acquired or traveler's diarrhea of <=7 days duration**

Strongly consider testing for:

- **Routine Bacterial Stool Culture** for:
  Salmonella, *Shigella*, *Campylobacter*, *E. coli* 0157:H7
- Testing for **Shiga toxin** when clinically indicated
  - Note: this test can replace the *E. coli* 0157:H7 culture as a component of the stool evaluation

Other Clinical Situations:

- ***Clostridium difficile* toxin assay** if patient with history of antimicrobial therapy or chemotherapy within recent weeks of onset
- **Giardia Ag EIA** in patient with history of day care (child), or a hiker/camper or immunocompromised patient
- Cryptosporidium by DFA only if outbreak known to be occurring

2. Community-acquired or traveler's diarrhea that is persistent or chronic (>7 days duration)

Strongly consider testing for:

- As in 1 above if not already performed
- **Giardia Ag EIA**

Other Clinical Situations:

- **Cryptosporidium by DFA** in known outbreak
- **Isospora and Cyclospora** only if outbreak in area

Full Ova and Parasite Studies should be requested ONLY on patients with diarrhea and relevant travel history, patients who have recently been residents of a developing country, and patients in an area of the United States where parasites other than *Giardia* are found

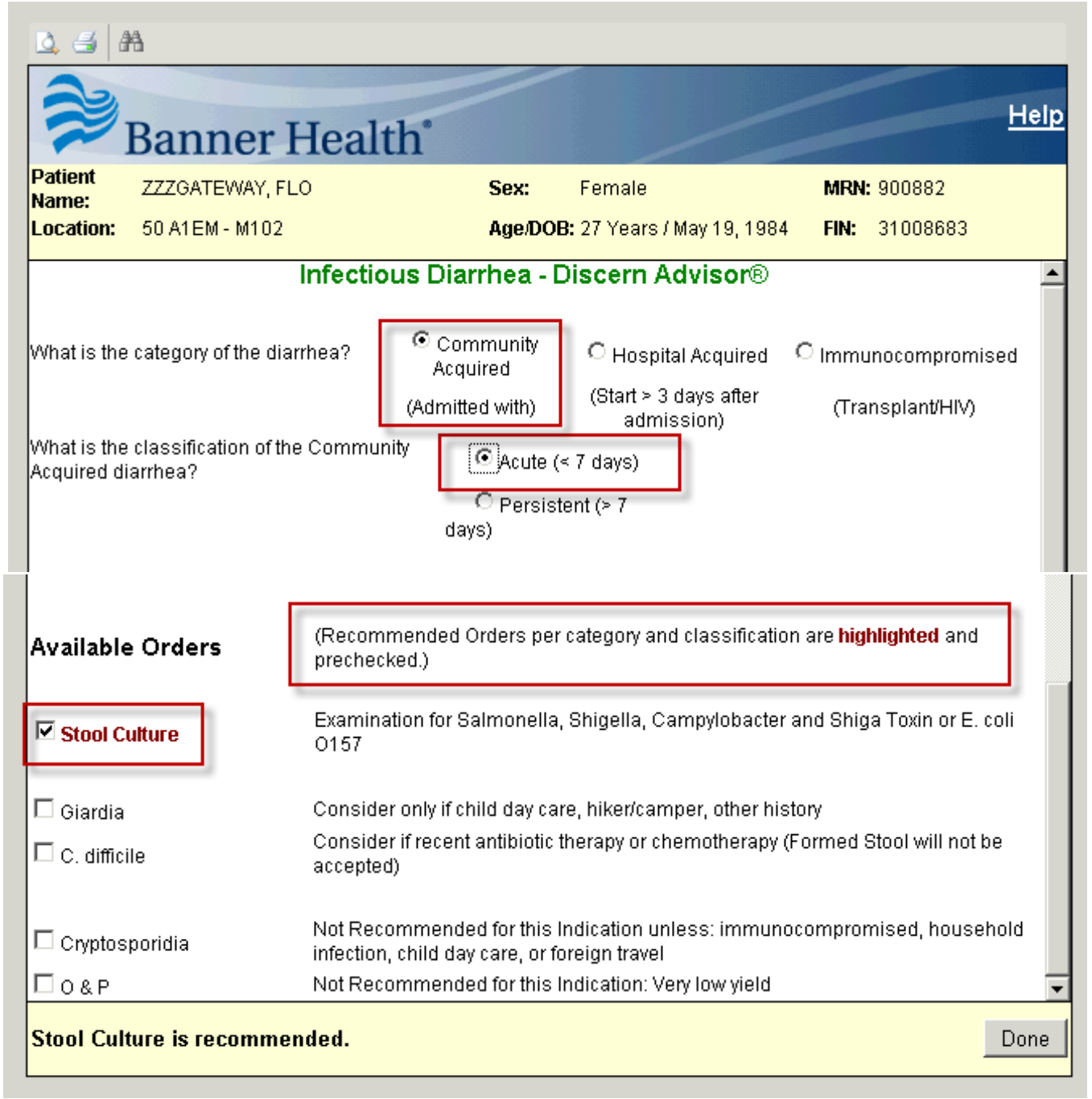
- **Single O & P full exam** only if tests above return negative and diarrhea persists (especially in immunocompromised patients or those who have been associated with developing countries)
- **Repeat O & P full exam X 2** (collected on separate days one to two days apart) if initial O & P exam is negative and symptoms persist

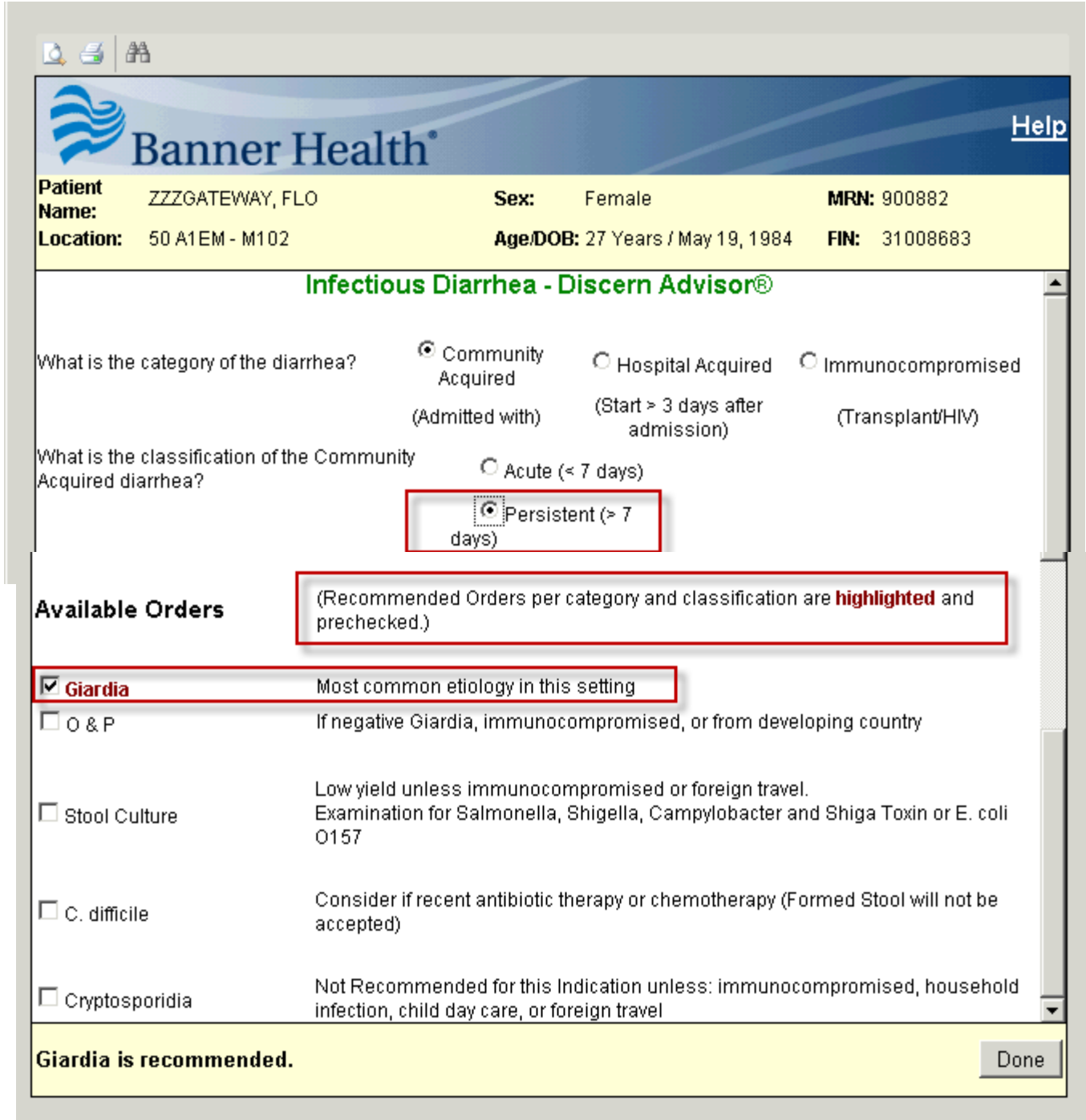
3. . Hospital-associated diarrhea (onset >3 days after admission)

- ***Clostridium difficile*** toxin assay
- As in I above only if patient with bloody stool, immunocompromised or infant and C. diff test negative

4. . HIV or severely immunocompromised patient

Depending on immune status of patient and his/her condition, more rapid progression of testing may have to be pursued and special situations may have to be evaluated. An Infectious Disease consult should be considered.


Banner Health
Help
Patient Name: ZZZGATEWAY, FLO
Sex: Female
MRN: 900882
Location: 50 A1EM - M102
Age/DOB: 27 Years / May 19, 1984
FIN: 31008683
Infectious Diarrhea - Discern Advisor®
What is the category of the diarrhea?
Community Acquired
(Admitted with)
Hospital Acquired
(Start > 3 days after admission)
Immunocompromised
(Transplant/HIV)
What is the classification of the Community Acquired diarrhea?
Acute (< 7 days)
Persistent (> 7 days)
Available Orders
(Recommended Orders per category and classification are highlighted and prechecked.)
Stool Culture
Examination for Salmonella, Shigella, Campylobacter and Shiga Toxin or E. coli O157
Giardia
Consider only if child day care, hiker/camper, other history
C. difficile
Consider if recent antibiotic therapy or chemotherapy (Formed Stool will not be accepted)
Cryptosporidia
Not Recommended for this Indication unless: immunocompromised, household infection, child day care, or foreign travel
O & P
Not Recommended for this Indication: Very low yield
Stool Culture is recommended.
Done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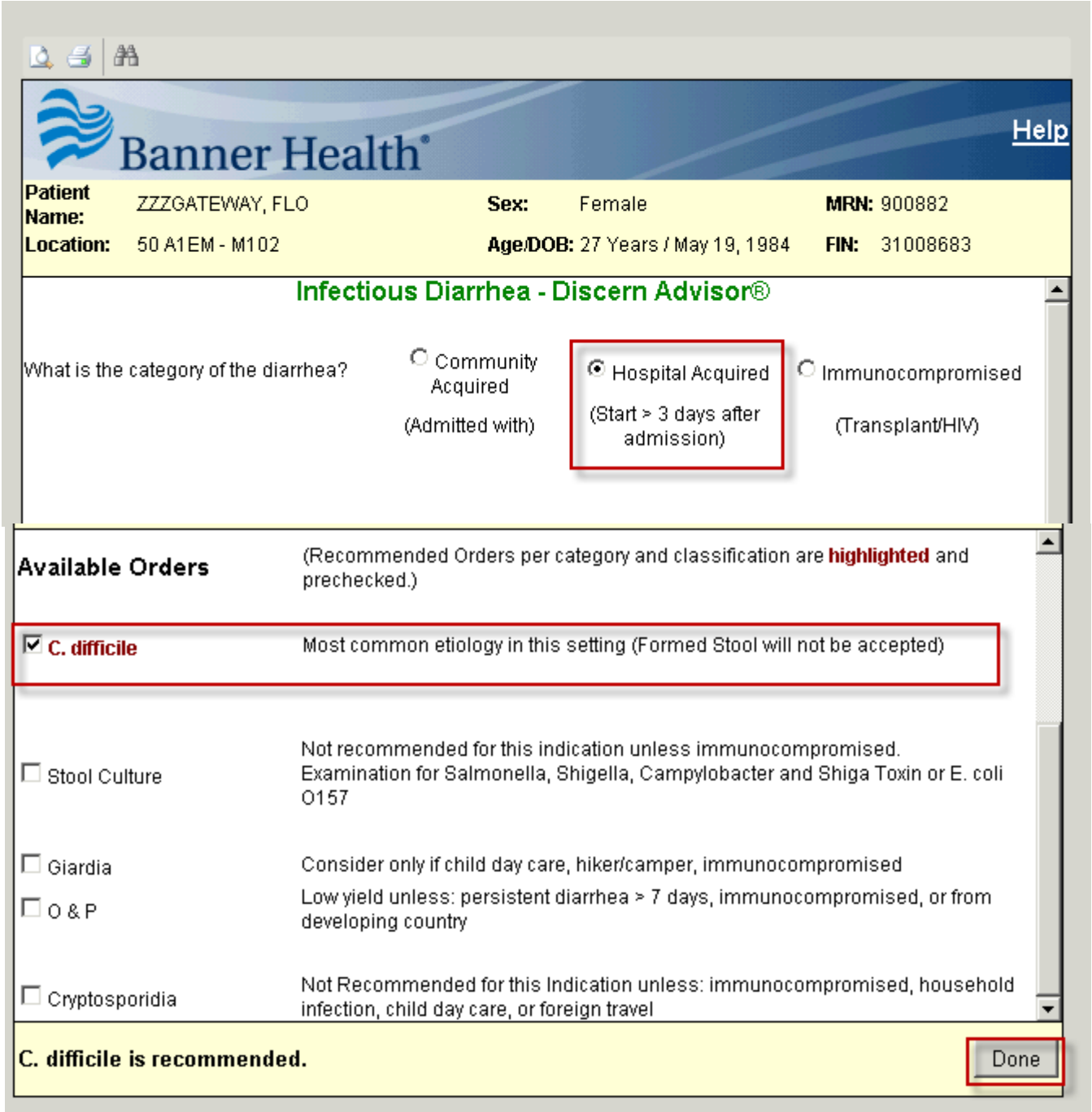
What is the classification of the Community Acquired diarrhea?
Acute (< 7 days)
Persistent (> 7 days)
Available Orders
(Recommended Orders per category and classification are highlighted and prechecked.)
Giardia
Most common etiology in this setting
O & P
If negative Giardia, immunocompromised, or from developing country
Stool Culture
Low yield unless immunocompromised or foreign travel.
Examination for Salmonella, Shigella, Campylobacter and Shiga Toxin or E. coli O157
C. difficile
Consider if recent antibiotic therapy or chemotherapy (Formed Stool will not be accepted)
Cryptosporidia
Not Recommended for this Indication unless: immunocompromised, household infection, child day care, or foreign travel
Giardia is recommended.
Done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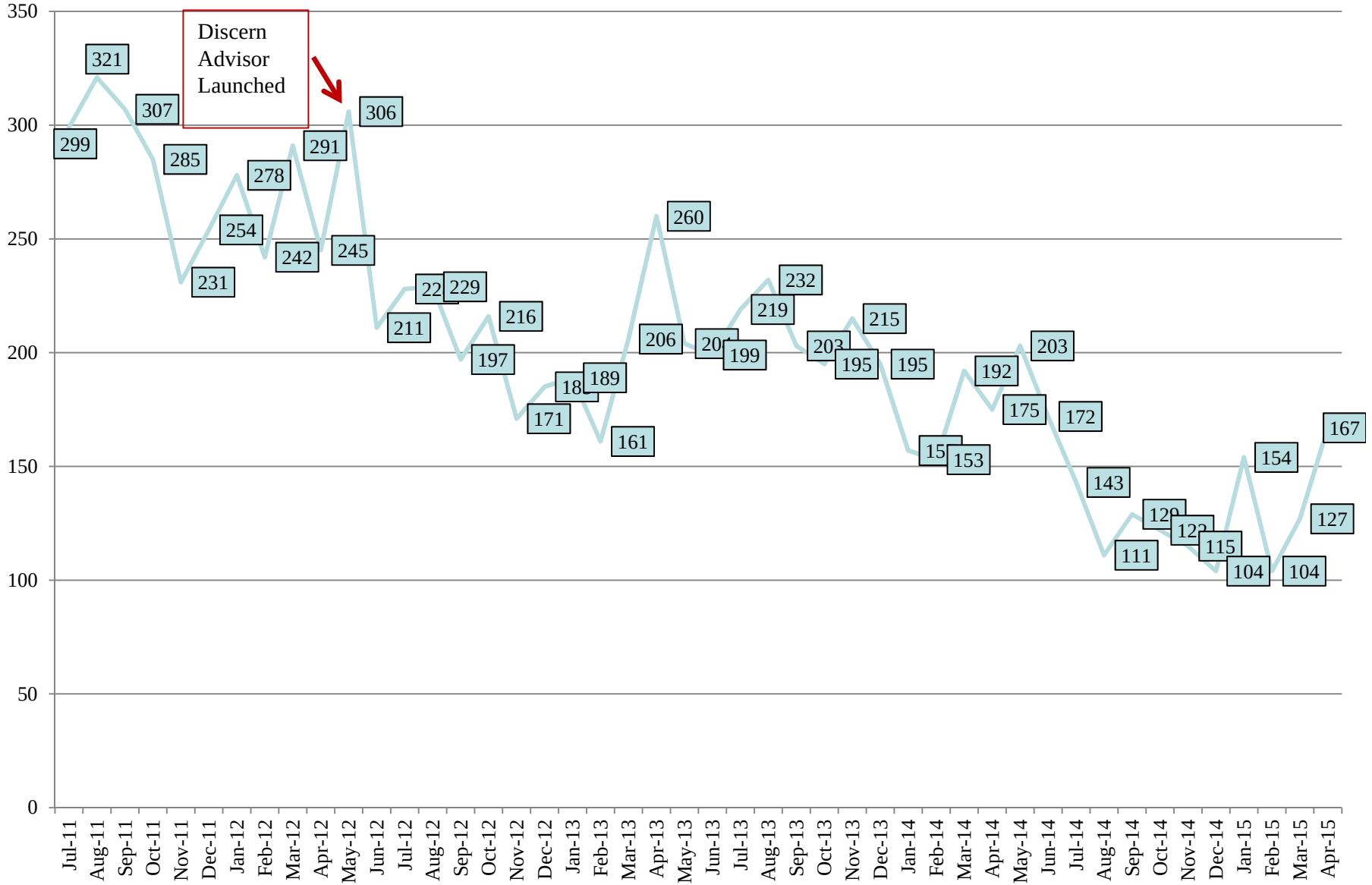
(Transplant/HIV)
Available Orders
(Recommended Orders per category and classification are highlighted and prechecked.)
C. difficile
Most common etiology in this setting (Formed Stool will not be accepted)
Stool Culture
Not recommended for this indication unless immunocompromised. Examination for Salmonella, Shigella, Campylobacter and Shiga Toxin or E. coli O157
Giardia
Consider only if child day care, hiker/camper, immunocompromised
O & P
Low yield unless: persistent diarrhea > 7 days, immunocompromised, or from developing country
Cryptosporidia
Not Recommended for this Indication unless: immunocompromised, household infection, child day care, or foreign travel
C. difficile is recommended.
Done

Monthly O andP Volumes- Banner Health System including Arizona and Western Region Hospitals



Urinary Tract

- Evaluate only symptomatic patients (unless immunocompromised, pregnant)
  - No symptoms – no UA or Culture
- Midstream, clean-catch urine collection (with cleansing of urethral meatus)
  - *E. coli* replication in room temp urine = one generation every 20 minutes
  - Transport immediately or place in special transport media (boric acid)
- Quantitative cultures
  - Difficult to interpret
  - Normally, urine from true UTI (symptomatic) grows >100,000 CFUs/ml of single organism (other interpretations abound for special case situations (pregnancy, etc) or if single catheter collected urine (>10,000)

Banner
University Medical Center
Phoenix

Typical FindingsClean-catch Urine in symptomatic UTI

Labs

Urinalysis: presence of nitrite and leukocyte esterase

Microscopy: WBCs but no casts seen

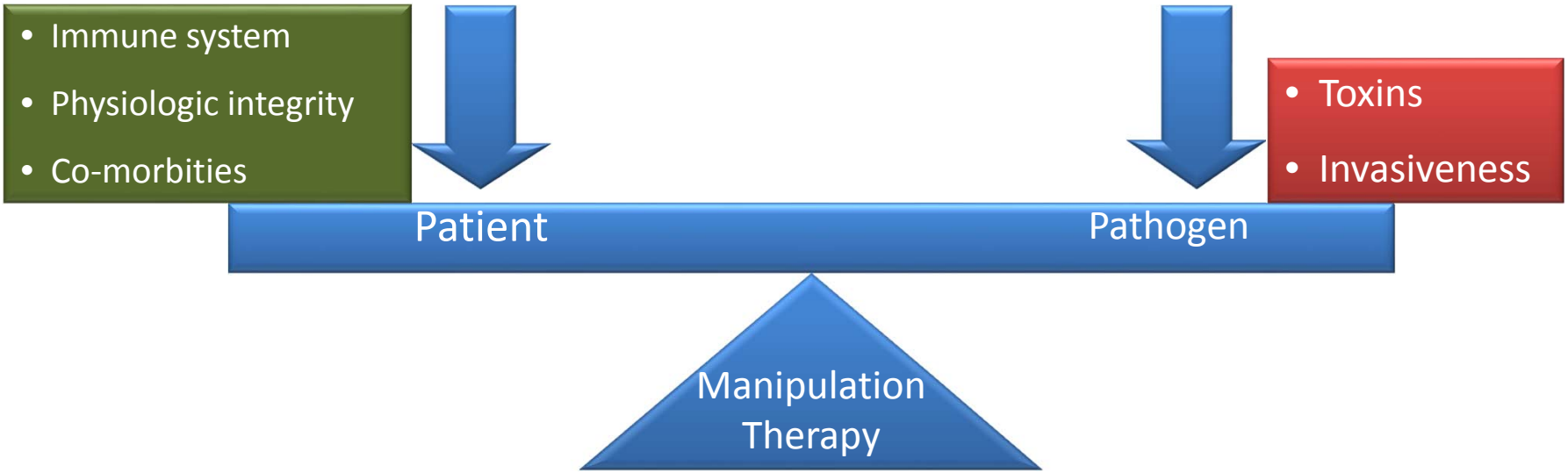
Bacterial culture:

- >10^5 cfu/ml
- GNR
- Indole-positive
- Oxidase-negative

Patient outcome depends on interaction of:

Parameters Influencing Outcomes

- Infectious process (microbiology)
- Patient's underlying condition (immunologic capability; co-morbidities)
- Simultaneous processes surrounding patient (environment, manipulation)



Thought:
How does this all relate to the practice of better medicine?

The End

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