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The Clinical Microbiology Laboratory and the Dx of Infectious Diseases

Michael A. Saubolle PhD DABMM FAAM FIDSA

Medical Director, Infectious Diseases Division,
Laboratory Sciences of Arizona – Banner Health;
Director, Infectious Diseases/Microbiology Curriculum,
Clinical Associate Professor of Medicine,
University of Arizona, College of Medicine, Phoenix/Tucson
Email: mike.saubolle@bannerhealth.com; tel: 602-839-3485

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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).
- In vitro antimicrobial susceptibility testing (AST) methods, their significance and interpretation of Clinical and Laboratory Standards Institute based resulting values.

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Laboratory Sciences of Arizona (BHS)
ID Division Metropolitan Phoenix Area

- 1 centralized Hospital Microbiology Laboratory in Tucson – B-UMCT
- 2 centralized Hospital Microbiology Laboratories in Phoenix metropolitan are
  - B-UMCP (also for West Valley hospitals; also all more esoteric or specialized testing)
  - Banner Gateway Medical Center (also for BBMC, BHH, BGMC)
    - Other Hospitals : Micro staining, set up of some specimens for culture, then sent to central labs for workup
- Sonora Quest Laboratories
  - (Banner Health [51%]; Quest Laboratories [49%]: for profit; commercial physicians' offices, clinics, nursing homes, etc.
    - Also provides virology, serology and molecular testing for the Banner Health Hospitals

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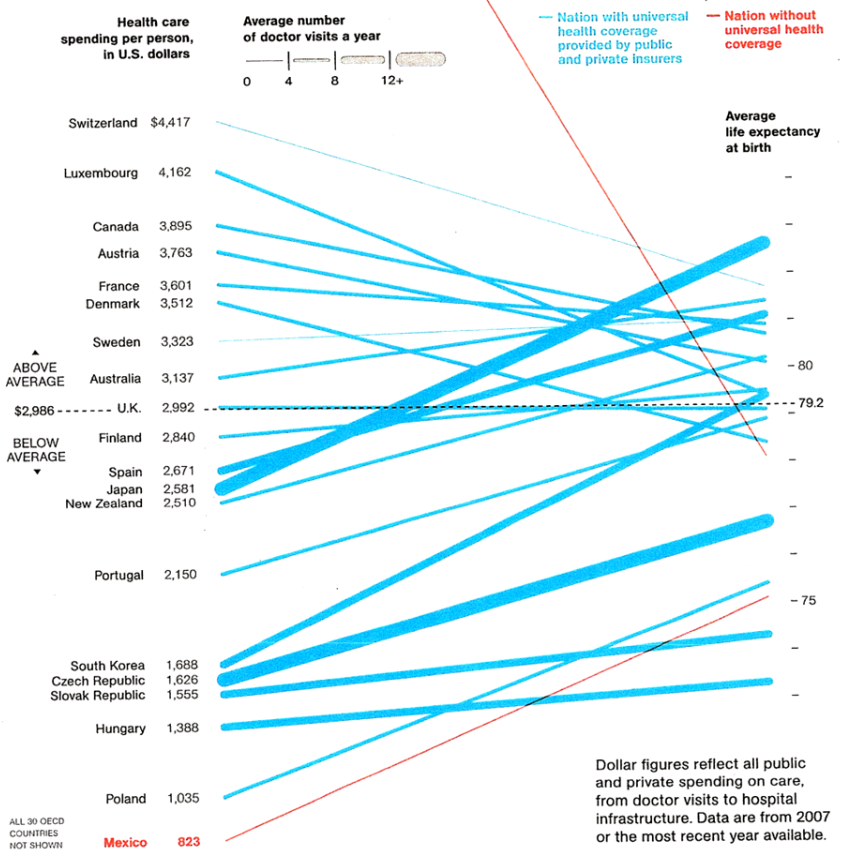
United States $7,290

HEALTH

The Cost of Care The United States spends more on medical care per person than any country, yet life expectancy is shorter than in most other developed nations and many developing ones. Lack of health insurance is a factor in life span and contributes to an estimated 45,000 deaths a year. Why the high cost? The U.S. has a fee-for-service system—paying medical providers piecemeal for appointments, surgery, and the like. That can lead to unneeded treatment that doesn't reliably improve a patient's health. Says Gerard Anderson, a professor at Johns Hopkins Bloomberg School of Public Health who studies health insurance worldwide, "More care does not necessarily mean better care." *—Michelle Andrews*



GRAPHIC: OLIVER UBERTI, NG STAFF. SOURCE: "OECD HEALTH DATA 2009," ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

National Geographic Jan, 2010

COUNTRY RANKINGS

Top 2*
Middle
Bottom 2*

	AUS	CAN	FRA	GER	NETH	NZ	NOR	SWE	SWIZ	UK	US
OVERALL RANKING (2013)	4	10	9	5	5	7	7	3	2	1	11
Quality Care	2	9	8	7	5	4	11	10	3	1	5
Effective Care	4	7	9	6	5	2	11	10	8	1	3
Safe Care	3	10	2	6	7	9	11	5	4	1	7
Coordinated Care	4	8	9	10	5	2	7	11	3	1	6
Patient-Centered Care	5	8	10	7	3	6	11	9	2	1	4
Access	8	9	11	2	4	7	6	4	2	1	9
Cost-Related Problem	9	5	10	4	8	6	3	1	7	1	11
Timeliness of Care	6	11	10	4	2	7	8	9	1	3	5
Efficiency	4	10	8	9	7	3	4	2	6	1	11
Equity	5	9	7	4	8	10	6	1	2	2	11
Healthy Lives	4	8	1	7	5	9	6	2	3	10	11
Health Expenditures/Capita, 2011**	$3,800	$4,522	$4,118	$4,495	$5,099	$3,182	$5,669	$3,925	$5,643	$3,405	$8,508

Notes: * Includes ties. ** Expenditures shown in $US PPP (purchasing power parity); Australian $ data are from 2010.
Source: Calculated by The Commonwealth Fund based on 2011 International Health Policy Survey of Sicker Adults; 2012 International Health Policy Survey of Primary Care Physicians; 2013 International Health Policy Survey; Commonwealth Fund *National Scorecard 2011;* World Health Organization; and Organization for Economic Cooperation and Development, *OECD Health Data, 2013* (Paris: OECD, Nov. 2013).

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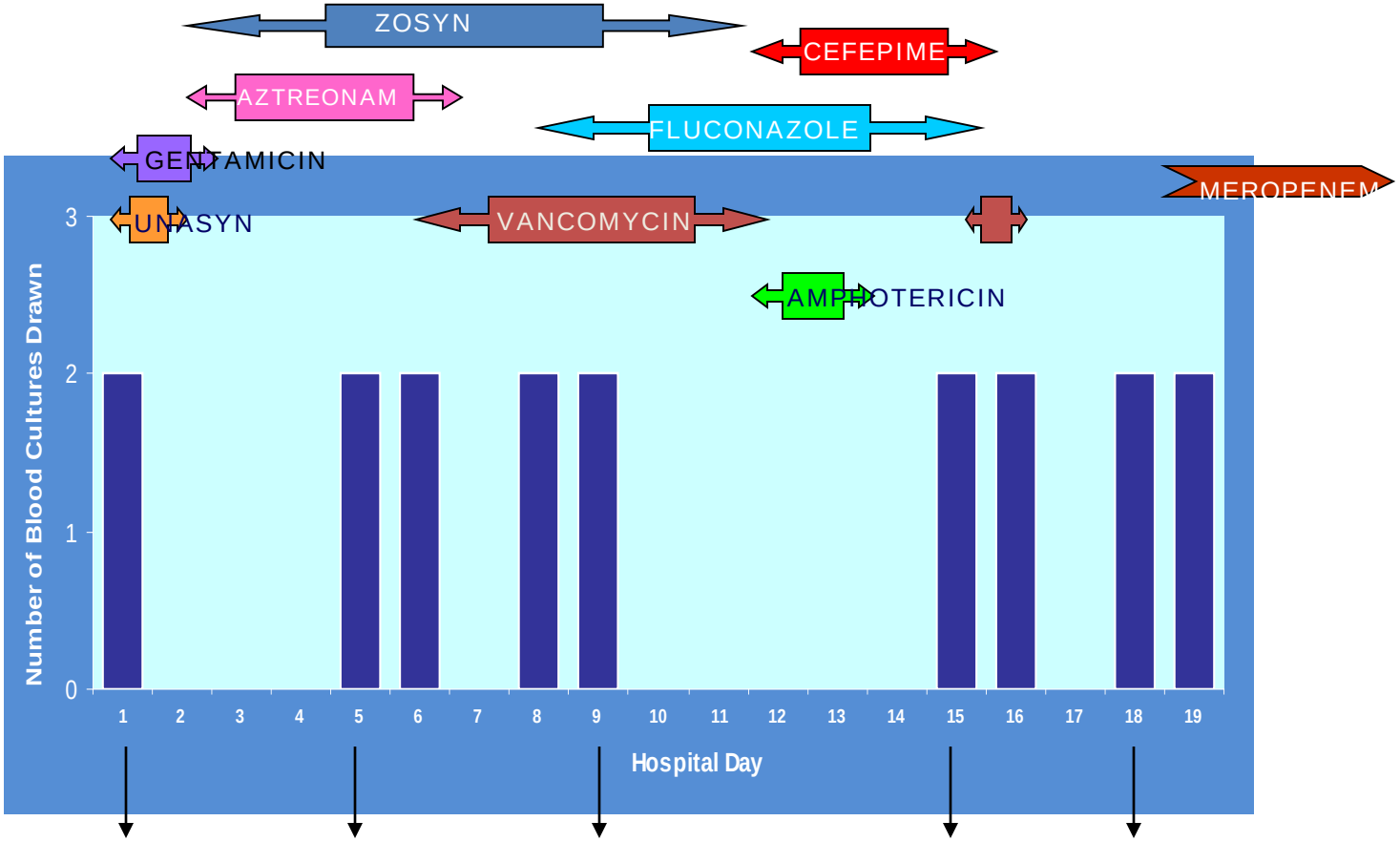
What NOT to DO

Patient Culture and Antimicrobic Rx History



Blood cultures x2:
E. coli
Urine: >10^5 *E. coli*
Enterococcus sp.
10^3

Blood cultures:
1 of 2
coagulase
negative *Staph*

Catheter tips:
Vancomycin-resistant
Enterococcus sp.
Candida albicans
coagulase negative
Staph

Blood cultures:
1 of 2
coagulase
negative *Staph*

Urine:
> 10^5VRE


phone
HELP CUT OUR COSTS PLAY HOSPITAL BINGO
FEEDING TIME 4 PM
PLEASE MAKE YOUR BED & MOP FLOOR BEFORE VISITING HOURS
NURSE
SAVE YOUR LINT FOR SNACKS
SLEEPING BAGS AIRED FIRST OF MONTH
BEDPANS EMPTIED THURSDAY
MOTEL 6
HEFTY OXYGEN TENTS
TONGUE DEPRESSORS
HOW TO CHANGE YOUR OWN BANDAGES
At a time when healthcare dollars are diminishing
"Cheer up—we're keeping our charges within the government ceilings!"

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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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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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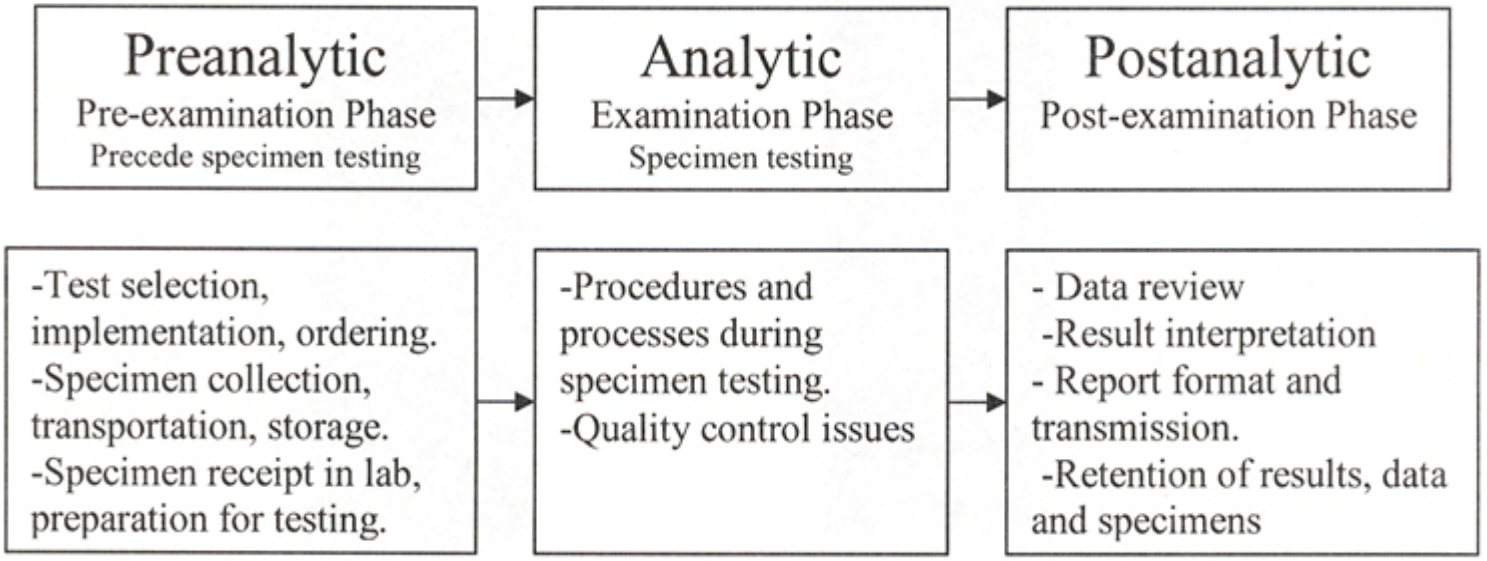
The Role of the Laboratory

The laboratory plays a central role in optimizing the care of patients with blood stream and other infections

- More than 70% of medical decisions based on lab results

Outcome of infection directly rated to the speed with which EFFECTIVE antibiotic therapy is instituted

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

The Laboratory

The function of a clinical laboratory is the provision of accurate, clinically significant data for the diagnosis and therapy of medical conditions in patients

- Data can be used to provide individual patient with a management plan to increase probability of achieving desirable outcome
- Production of laboratory data is culmination of sequential processes including preanalytic, analytic and postanalytic laboratory activities ("path of workflow")
- Activities begin with a clinician's request for specific studies on an individual patient (or in some states patient can order tests, **BUT**
- **Laboratory must adhere to regulatory and financial oversight**

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Regulatory Issues and the Laboratory

- **Clinical Laboratory Improvement Amendments (CLIA) passed by Congress in 1988:**
  Established quality standards for laboratory testing and to ensure the accuracy, reliability and timeliness of patients test results

  - Categorized tests into **"waived" and "nonwaived"** (2003)

  - **Waived tests**: simple lab procedures and examinations which are cleared by the FDA and have easy methodologies with little or no harm to patient if performed incorrectly

  - **Nonwaived tests**: more difficult methodologies requiring greater personnel training; labs performing such testing require CLIA certification or licensure and must follow strict requirements and criteria

Regulatory Issues and the Laboratory

- Must have written guidelines and procedures for all work being performed, including preanalytic, analytic and postanalytic activities. Must follow all procedures.
- Tests using commercial reagents: **FDA approved, validated, verified** (sensitivity, specificity, reference ranges, outcomes); **note: only FDA approved are reimbursed by CMS**
- Adequate personnel (and appropriate level of education for each task performed)
- Quality Control and Quality Assurance programs in place with documentation
- **For Licensure must be certified and inspected by a deemed Regulatory Agency (e.g. State Health Dept, College of American Pathologists, Federal-CLIA)**

CMS - Centers for Medicare and Medicaid Services

Laboratory Billing Issues (presently pay-for-service in most instances)

- Reimbursement by CMS or other third-party payers
- Only FDA approved tests usually reimbursed by CMS and many other third-party payers
- Tests must be recognized by the American Medical Association and must be assigned a Current Procedural Code (CPT-10) by the AMA's CPT Committee
- Cost-effectiveness of test and reimbursement must be considered when a lab provides tests
- Tiered reimbursement:
  - CMS (regional differences)
  - Third-party payers (contractual basis)
  - Private payers (usually the highest cost)

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Billing for Inpatient Laboratory Procedures

Procedure	Amount Billed
• Aerobic Bacterial Culture	$260
– Each identification	$24
– Each susceptibility	$140
• Anaerobic Culture	$250
• Fungus Culture (ID, suscept. additional)	$250
• AFB Culture (ID, suscept.additional)	$260
• Ova & Parasitology	$212
• Virus Culture (full)	$254
• Virus Shell-Vial Culture	$143

Note: CMS and third party payers do not pay these prices; CMS pays by Diagnostic Related Groupings or DRGs; CMS no longer pays for many "nosocomially acquired conditions."

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Lab Billing Issues: Reimbursement for Outpatient Lab Procedures

Procedure	Amount billed	Paid by 3rd Party
1	$6.30	$3.06
2	$113.00	$9.12
3	$31.00	$11.56
4	$12.03	$6.14
5	$10.07	$5.14
6	$31.00	$15.81
7	$36.00	$5.53
Total	$239.40	$45.08

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.

Infectious Diseases
Crucial Points in Diagnosis

Pre-analytic

- PE, history (including travel), 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.

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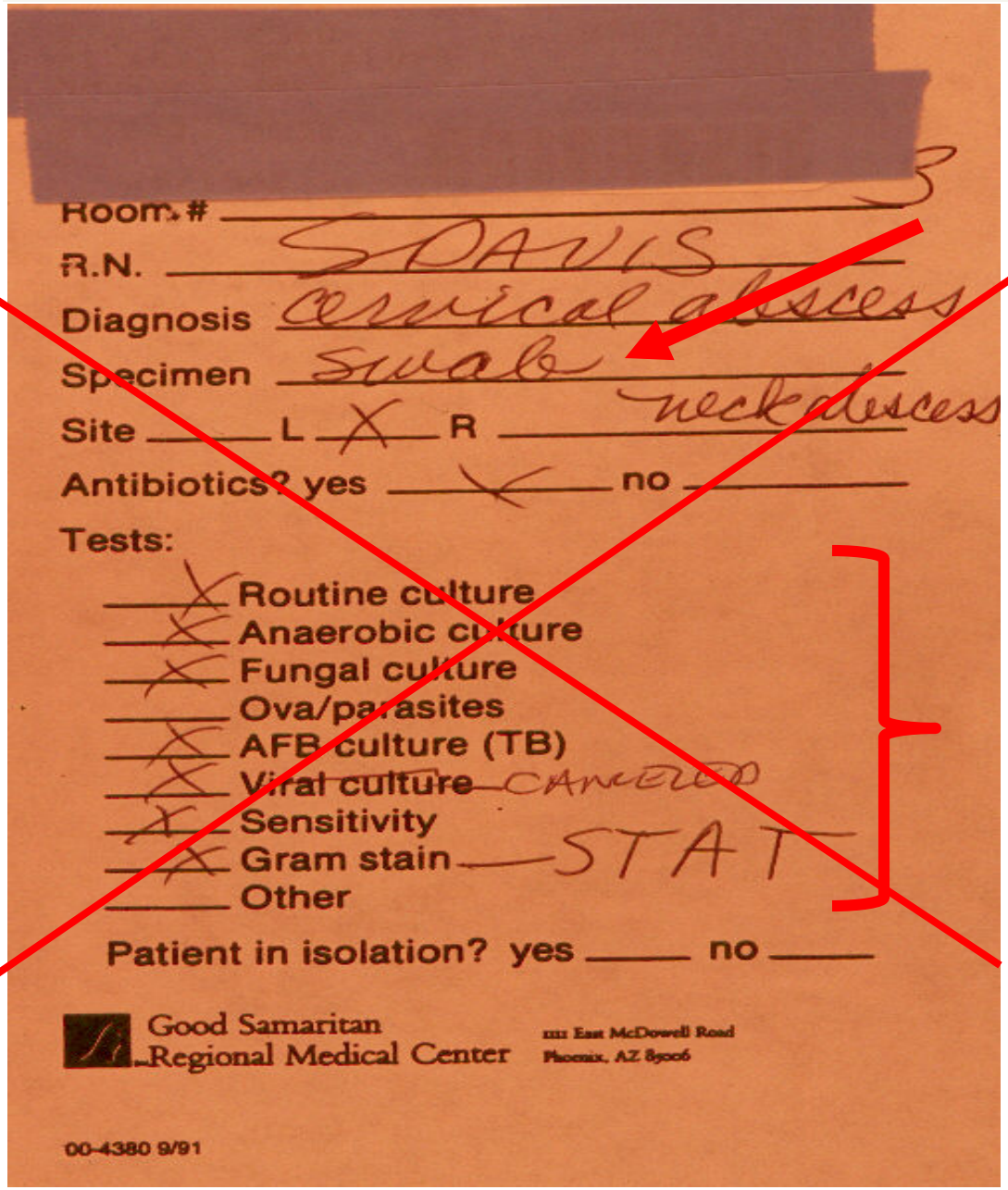
Clinical Relevance vs. Cost of Laboratory Testing and Results

- 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)**

Utilization – the shotgun method



Room # 3

R.N. DAVIS

Diagnosis cervical abscess

Specimen swab

Site ___ L X R ___ neck abscess

Antibiotics? yes X no ___

Tests:

- X Routine culture
- X Anaerobic culture
- X Fungal culture
- ___ Ova/parasites
- X AFB culture (TB)
- X Viral culture CANCELED
- X Sensitivity
- X Gram stain — STAT
- ___ Other

Patient in isolation? yes ___ no ___

Good Samaritan Regional Medical Center

1111 East McDowell Road
Phoenix, AZ 85006

00-4380 9/91

Case

Patient with para-spinal abscess is debrided and surgical specimens submitted for microbiologic evaluation:

- 6 specimens submitted; all from around spinal column with abscess
- Surgeon ordered 1. Bact Cult & sens; 2. Anaerobic Cult and Sens; 3. AFB Cult; 4. Fungus Cult; 5. O & P Exam on EACH SPECIMEN submitted
- Direct Gram stain showed GPC in Clusters and previous BCBs were already positive for *Staphylococcus aureus*
- Surgeon would not allow changing orders, so we went to attending who canceled all except for the original Bact/Sens on a single specimen ($260)
- Total would have been $1,232 x 6 = $7,392

Differences in Human Microbiome Composition by Body Site

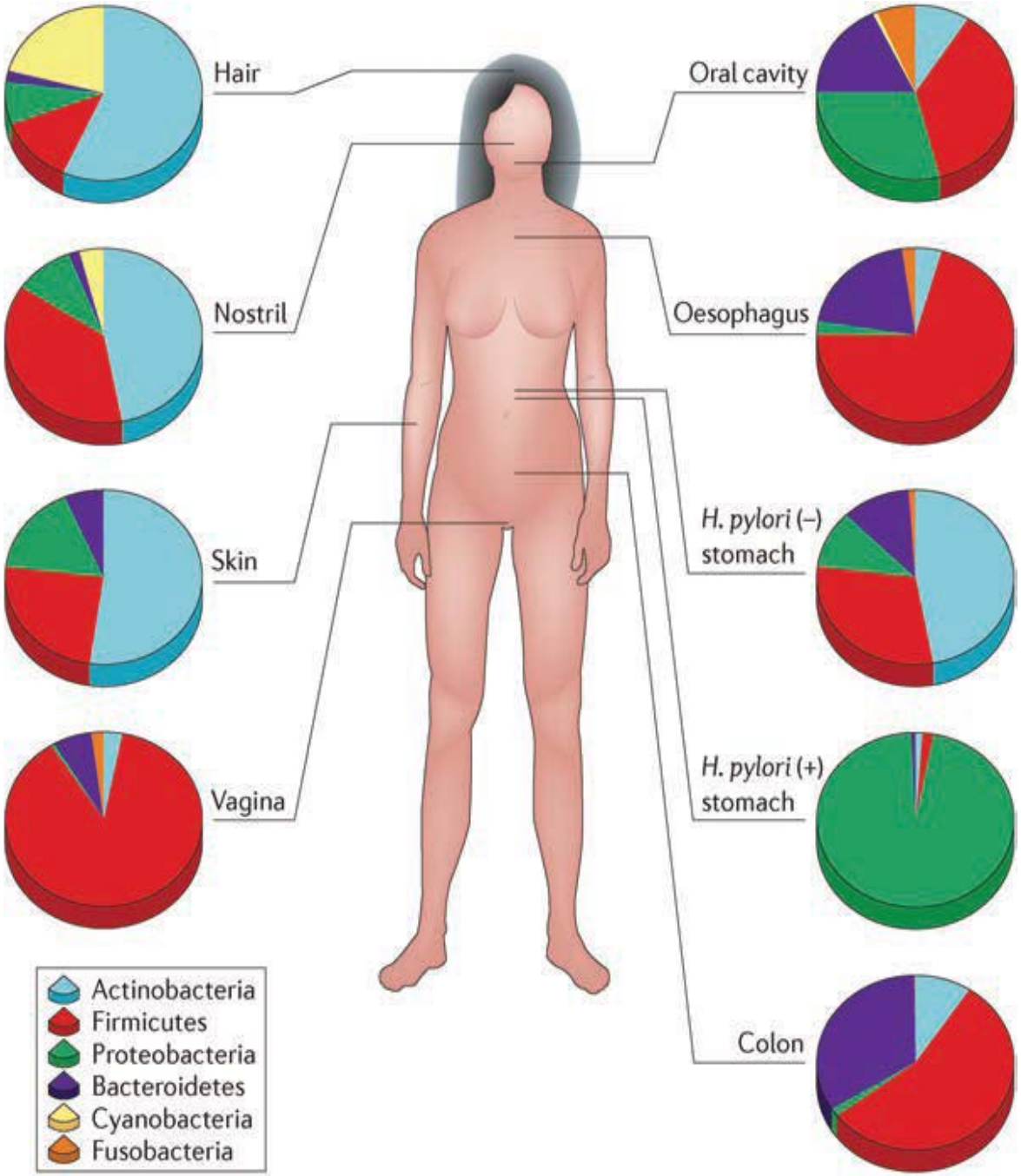


Table 1. Approximate numbers of bacteria inhabiting various regions of the human body, as collated from various printed sources[1–6,8,9,48,87]

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

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."

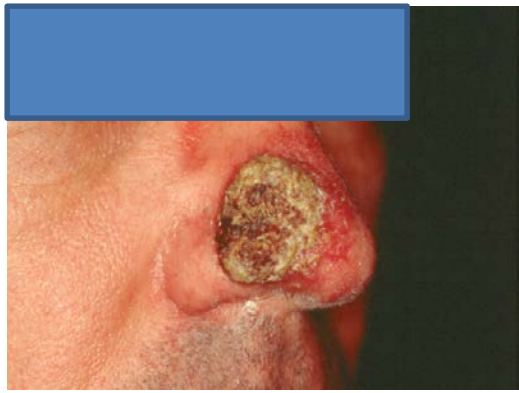
Catherine D. DeAngelis, MD
Arch Pediatr Adolesc Med 1994; 148:1277.

Pre-analytic: Test Ordering

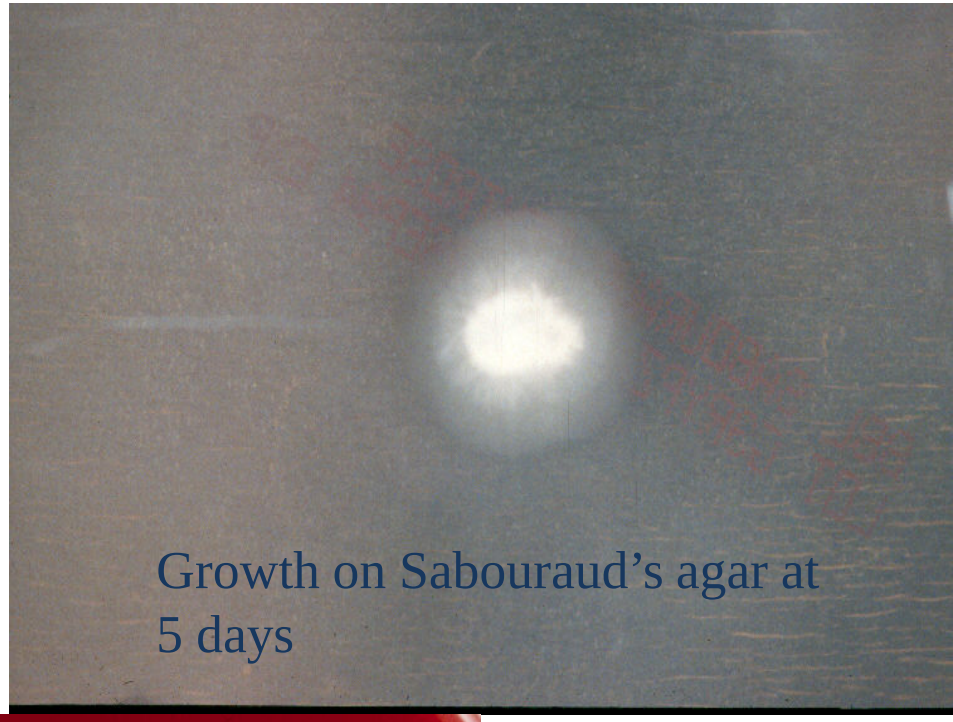
- Know what test(s) to order; understand ordering process (may differ between institutions) – get trained
  - Bacterial Cultures (routine, anaerobic, screens)
  - Other: Fungus, AFB, O&P, Viral cult
- Know when to order special studies:
  - Pertussis
  - GC (genital, throat, perirectal)
  - Legionella
  - Borrelia, etc
- Know when to order Molecular or Serologic studies
- Never order a test which you don't know how to use or whose results you don't understand (call for help if need be)

Case:

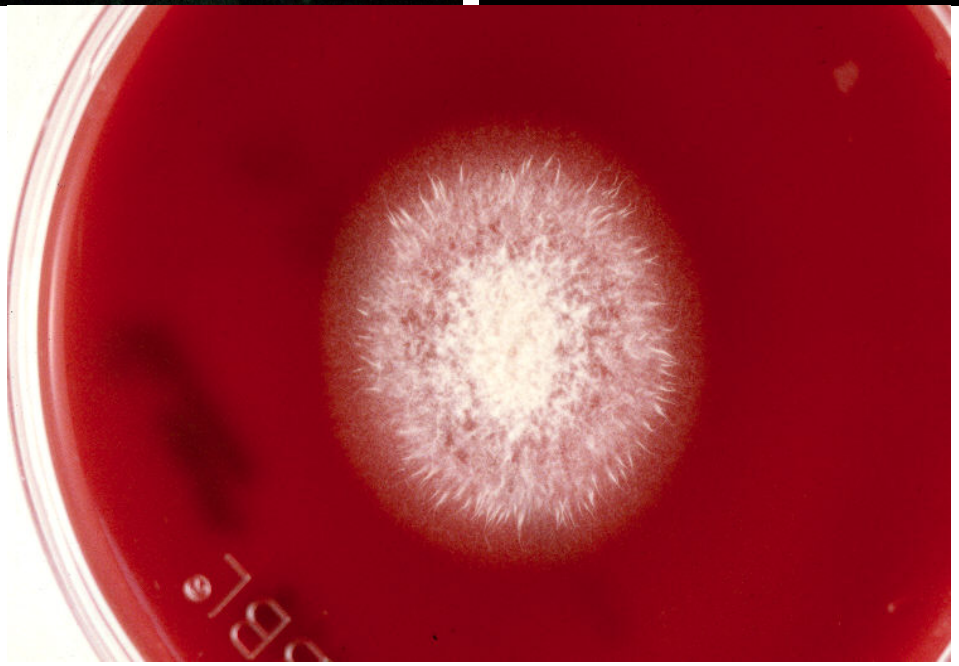
- 39 y/o male presents with 2 month history of fever, night sweats and weight loss.
- His peripheral WBC count is elevated slightly as is his sed rate
- He has a large lesion on his nose
- A swab culture of the lesion grew MRSA, coag negative staph and diphtheroids



What else can and should be done for this lesion?



Growth on Sabouraud's agar at 5 days



Growth on BAP at 10 days

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

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

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

Clinical Relevance of Laboratory Results

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

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

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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Test (Rapid Flu) characteristics based on patient population tested

Age Group	Sensitivity of Test
< 6 years	100% (17/17)
6 to 21 years	100% (19/19)
22 to 59 years	87% (21/24)
>= 60 years	78% (14/18)
Pediatrics to 59 years	98% (57/60)
All ages	91% (71/78)

Test technical insert asserted sensitivity was 90%

Sources and Types of Specimens

- Focus on clinical presentation / syndrome / patient history
  - Consider bacteremia (blood culture/peripheral smears)
  - Wound (type, location – normally sterile or topical site)
  - Respiratory tract (upper, lower)
  - Gastrointestinal (stool, fecal)
  - Urogenital
  - Urinary

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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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Value of Smear and Culture of Needle biopsy of solitary lung nodules

Forseth et al. *Arch Intern Med* 1986; 146: 319-20.

Dx by Smear	# Patients	Fungal Culture	AFB Smear	AFB Culture
Granuloma				
Spherules	49	1/33 (3%)	0/33	0/33
No Spherules	48	0/41	3/41 (7.3%)	1/41 (2.4%)
Nondiagnostic	94	2/55 (3.6%)	0/55	1/55 (1.8%)
Carcinoma	149	0/26	0/26	0/26
Benign	8	0/1	0/1	0/1
Total	348	3/156 (1.9%)	3/156 (1.9%)	2/156 (1.3%)

- Only 3 cultures yielded new evidence (2 cocci, 1 AFB)
- Cost per Dx was $3,200
- Thoracotomy cost was > $6,000, so cultures cost-effective
- However, each of these 3 patients underwent a thoracotomy before cultures turned positive within 10 days

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Resubmission of sputum after initial specimen rejection

B-UMCP Unpublished Data

Originally added comment "resubmit if clinically warranted" after each rejected sputum

- 93 patients with rejected specimens studied
- 45/93 (48%) sputum not resubmitted
- 18/93 (19%) resubmitted but still inadequate
- 27/93 (29%) satisfactory, failed to yield pathogen
- 3/93 (3%) satisfactory, yielded potential pathogen

All 3 patients evaluated and already on appropriate Rx, resubmission had no effect on patient care

1. Effectiveness of PCR testing at Banner Medical Centers in the Phoenix area (2015)

166 consecutive pts with PCR ordered

(20 CSFs - 12%; 146 BALs – 88%)

- 162/166 (98%) negative by PCR
- 4/166 (2%) positive by PCR
- 0 CSF positive by PCR
- 4 BALs positive by PCR
  - 3 also positive by serology but 2-8 days sooner
  - 1 positive by PCR, negative by serology, but no follow up
- 8 BALs positive by sero, but negative by PCR

(Saubolle, LSA – unpublished data)

2. Review of Utilization *Coccidioides* PCR reference testing by one of Banner Medical Centers in 2016

Duration of study: Jan-Dec 2016 ; Total Patients tested: 101

PCR neg: 99 (98%)

PCR pos: 2 (2%)

PCR FNeg: 3 (3%)

A. The 2 **positive** patients had:

1. PCR collected on 12-6-16 and reported as positive on 12-8-16 (2 day TAT); Serologies: only CF was ordered and was anticomplementary; Cultures collected on 12-4-16 were reported growing a mould on 12-7-16 and finaled as *Coccidioides* spp on 12-9-16

Summary: Positive PCR did not contribute to patient care.

1. PCR **positive** on BAL 3-18-16; serologies and cultures all negative. Quantiferron psotive for tb; AFBV cultures negative; fungal cultures negative; patient responded to fluconazole.
   Summary: possible coccy case – PCR may have been valid.

B. The two PCR **negative** patients had: positive serologies and one had positive cultures as well.

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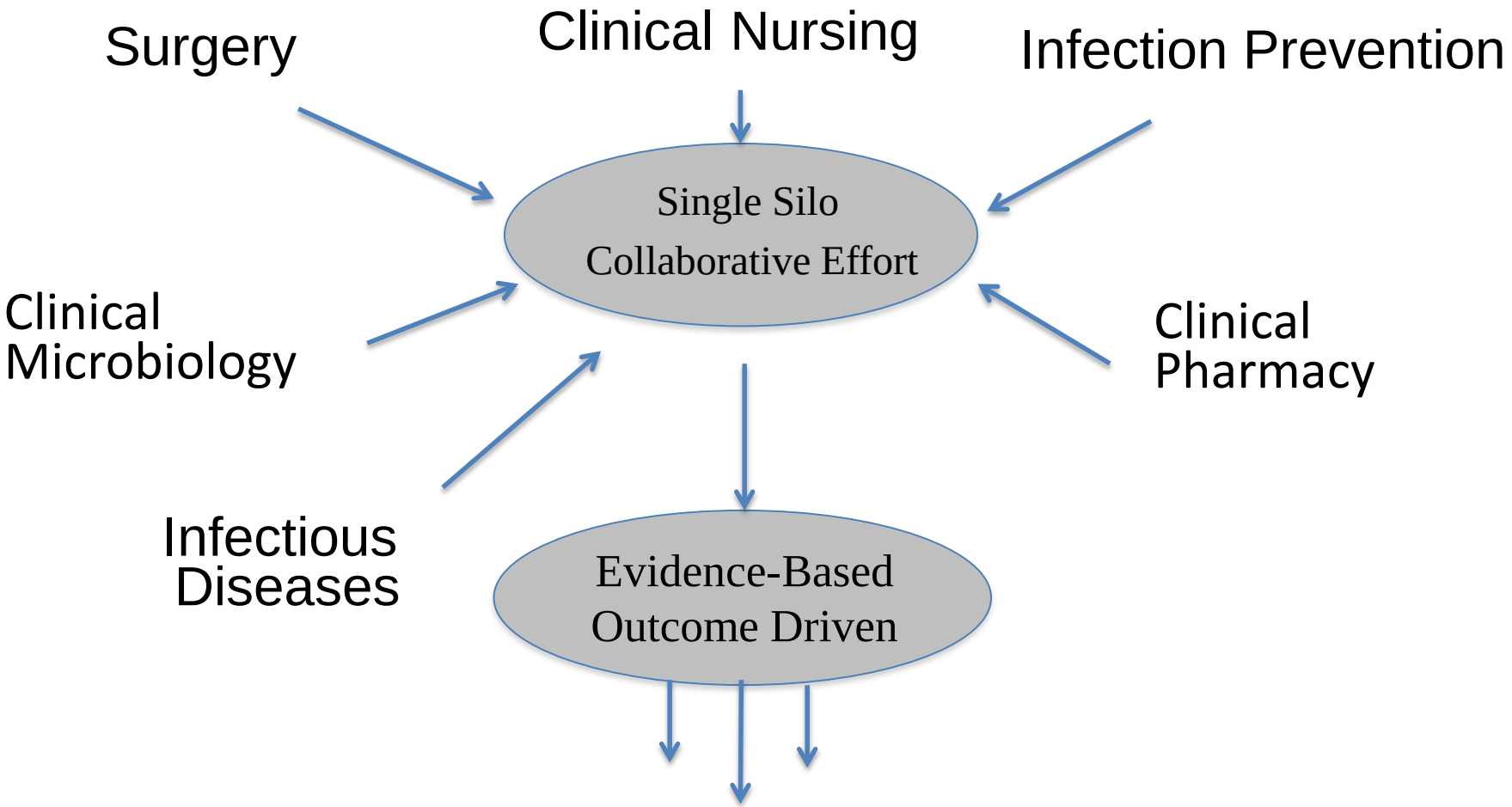
New paradigm in clinical microbiology

- 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

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

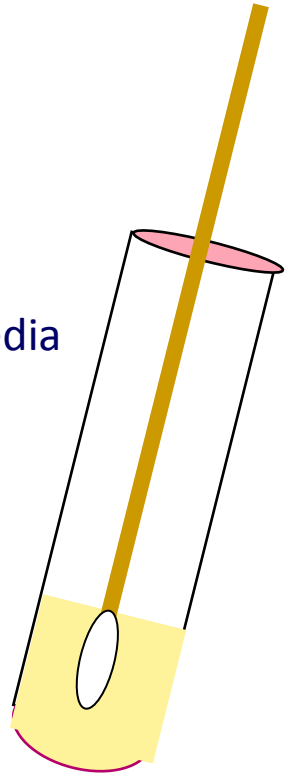
Swab **WORST** type of sample

A. Picks up extraneous microbes

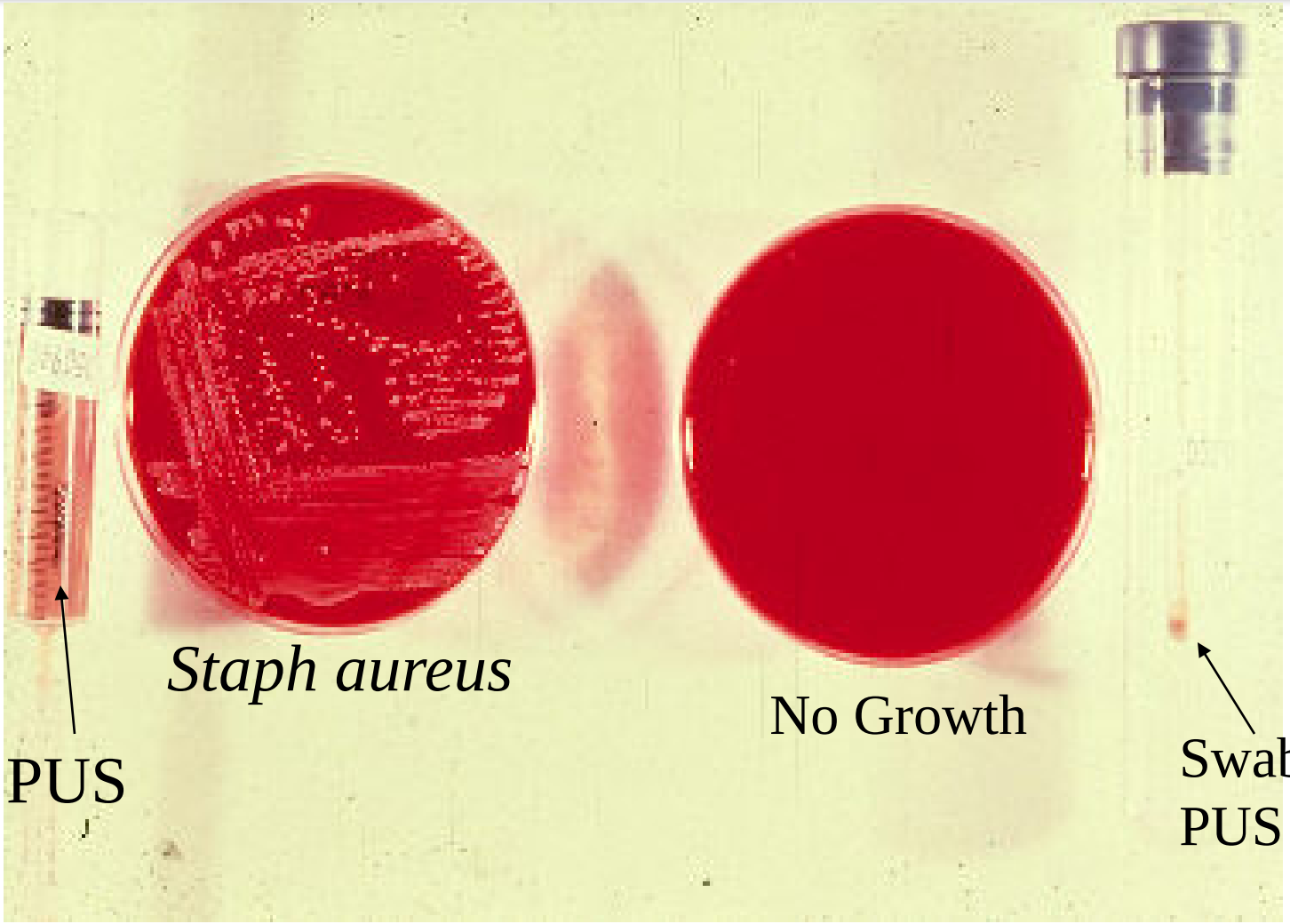
B. Holds extremely small volume of specimen

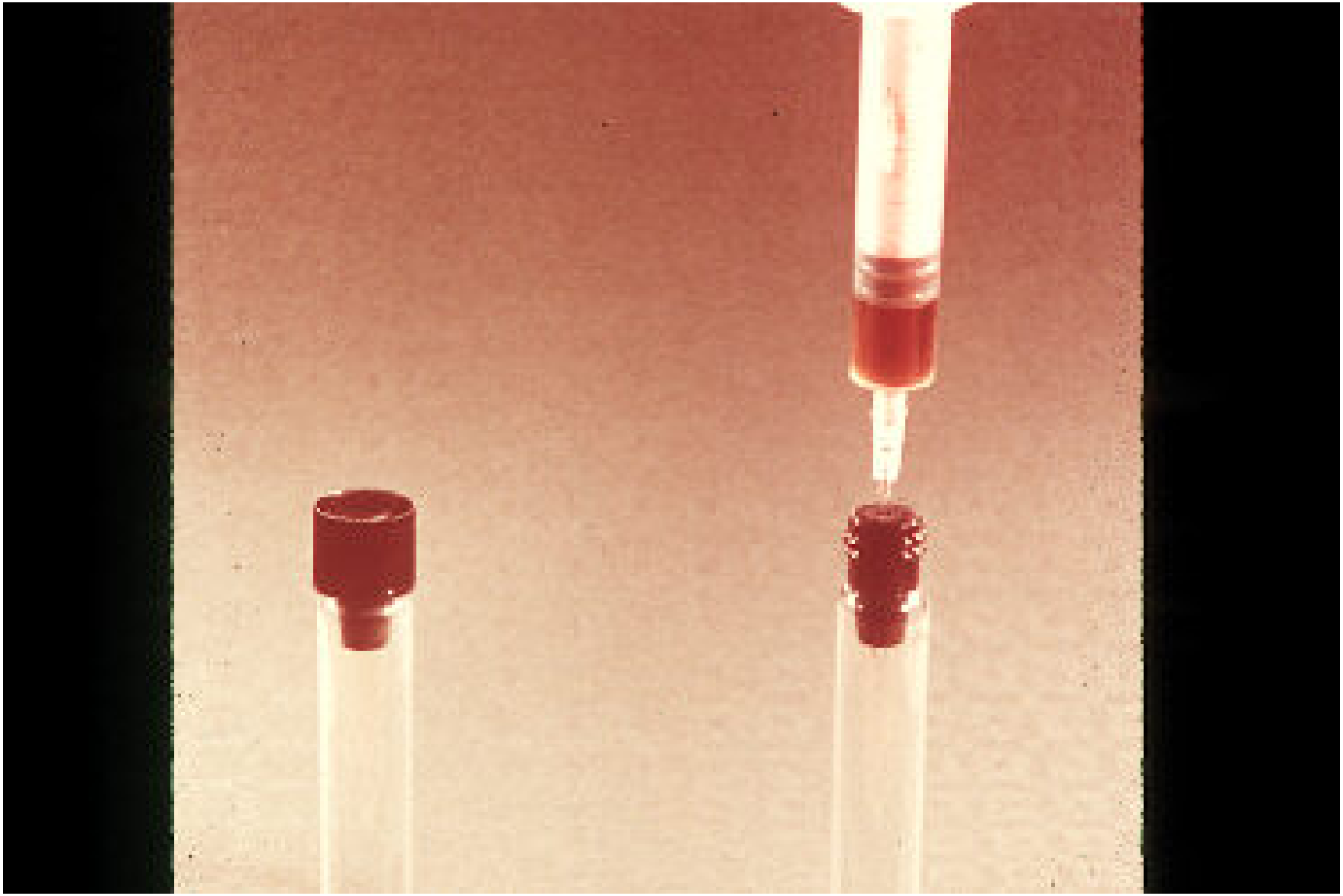
C. Hard to get bacteria or fungi away from fibers and onto media

D. Inoculum not uniform across several different agar plates



Slide from: Ellen-Jo Baron, Stanford


Staph aureus
No Growth
PUS
Swab of PUS




If there is an Issue, Get Some Tissue

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

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

Growth Quantitation

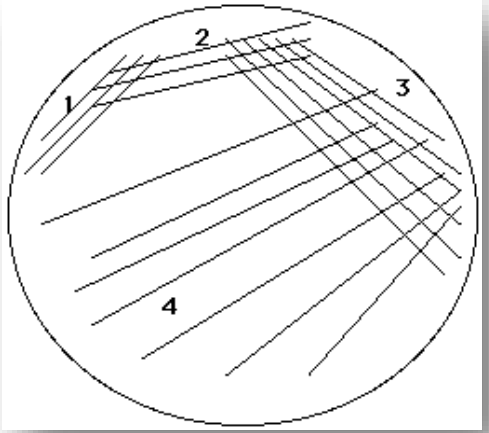
- Depending on the type of inoculation method used, the microbiologist will determine the actual number of colonies present or an estimate of the bacteria present.
  - When plates are inoculated with a measured amount of specimen as in **quantitative** inoculation, the colonies can be counted and the 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



Blood Cultures

Routine (includes the yeast)

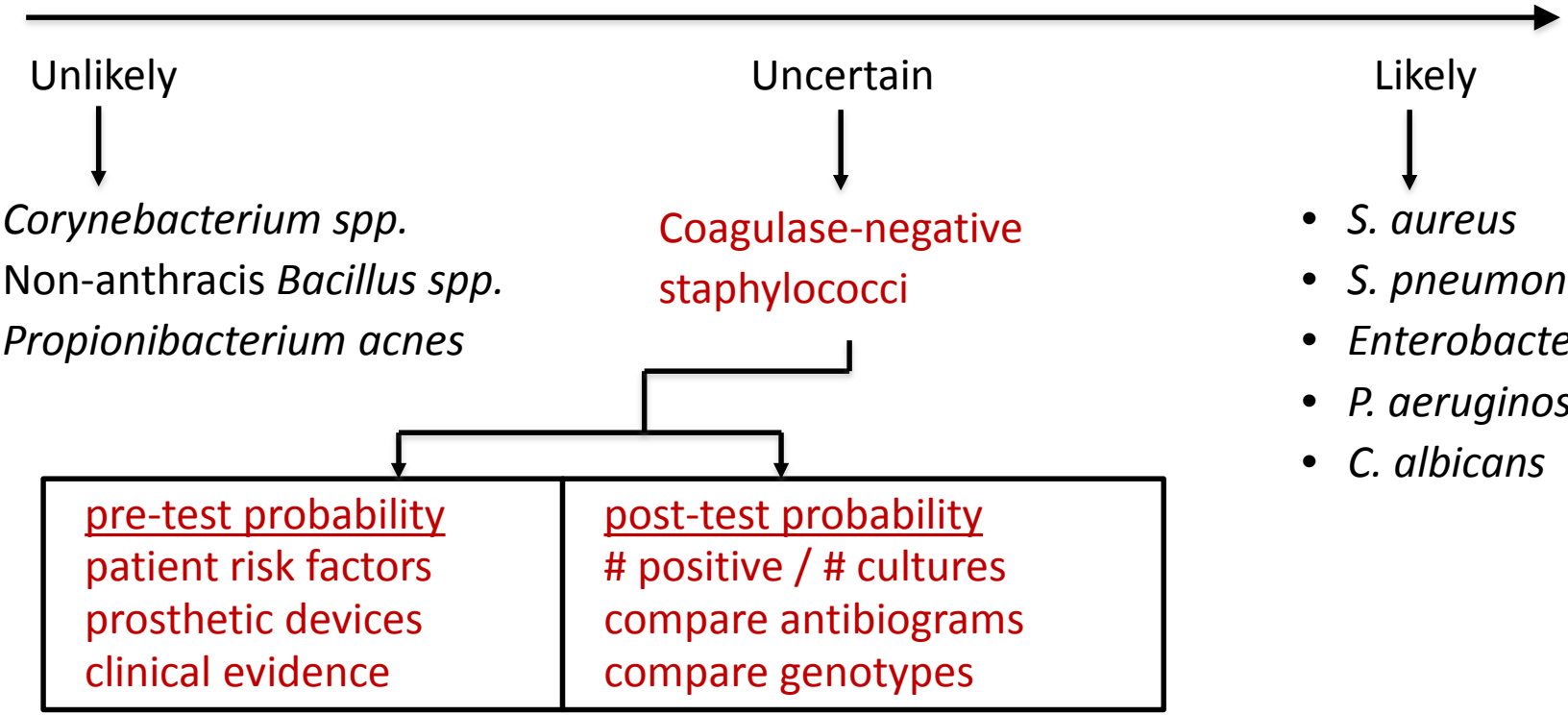
- Number (x 2-3; 2 bottles/culture),
- 20 mls/culture of blood
- Prior to Rx; one after another in sepsis; peripheral venous draw; spaced about 1 or more hrs if abscess suspected
- Contamination
  - skin disinfection (following contamination rates, $2-6 thousand/case)
  - interpretation: isolate types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

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

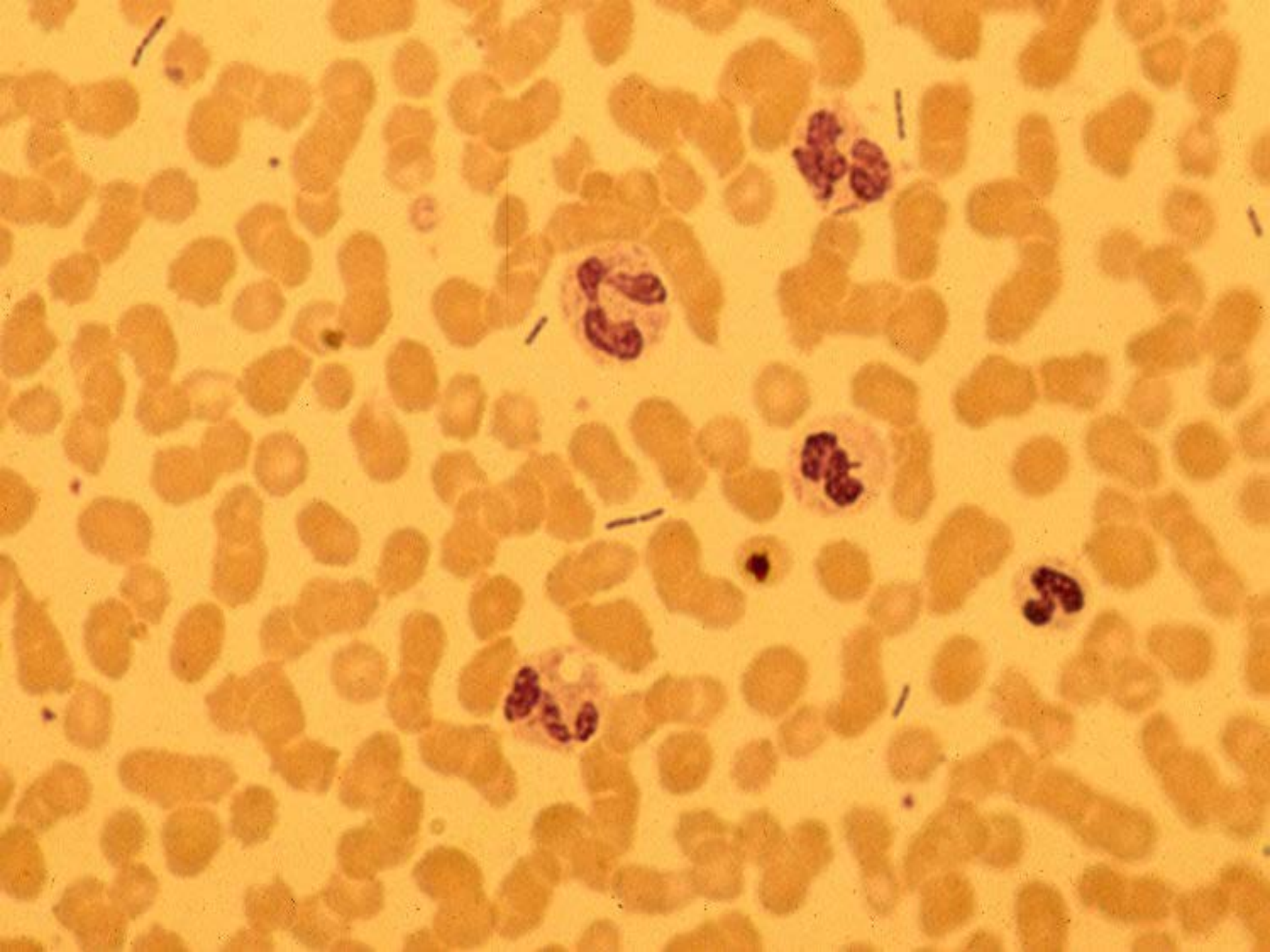
AFB (Rapid growers: MAC, TB, other NTM in Icpts - non-IC should not be cultured for TB)

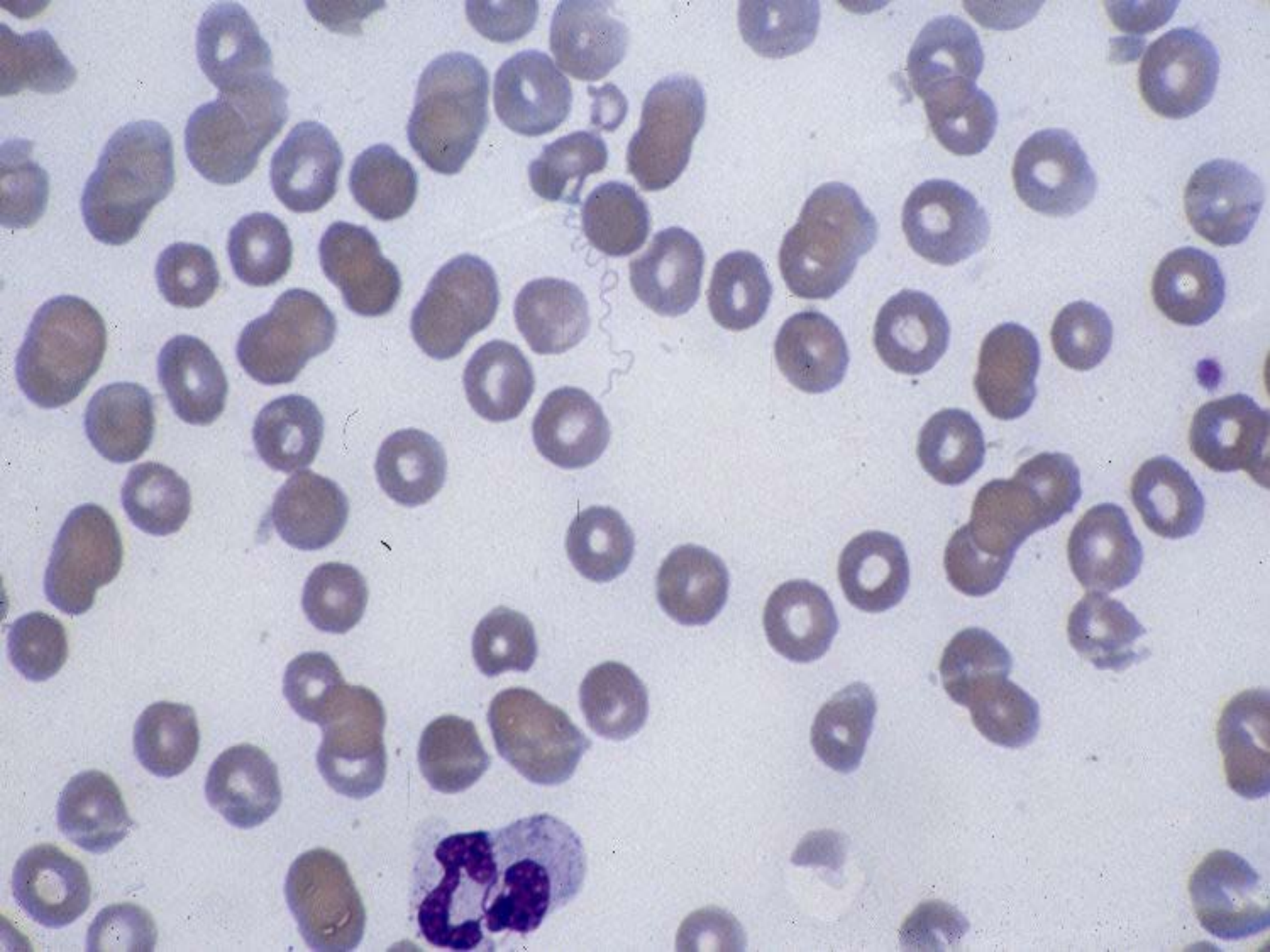
Interpreting a "Positive" Blood Culture

True Bacteremia:



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





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

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)

Soft Tissue Specimen Collection

When

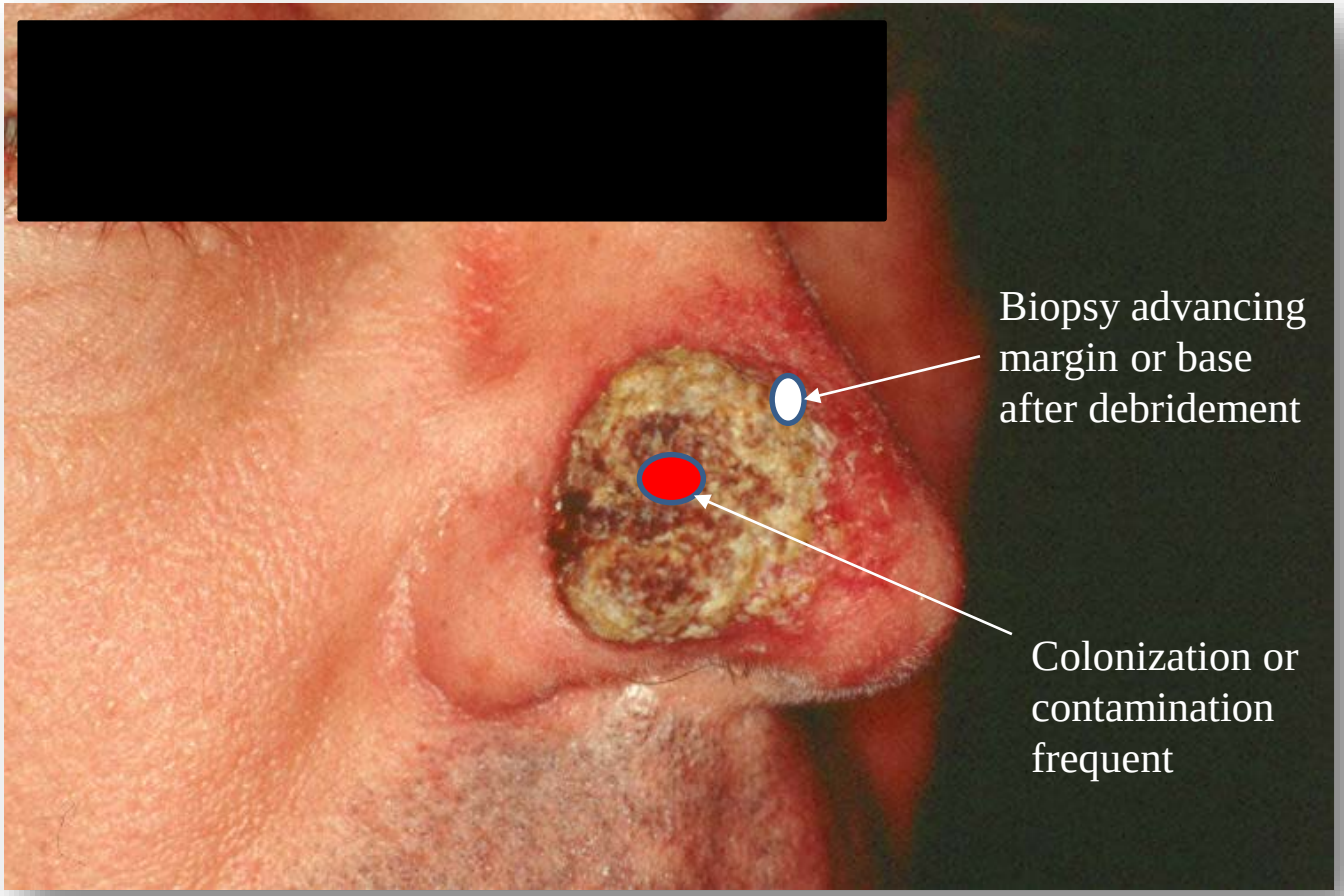
- Culturing clinically *uninfected* lesions is unnecessary, unless done as part of an infection-control surveillance protocol (C-III).
- Cultures of infected wounds are valuable for directing antibiotic choices, but may be unnecessary in cases of acute mild infection in an antibiotic-naive patient (B-III).
- 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.

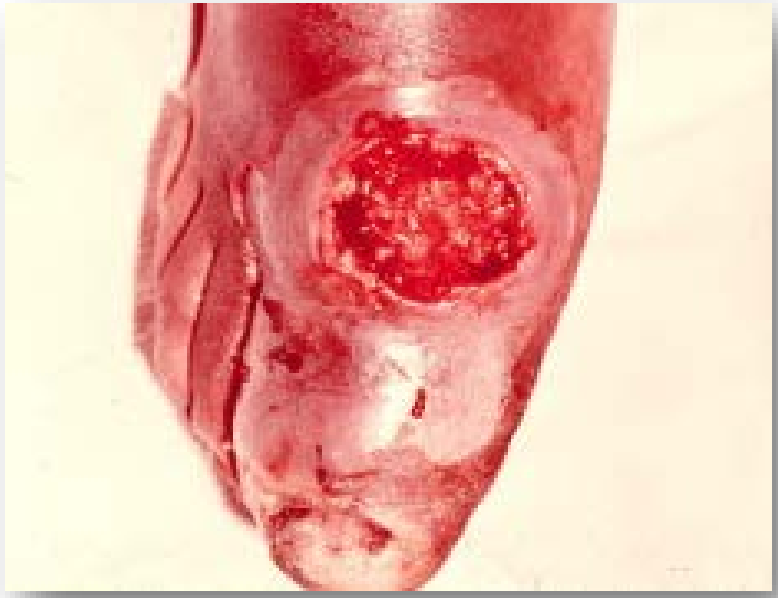
From: Lipsky, et al. Clin Infect Dis 2004; 39:885

Ulceration due *Coccidioides spp.*



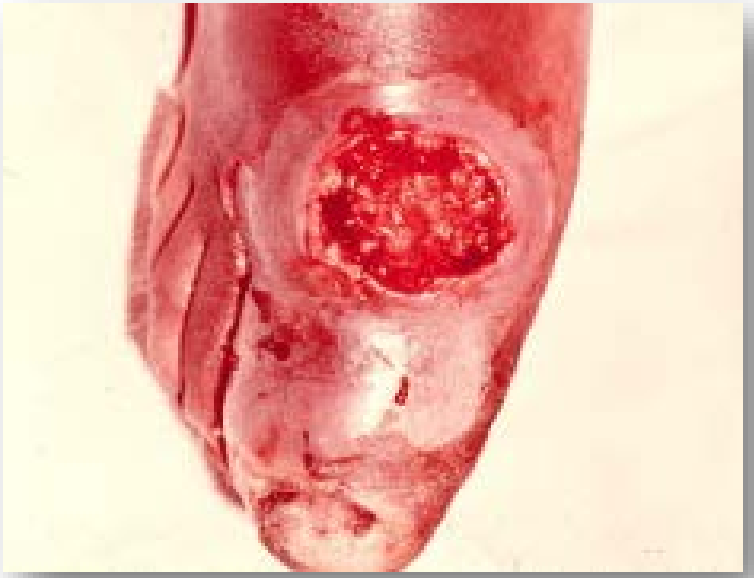
A patient with a diabetic foot ulcer is seen in clinic and the ulcer looks ugly but otherwise has no red streaking outside the borders. It is best to:

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?



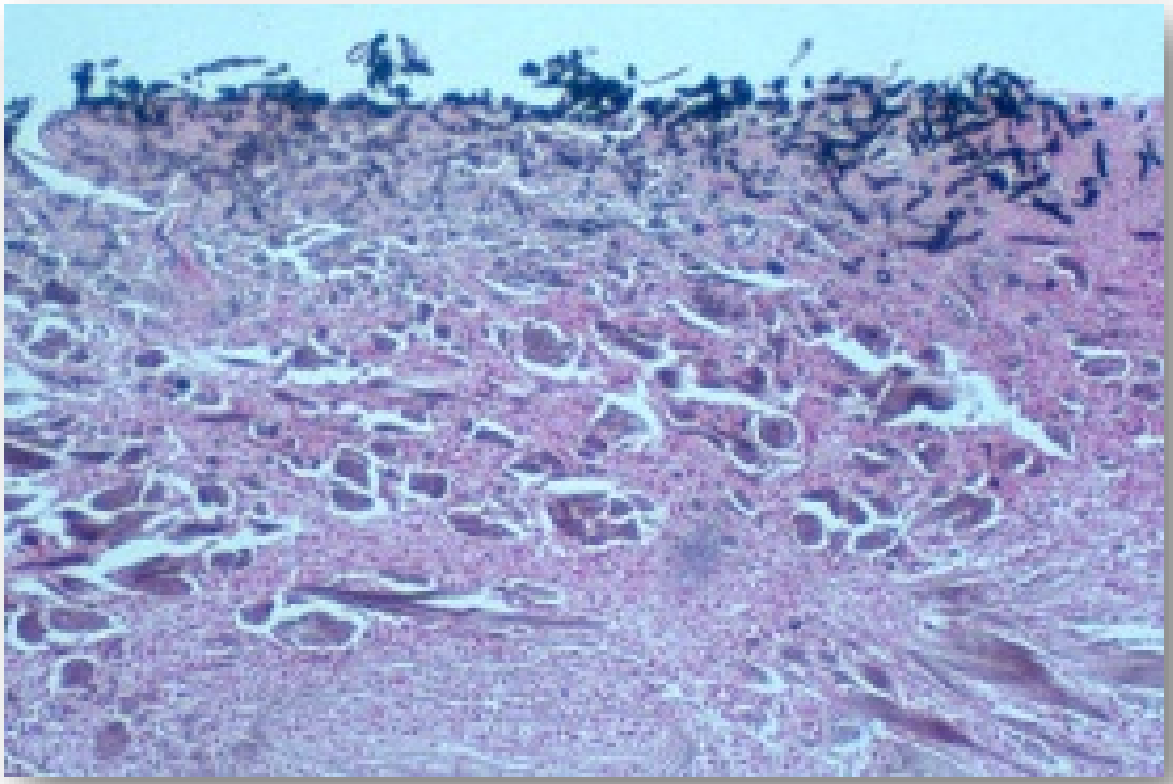
A patient with a diabetic foot ulcer is seen in clinic and the ulcer looks ugly but otherwise has no red streaking outside the borders. It is best to:

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



- 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



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

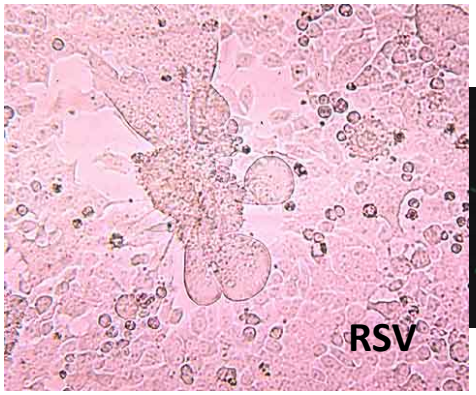
Upper Respiratory Tract Pathogens

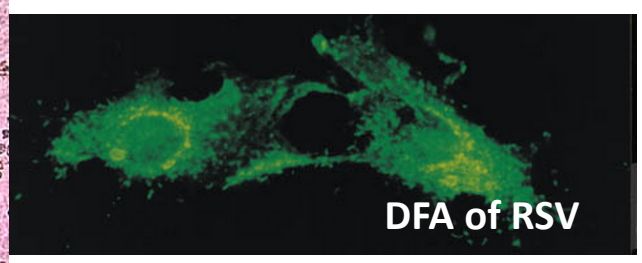
Respiratory Syncytial Virus

- 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

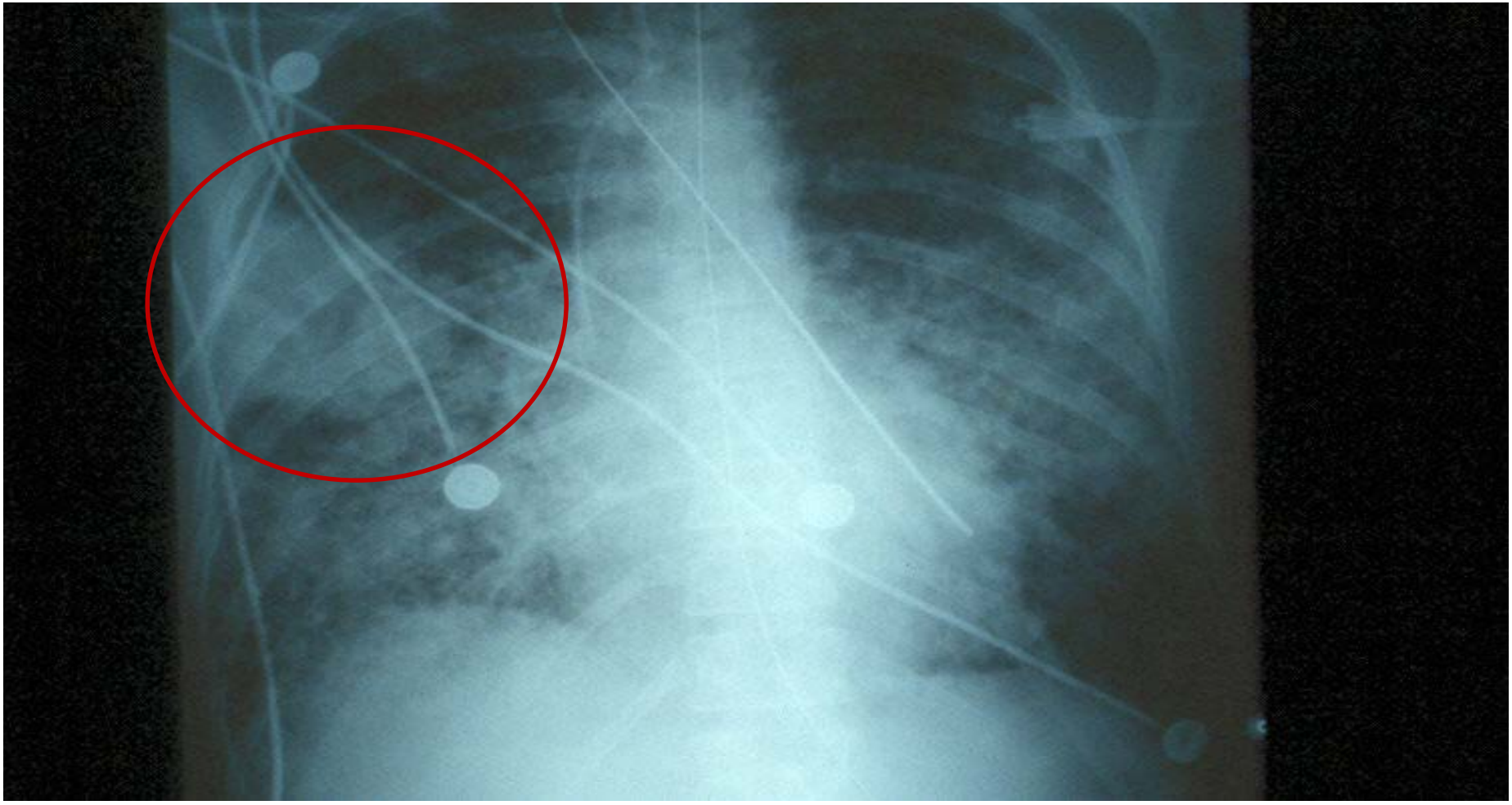
Clinical and Microbiological Relevance

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)

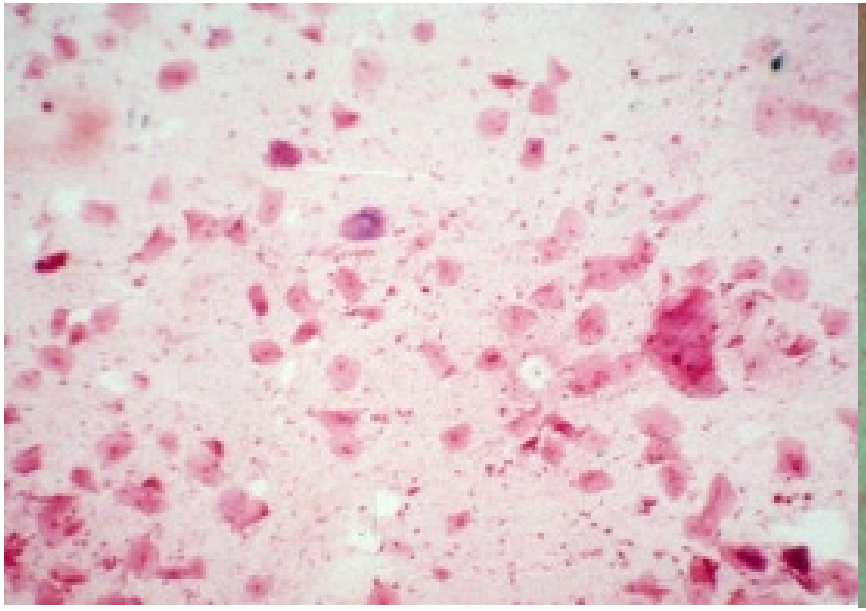
Banner
University Medical Center
Phoenix

Dx of CAP - X-ray

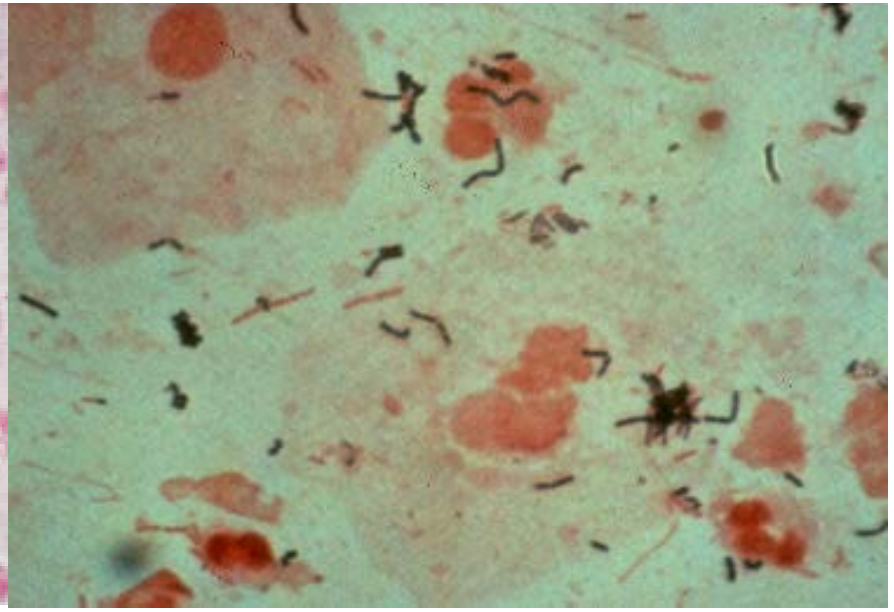


Banner
University Medical Center
Phoenix

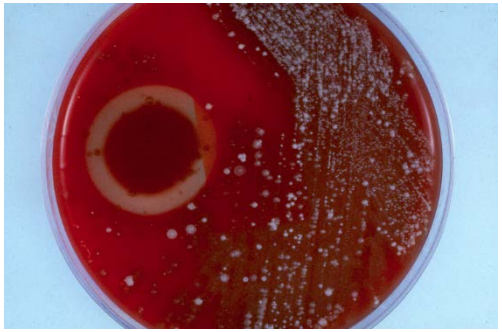
Sputum Screening: Gram stain of sputum contaminated with saliva



(120 X)



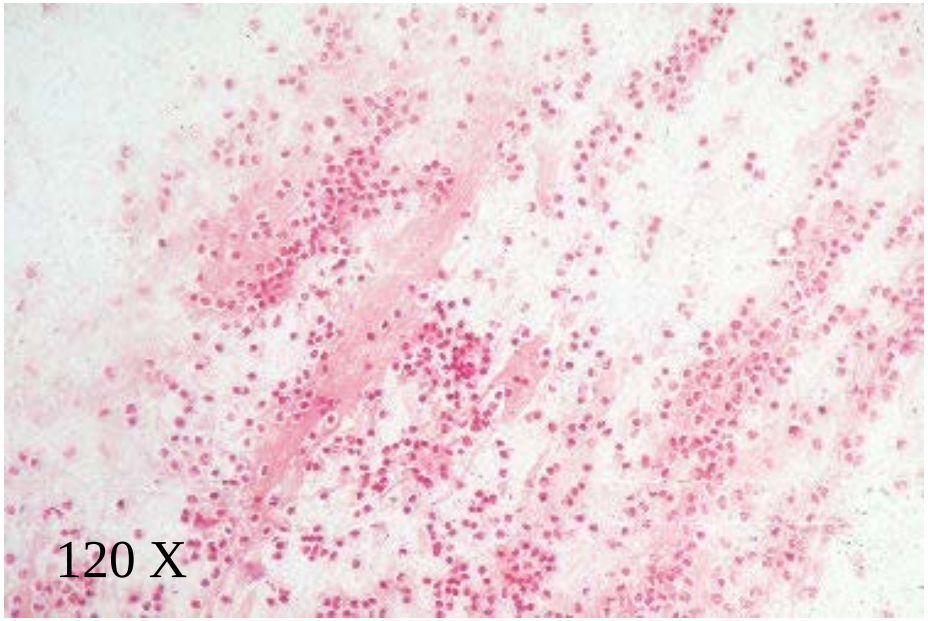
(1,200X)

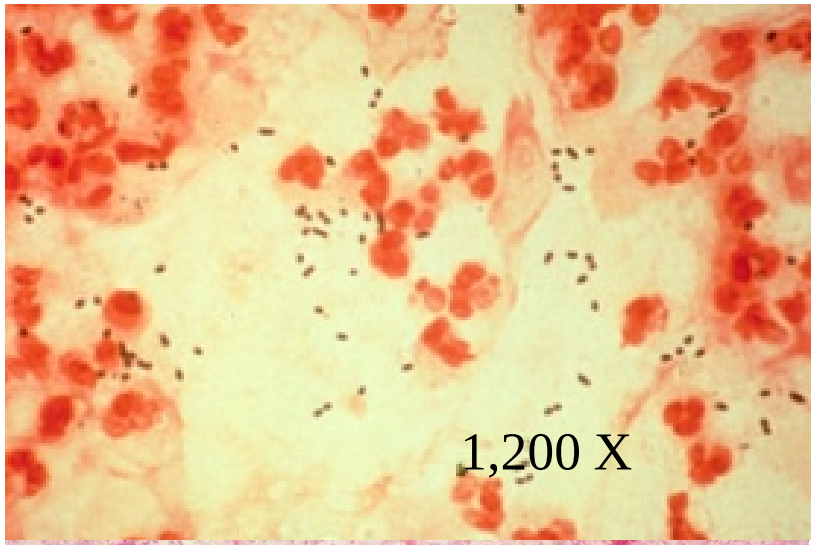


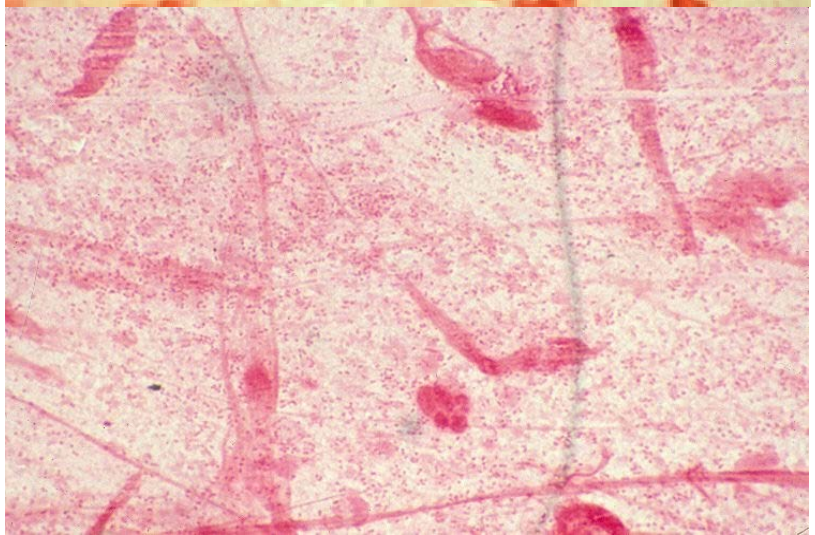
Growth of mixed flora on culture

Banner
University Medical Center
Phoenix

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

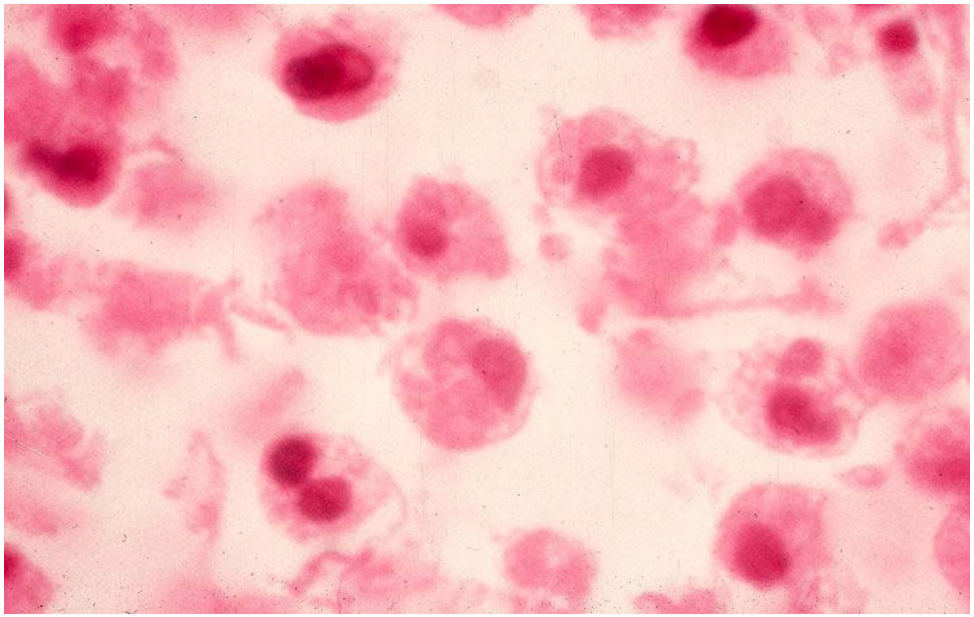






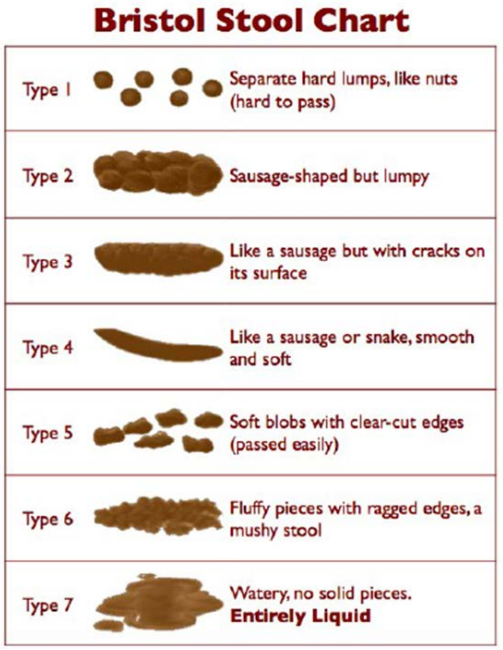
Gram stain of sputum with PMNs but no pathogenic organisms

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



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

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* (routine GDH + Toxin)
  - 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

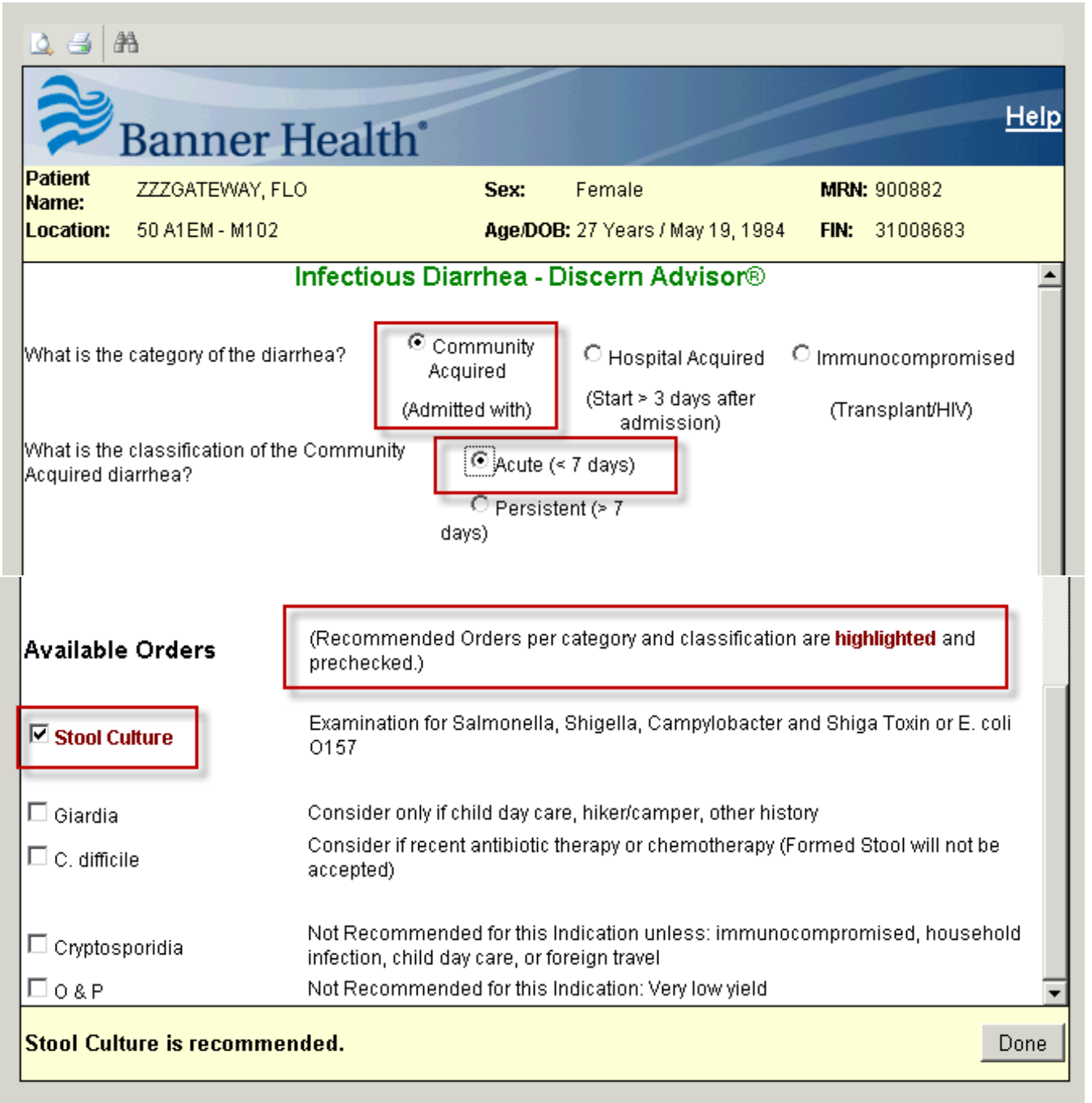
- **Single O & P full exam** only if tests above return negative and diarrhea persists (especially in immunocompromised patients or those who have been associated with developing countries)
- **Repeat O & P full exam X 2** (collected on separate days one to two days apart) if initial O & P exam is negative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

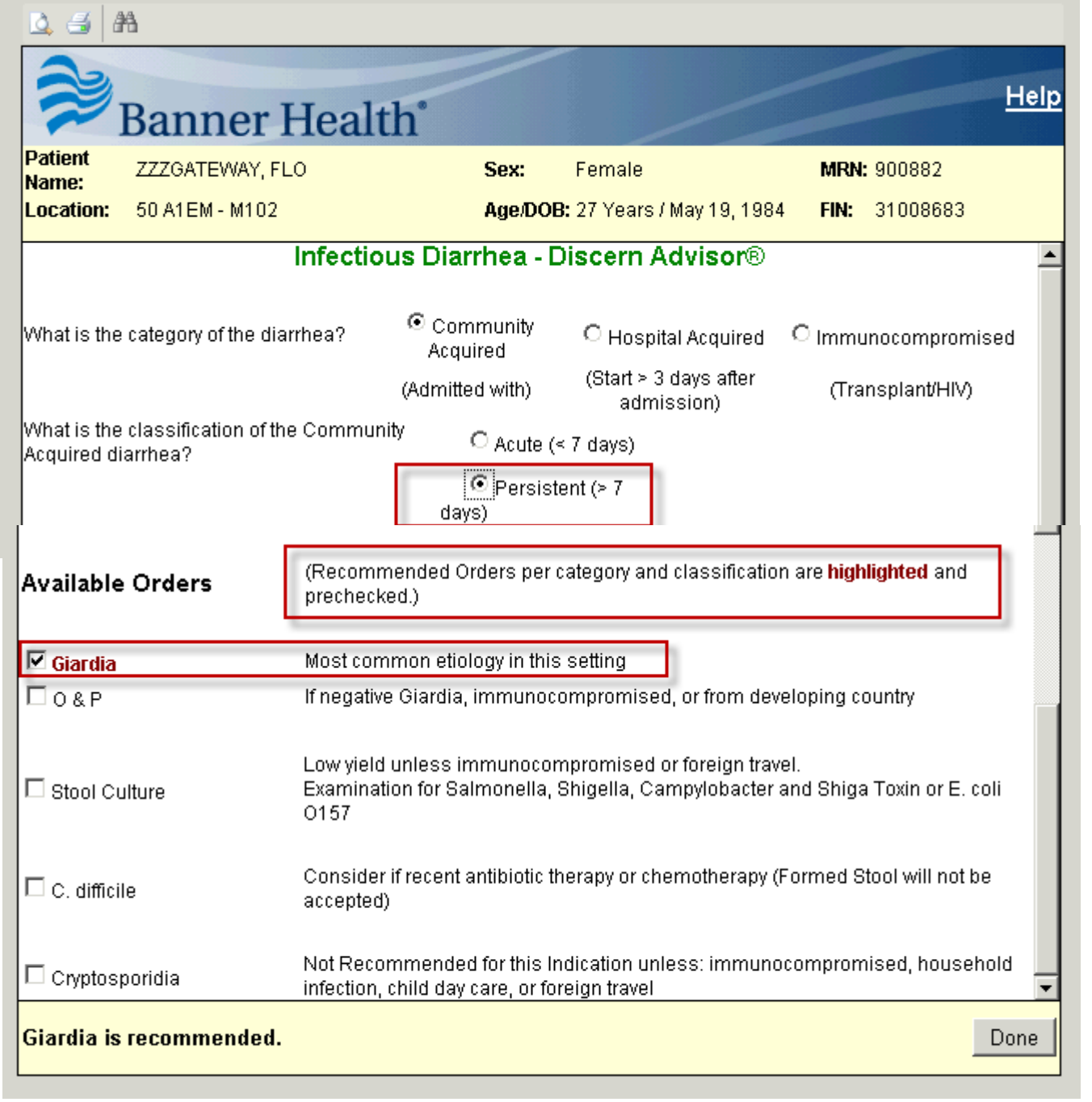
3. . Hospital-associated diarrhea (onset >3 days after admission)

- ***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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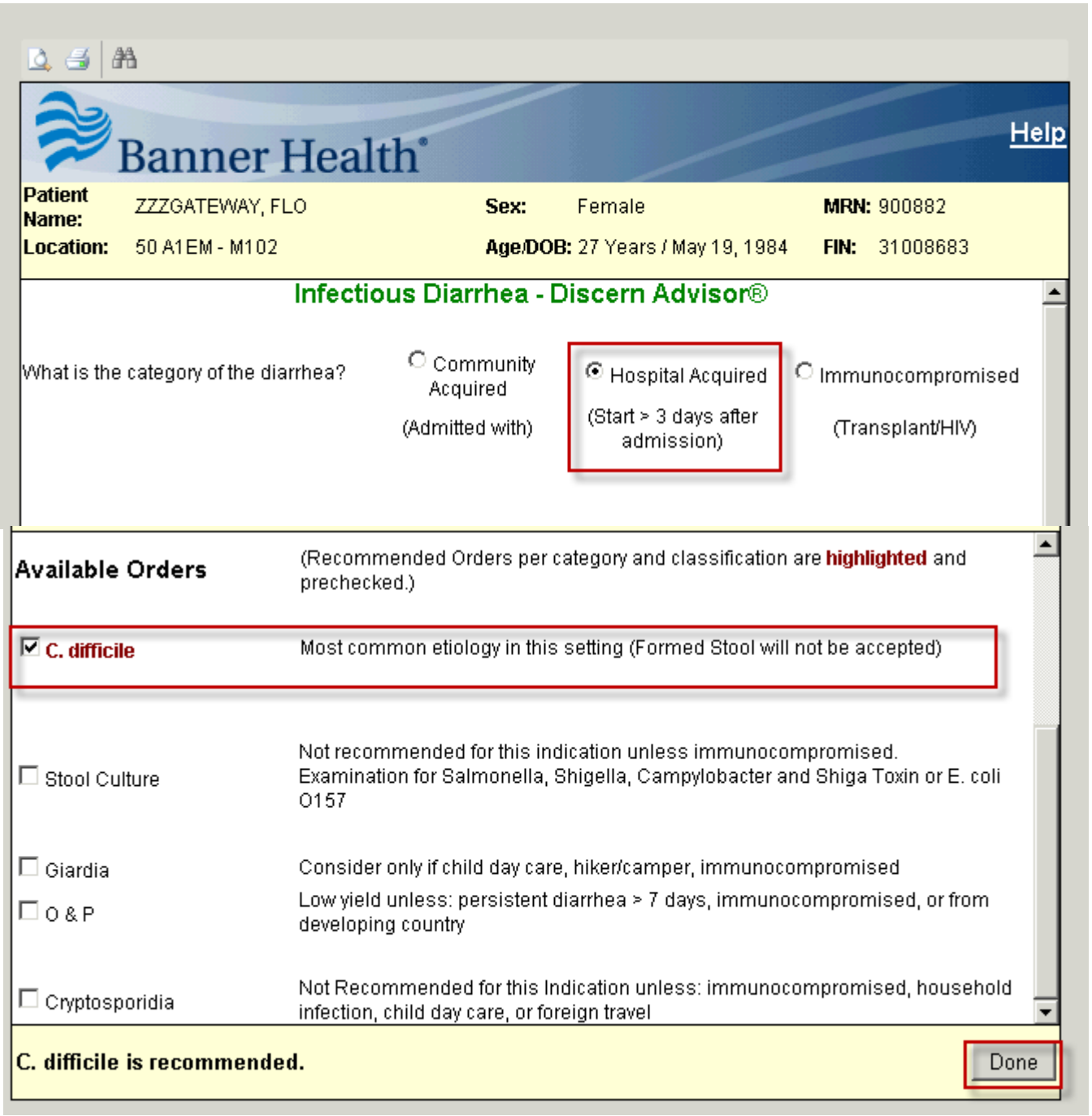
(Transplant/HIV)
What is the classification of the Community Acquired diarrhea?
Acute (< 7 days)
Persistent (> 7 days)
Available Orders
(Recommended Orders per category and classification are highlighted and prechecked.)
Giardia
Most common etiology in this setting
O & P
If negative Giardia, immunocompromised, 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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Help
Patient Name: ZZZGATEWAY, FLO
Sex: Female
MRN: 900882
Location: 50 A1EM - M102
Age/DOB: 27 Years / May 19, 1984
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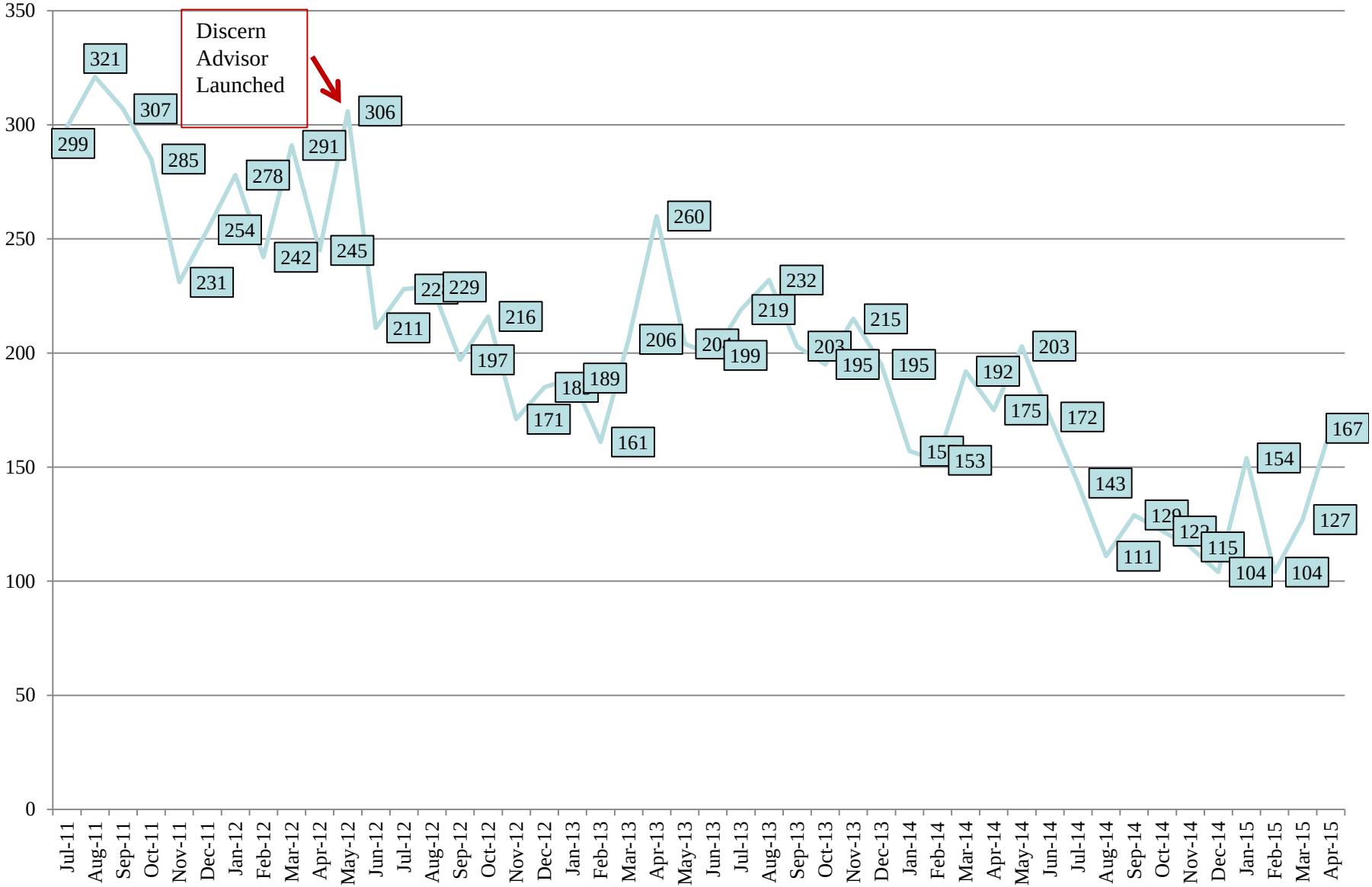
Infectious Diarrhea - Discern Advisor®
What is the category of the diarrhea?
Community Acquired
(Admitted with)
Hospital Acquired
(Start > 3 days after admission)
Immunocompromised
(Transplant/HIV)
Available Orders
(Recommended Orders per category and classification are highlighted and prechecked.)
C. 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)

Typical FindingsClean-catch Urine in symptomatic UTI

Labs

Urinalysis: presence of nitrite and leukocyte esterase

Microscopy: WBCs but no casts seen

Bacterial culture:

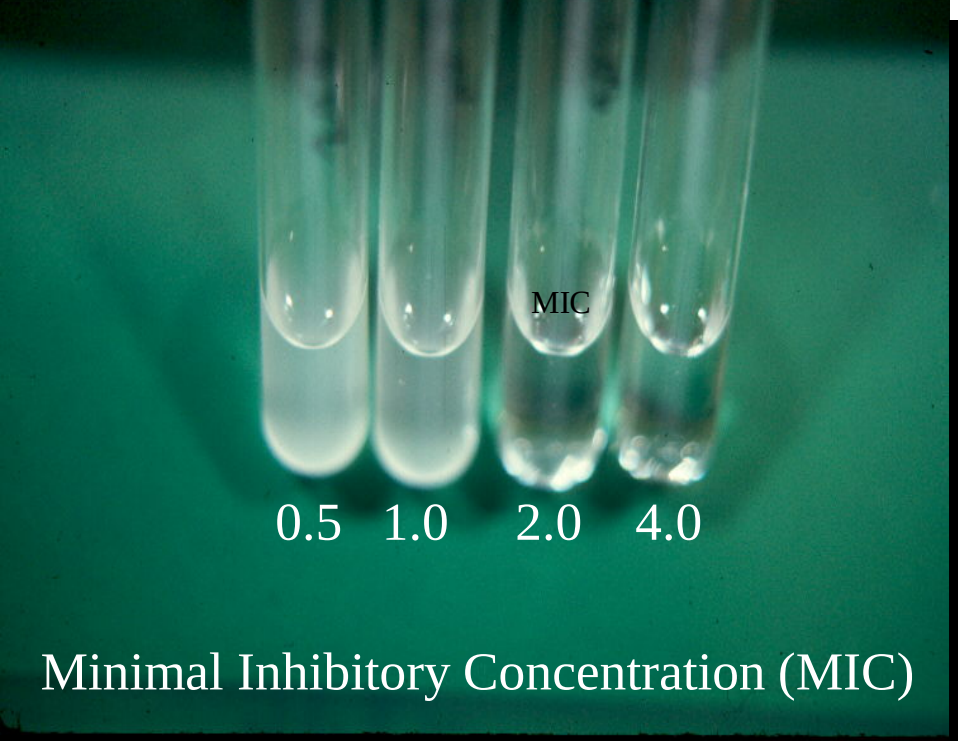
- $>10^5$ cfu/ml
- GNR
- Indole-positive
- Oxidase-negative

Goals of Susceptibility Testing

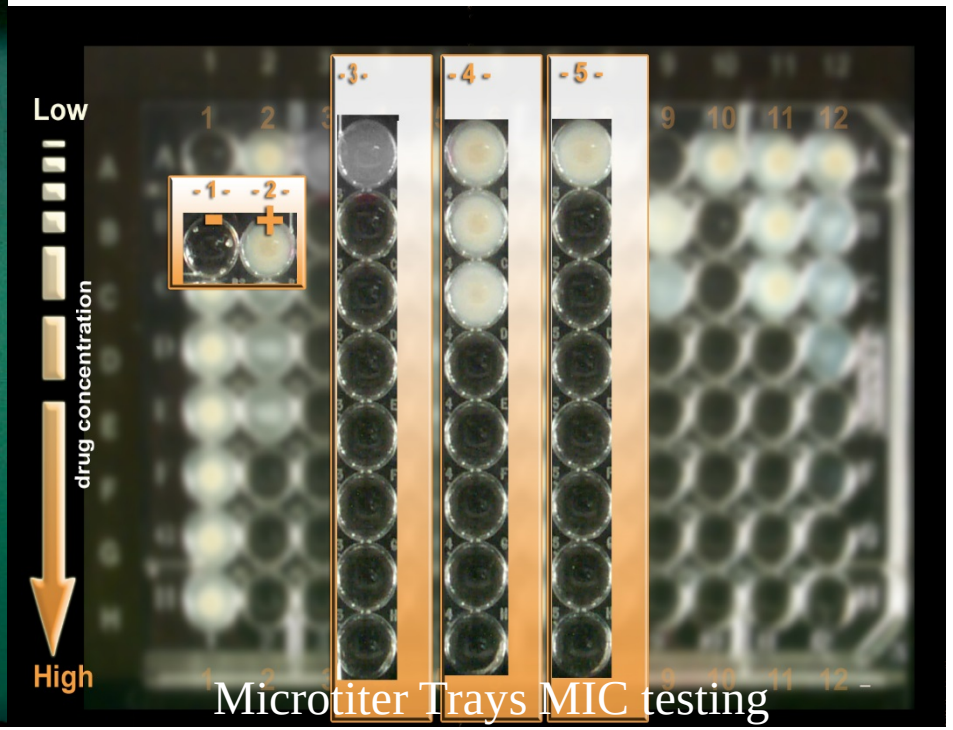
- Detection of antimicrobial resistance in individual pathogens
- Guidance of antimicrobial therapy (appropriate, cost-effective)
- Surveillance of emerging resistance in community
- Evaluation of new antimicrobial agents

Primary Clinical Laboratory Options for Susceptibility Testing

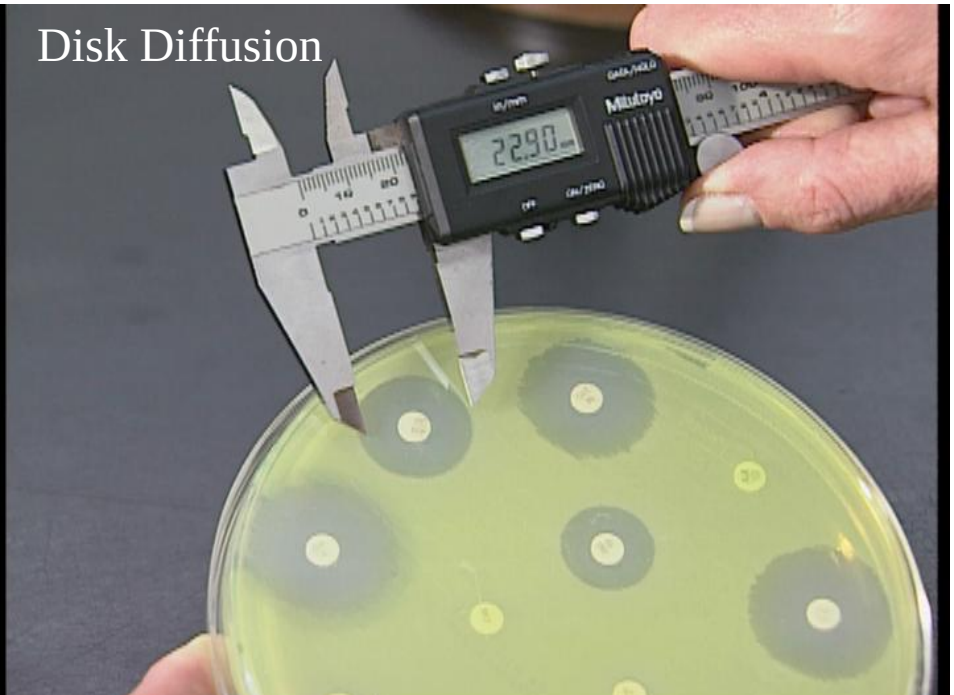
- Disc diffusion tests (Kirby Bauer-semiquantitative)
- Broth dilution tests (usually microdilution-quantitative)
- Antimicrobial gradient diffusion tests (E-Test)
- Specialized screening tests (single drug concentration, spot tests)
- Automated susceptibility testing (usually quantitative results)



Minimal Inhibitory Concentration (MIC)

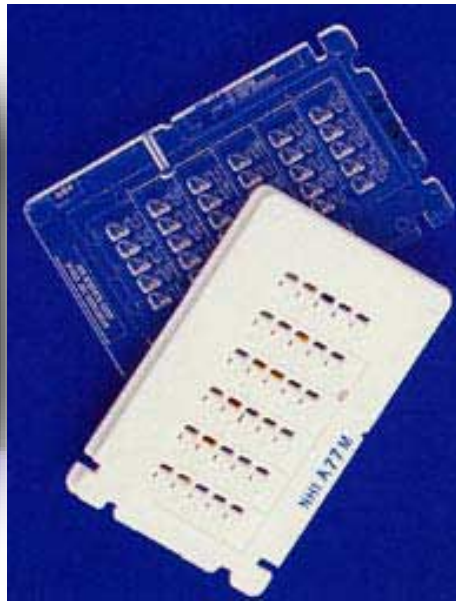


Microtiter Trays MIC testing



Epsilometer Gradient Strip (Etest)


Vitek
Vitek Automated MIC System




MicroScan
MicroScan Automated MIC System

How is Resistance Defined?

- MIC determinations represent the most refined means of measuring in vitro antibacterial activity (reproducible ↑↑)

- Establishment of MIC breakpoints
  - Clinical and Laboratory Standards Institute (CLSI)
  - Food and Drug Administration (FDA)

- MIC breakpoints (interpretive criteria)
    - Susceptible (S)
    - Intermediate (I)
    - Resistant (R)
    - Non-susceptible

What's in an interpretation?

- **Susceptible** – Implies that an isolate is inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.
- **Intermediate** – An isolate that approaches the usually attainable blood and tissue levels and for which response may be lower than for a susceptible isolate. Also includes a buffer zone to account for small differences in testing that would otherwise lead to a major interpretive discrepancy.
- **Resistant** – Implies that an isolate is not inhibited by the usually achievable concentrations of the agent with normal dosages.
- **Non-susceptible** - Category used for organisms that only have a susceptible category. This designation does NOT necessarily mean that an isolate has a resistance mechanism. It only means that the result falls outside the range that has been defined for the wild-type distribution.

Question

A patient has an *E. coli* isolated from blood which is resistant to the antimicrobial Cefotaxime with which the patient has been treated for the past 4 days. Being otherwise a normal host, the patient's chances of a good outcome while on this regimen is approximately which of the following?

A. 20%
B. 40%
C. 60%
D. 80%
E. 90%

Question

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Banner
University Medical Center
Phoenix

So how good are we?

IDEAL

- SUSCEPTIBLE
  - % Success --- 100%
- RESISTANT
  - % Success --- 0%

REALITY

- SUSCEPTIBLE
  - % Success --- 90%
- RESISTANT
  - % Success --- 60%

Why is this?

Doern

Why not the 100 – 0% Rule?

1. Technical errors?
2. Wrong test?
3. Patient factors?

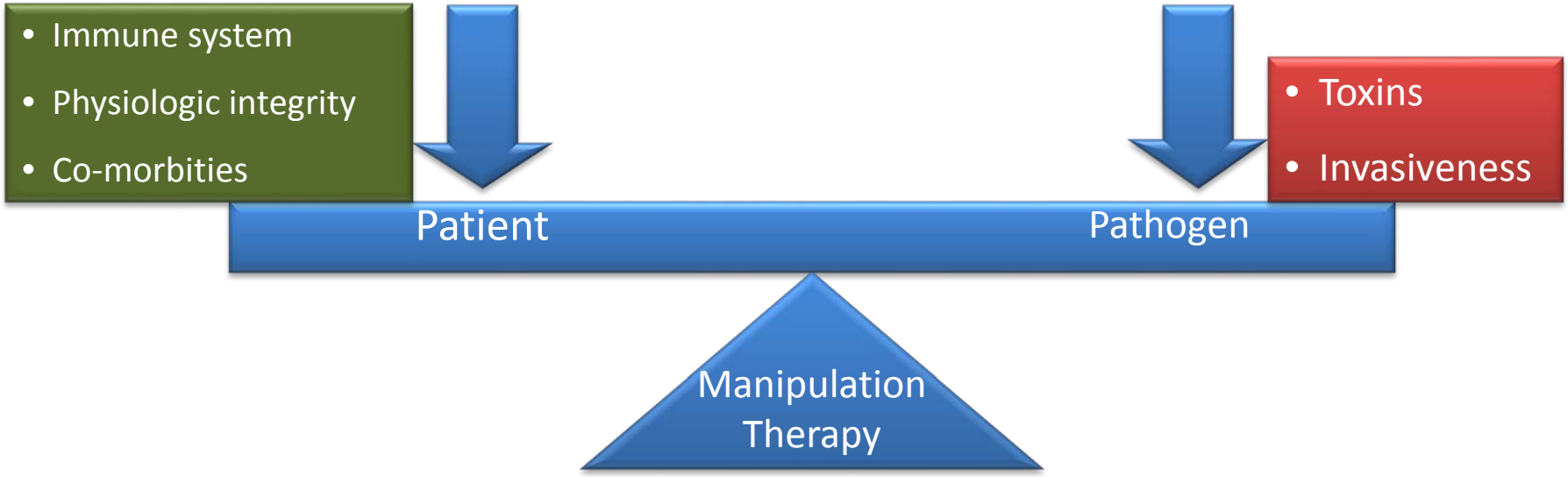
Things not accounted for by susceptibility testing

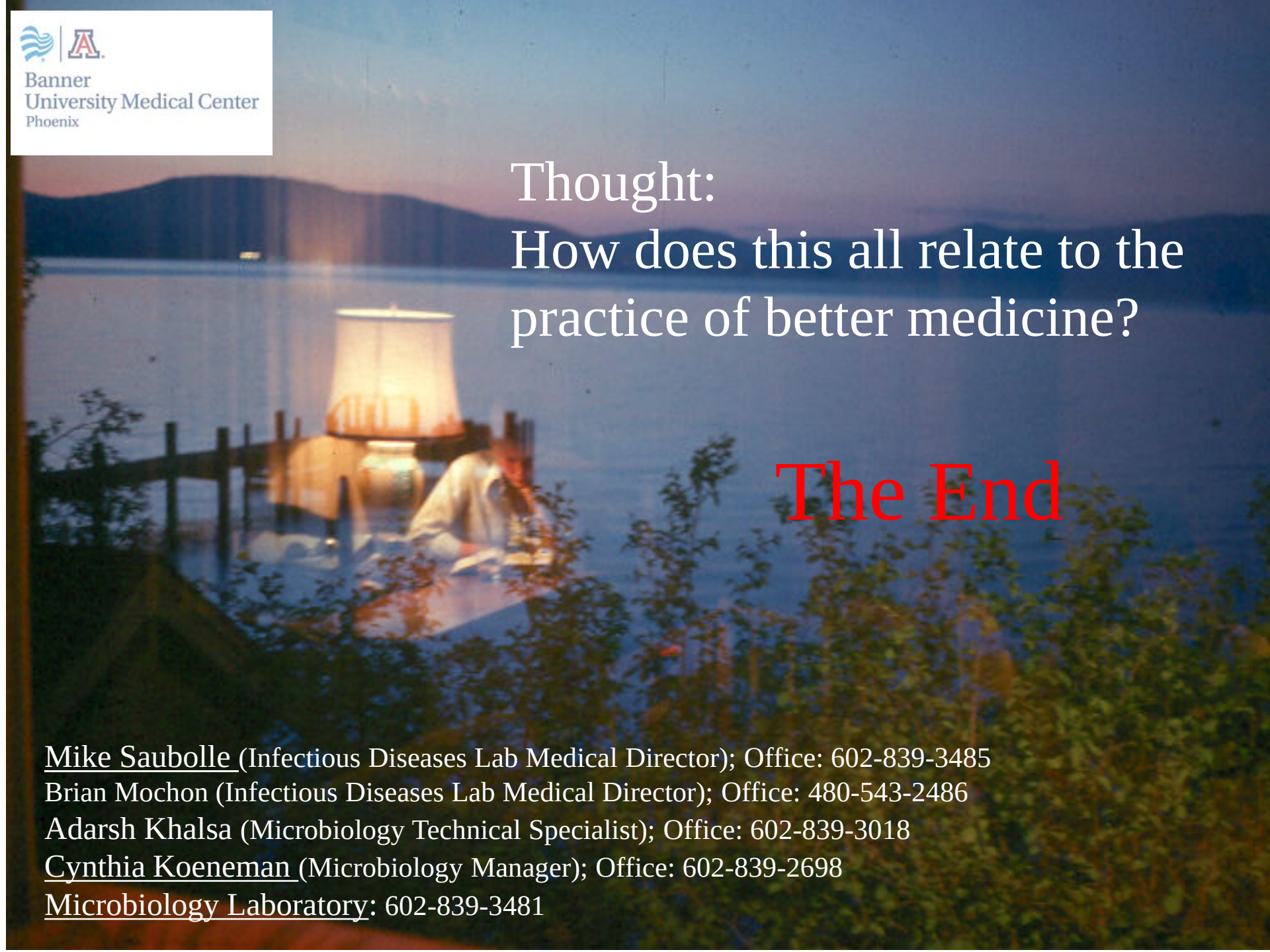
- **Drug pharmacokinetics**
- **Drug delivery to site of infection**
- **Host response (or lack of)**
- **Toxin production**
- **Polymicrobial interactions**

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)





Mike Saubolle (Infectious Diseases Lab Medical Director); Office: 602-839-3485
Brian Mochon (Infectious Diseases Lab Medical Director); Office: 480-543-2486
Adarsh Khalsa (Microbiology Technical Specialist); Office: 602-839-3018
Cynthia Koeneman (Microbiology Manager); Office: 602-839-2698
Microbiology Laboratory: 602-839-3481