

Bloody fast microbiology

SWACM 2010

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Traditional and molecular methods
for faster positive blood culture ID
and susceptibility

Disclosures

- I will mention many products by brand name. Mention does not equal endorse.
- I have used some of these products but not all.
- I have not taken money, pizza, pens, or note pads from any of the companies mentioned.
- My lab does not use any of these products...yet.
- Statements in “quotation marks” reflect info directly from corporate websites
- Most of the images are from corporate websites.

Objectives for this talk

- Review classic rapid ID and susceptibility methods from positive blood cultures
- Describe new molecular ID and susceptibility methods from positive blood cultures
- Examine technical and financial barriers to implementation
- Review a case where faster information leads to a better patient outcome

Central questions of this talk

- Why this topic is of interest to me?
 - My first positive blood culture way back when
- Does what we do in the lab matter?
- If we do it faster does it matter more?

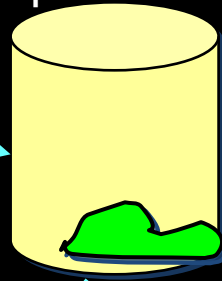
Before we start

- If speed does matter, and it does, consider that a positive blood culture is already relatively old information, compared to a positive direct specimen test
- We need fast, easy, cheap, multiplex identification and resistance determination
- We need to play “catch-up”

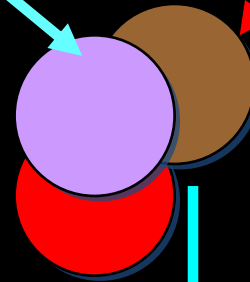


Doug

Specimen

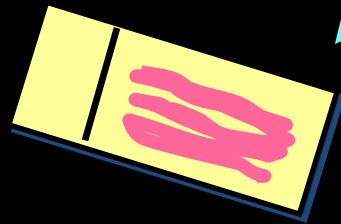


Bug



Culture, 24 hours

Drug



Gram stain, 15 minutes



Rapid flu test, 15 minutes



Antibiotics susceptibility 6 to 24 more hours

Detect, identify, eliminate. A progression of actionable information leading to treatment and cure!

This article always bothers me

Detection and Treatment of Bloodstream Infection: Laboratory Reporting and Antimicrobial Management

Erik L. Munson,¹ Daniel J. Diekema,^{1,2} Susan E. Beekmann,¹
Kimberle C. Chapin,³ and Gary V. Doern^{1*}

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We analyzed antimicrobial use in 509 episodes of clinically significant bloodstream infection to assess the impact that microbiology laboratory reporting had on antimicrobial management. Most therapy interventions occurred at the time of phlebotomy and after notification of Gram stain results by telephone. Release of antimicrobial susceptibility data had the least impact on antimicrobial management.

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2003, p. 495–497
0095-1137/03/\$08.00+0 DOI: 10.1128/JCM.41.1.495–497.2003
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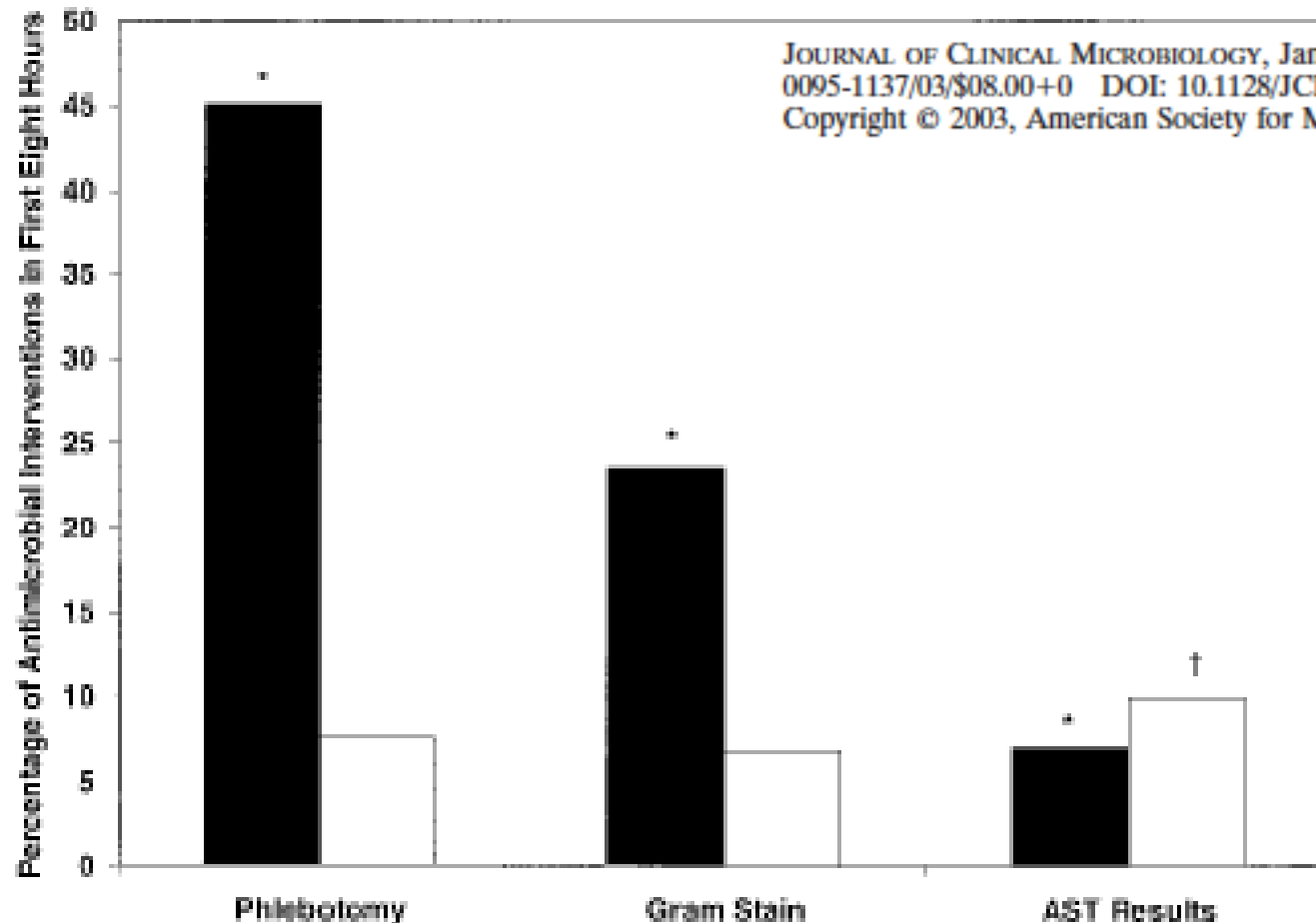


FIG. 1. Percentage of all antimicrobial interventions occurring within the first 8 h (initiations, solid bars; discontinuations, open bars) after each event of interest. *, $P < 0.001$ for differences noted in therapy initiations. †, $P < 0.05$ for differences noted in therapy discontinuations.

The big WHY?

- Automated blood culture systems have revolutionized the detection of bacteremia
- Detection is only the first step. We need faster identification and susceptibility to make a real impact
- To improve mortality, morbidity, lower antibiotic costs, prevent resistance, get patients out of the hospital, save \$\$\$

Traditional but faster:

- Based on gram stain:
 - Drop A and P disks
 - BEA agar or BEA/Vanc
 - Wet mount motility if GPR
 - Beta strep and Strep pneumo latex tests
 - Coagulase
 - If yeast, germ tube, India ink, urea

What about when day shift leaves?

Media selection

- Chromagars, based on gram stain:
 - MRSA
 - VRE
 - *Candida*
- For anaerobes:
 - Sub to BBE/LKV and Bruc or CDC and use CLSI M35-A2 abbreviated ID procedures

Remember the Isolator?

- Lysis-centrifugation
- Identifiable colonies in 18-24 hrs
- Very labor intensive
- Contaminant-prone
- Safety issues, breakage



Save-a-day: positive bottle to Vitek

- Remove 3 ml from bottle using a sterile 3 ml syringe.
- Dispense the blood into a sterile labeled tube.
- Centrifuge the specimen for thirty (30) seconds.
- Using a sterile transfer pipette, transfer the supernatant to a second sterile tube. Discard the pellet.
- Centrifuge the supernatant for three (3) minutes.
- Use a sterile pipette to remove the supernatant and discard.
- Resuspend the pellet in 0.45% sterile saline.
- Centrifuge for 3 minutes.
- Use a sterile pipette to remove the supernatant and discard.
- Re-suspend the pellet in 1.8 ml of sterile 0.45% saline in a 12x75 mm sterile plastic tube labeled with the accession number. Use this suspension to set up Vitek susceptibility and identification tests
- Or...disk diffusion susceptibility

Clin micro proc handbook, 3rd Ed.

- Section 3.4.1 blood cultures
- Centrifuge pos blood culture broth in serum separator tube, adjust pellet on top of silicone layer to 0.5 McFarland – set up ID and suscep
- Table 3.4.1-2
 - Initial processing based on gram stain

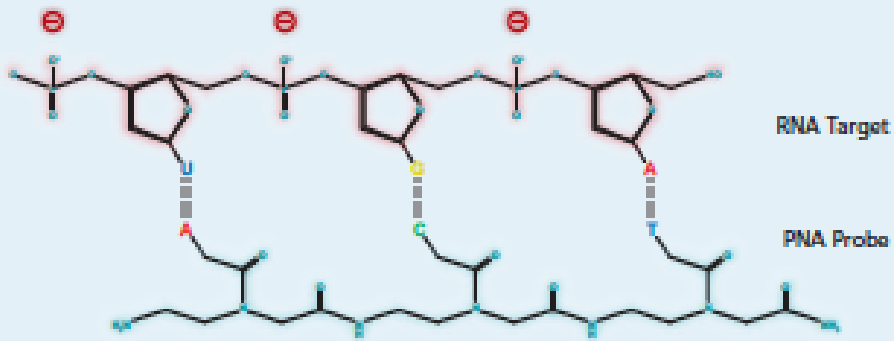
Non-amplified molecular approaches

- A question about PCR: If we can already see it in a Gram stain, why should we have to amplify it?

Advandx PNA FISH

- Like DFA but using peptide nucleic acid fluorescent in-situ hybridization
- Need special covered heat block, water bath,
- Fluorescent scope and special filter
- No amplification step needed
- About 90 minute TAT, but hands on time is about ten minutes
- Choose kit based on gram stain

How it works



PNA Probe Binding to RNA Target

Prepare Smear



5-20 min.*

Hybridize



30 min.

Wash



30 min.

Examine



View Results

PNA FISH workflow

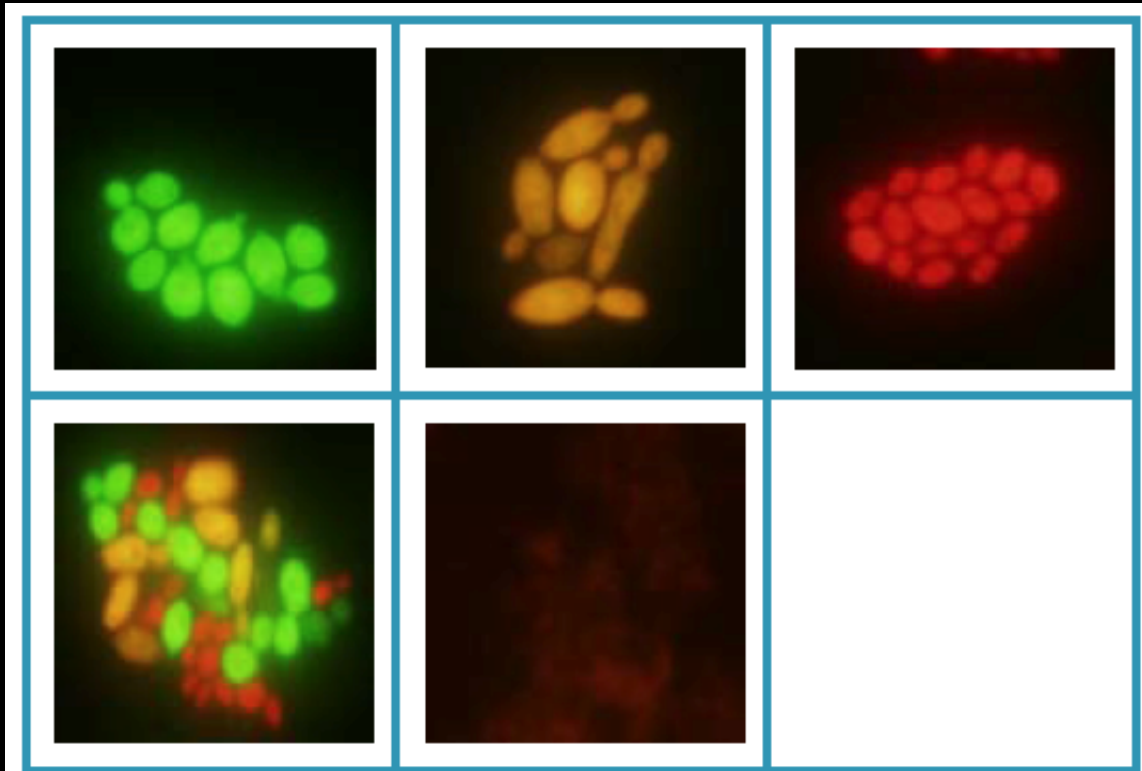
- Gram stain, fix, add reagent, hybridize, rinse, and read
- Ideally, do as needed
- Practically....once or twice per shift?
- What about second and third shift?
- Weekends?
- It is hard enough to get second shift to do gram stains, now fluorescent microscopy too?

PNA FISH

- Practical example: blood cultures 2 of 2 positive in all four bottles, drawn in ED by nurse, positive for GPC in clusters
- Patient has pneumonia, looks like *H. influenzae* based on sputum gram stain
- *S. aureus* versus Coag neg Staph (CNS)
- What does doc do with results?
- Hopefully interpret *S. aureus* as pathogen and CNS as contaminant, get patient off antibiotic

Yeast Traffic Light FISH

- Can help adjust antifungal therapy



Representative examples of green-positive *C. albicans* (top-left), yellow-positive *C. tropicalis* (top-middle), red-positive *C. glabrata* (top-right), mixture of green-positive *C. albicans*, red-positive *C. glabrata* and yellow-positive *C. tropicalis* (bottom-left) and negative (bottom-middle) test results.

Future FISH



AdvanDx

GNR Traffic Light™ PNA FISH®

Escherichia coli, *Klebsiella pneumoniae*
and *Pseudomonas aeruginosa*
Culture Identification Kit

Acinetobacter too?

BinaxNOW PBP2a

- Inverness Medical BinaxNOW® PBP2a immunochromatographic assay detects penicillin-binding protein 2a in blood cultures positive for *S. aureus*. “30 minute TAT from blood culture.”
- PNA FISH first, if *S. aureus* then BinaxNOW PBP2a?
- Or BinaxNOW *S. aureus* followed by BinaxNow PBP2a?



Microphage MRSA/MSSA

- “MicroPhage's MRSA/MSSA blood culture test enables clinicians to direct the most appropriate therapy by identifying and determining antibiotic susceptibility or resistance of *S. aureus* from blood cultures in four hours.”
- bacteriophage amplification
- www.microphage.com
- Not FDA-approved yet



What about...

- 3:25 pm Friday afternoon?
- Nights?
- Weekends?
- Do these patients get short-changed?
- Sometimes it is hard to even get anyone to take the gram stain report, how will they respond to calling them twice?

Some obstacles to implementation

- Cost
- Equipment
- Workflow
- Expertise
- Billing, CPT codes
- Will the results be acted upon?
- Did I mention cost?
- New is hard. Extra work is hard.

Here are the real kickers

- Convincing lab and hospital administration that spending more money in the lab is worth it can be difficult
- Often they do not see the big picture, only the lab budget
- They may not believe diagnostic company financial savings data
- Don't take a lone ranger approach, must get physicians, pharmacy, and infection control involvement

Model of how this might work?

- Positive blood culture
- Gram stained and results called to inpatient pharmacist, tell him/her you are going **FISHing**
- PNA FISH performed ASAP and results called inpatient pharmacist who can adjust therapy
- How long after antibiotic change order does it take for pharmacy to fill order and nurse to administer drug? Out of the lab's control
- Again, what about at night and weekends?

It takes a village?

- More like a pharmacist/interventionist
- Faster results will mean nothing without follow-through
- Will most likely be a pharmacist who can make therapy changes

Will you get paid?

- CPT codes are there.
- Consult with rep AND look them up in the CPT code book
- Run an audit to make sure billing is working
- Inpatient billing is very different than outpatient
- It is likely that money will be saved, not made

Amplified approaches

- Again, to me, does not make sense to amplify what I can already see
- Ideally, we should be able to amplify a raw blood specimen and do PCR, bypass the blood culture entirely
- Advantage is that instrument can be used for other things, especially if you already have one

BD GeneOhm™ StaphSR Assay

- Differentiates MRSA and MSSA using SmartCycler platform, TAT less than 2 hours
- Makes sense if you already have a SmartCycler
- BD has a new platform on the market with one FDA-approved test



BD MAX System

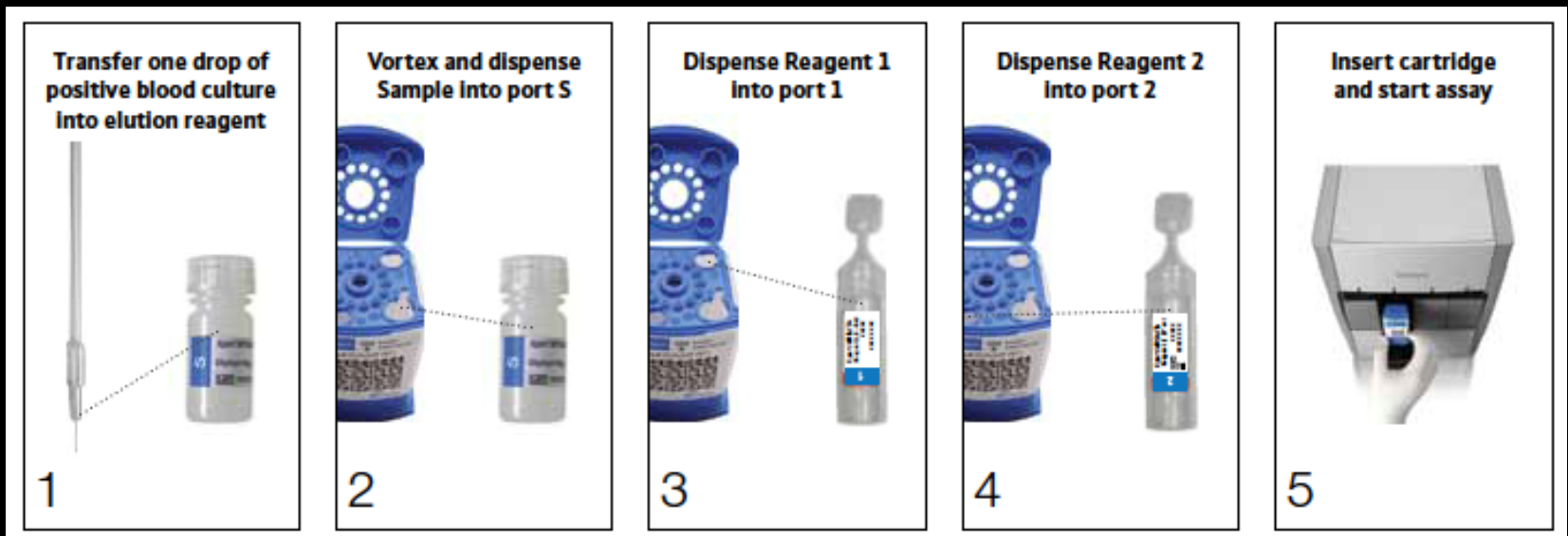


- Bloodstream Infection Panel – positive blood cultures
- “All reagents and consumables required for lysis, nucleic acid extraction and PCR (optional) are loaded into a Unitized Reagent Strip (URS),



Cepheid Xpert MRSA/SA BC

- “Allows de-escalation from broad-spectrum therapy to a targeted antimicrobial approach 18-48 hours sooner, enhance patient outcomes, reduce length of stay, improve antimicrobial stewardship and help ensure appropriate infection control procedures”. Results in less than one hour
- Makes sense to do this one if you already have an instrument



Product recall

Dear Valued Customer:

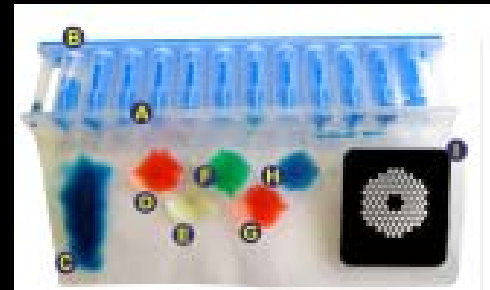
Based on the recall announced July 1, 2010, Cepheid is initiating a recall of all lots of Xpert MRSA/SA BC (blood culture) product. The recall is a corrective action, which does not require return of product to the manufacturer. Customers may continue to use the product; however, when a MRSA negative/SA positive result is obtained, the results should be interpreted as MRSA indeterminate/*Staphylococcus aureus* positive, antimicrobial susceptibility testing pending. Further testing should be performed using an FDA-cleared, phenotypic antimicrobial susceptibility testing method on isolated colonies recovered from the blood culture bottle. MRSA positive/SA positive results can still be reported as such. The new instructions will be incorporated in the product labeling. The Xpert MRSA/SA BC product produces false-negative MRSA results, which could potentially contribute to incorrect treatment of an MRSA infection.

The following statement will be added to the Interpretation of Results section of the package insert for "MRSA Negative/SA Positive" results:

The results should be interpreted as "MRSA indeterminate/SA Positive, antimicrobial susceptibility testing pending". Further testing should be performed using a FDA-cleared, phenotypic antimicrobial susceptibility testing method on isolated colonies recovered from the blood culture bottle.

FilmArray blood culture panel

- Idahotech FilmArray blood culture panel, two posters at ASM, **not FDA-approved**
- Claim detection of 22 species and resistance determinants including *mecA*, *vanA,B,C*, KPC, TEM, SHV, CTX-M, and OXA-58

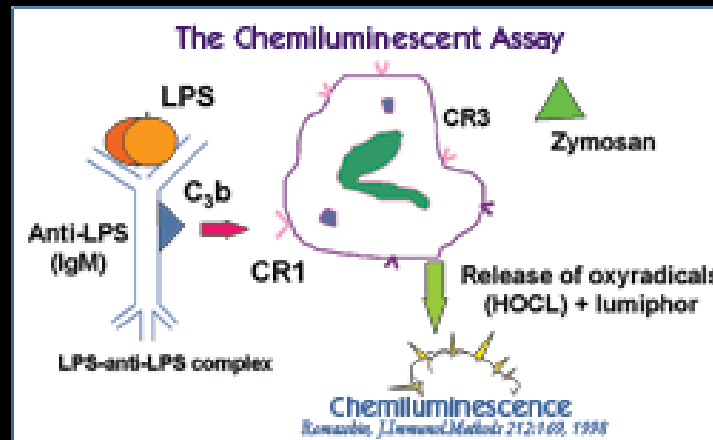


More questions

- If you get a definitive molecular ID, do you still confirm ID and resistance with classic methods?
- If so, are you going to bill for both?
- If discrepant, which will you report?
- Example: PNA FISH says isolate is *C. albicans*. Do you even bother to sub it out?

Whatever happened to?

- Endotoxin assays? (biomarker) “the EAA™ assists physicians in stratifying patients at high risk for severe sepsis.”, calculates an odds ratio
- Endotoxin activity assay system from Spectral Diagnostics, , <http://www.spectraldx.com/endotoxin.htm>





Procalcitonin

- Biomerieux Vidas B.R.A.H.M.S. PCT
- “The blood plasma level of PCT is a reflection of the severity of bacterial infection, ranging from slightly elevated concentrations in infections with minor systemic inflammatory response to very high values in cases of severe sepsis and septic shock.”

So who benefits from faster TAT?

- A case example

Conclusion

- With faster positive blood culture diagnostics, we should be able to change this graph

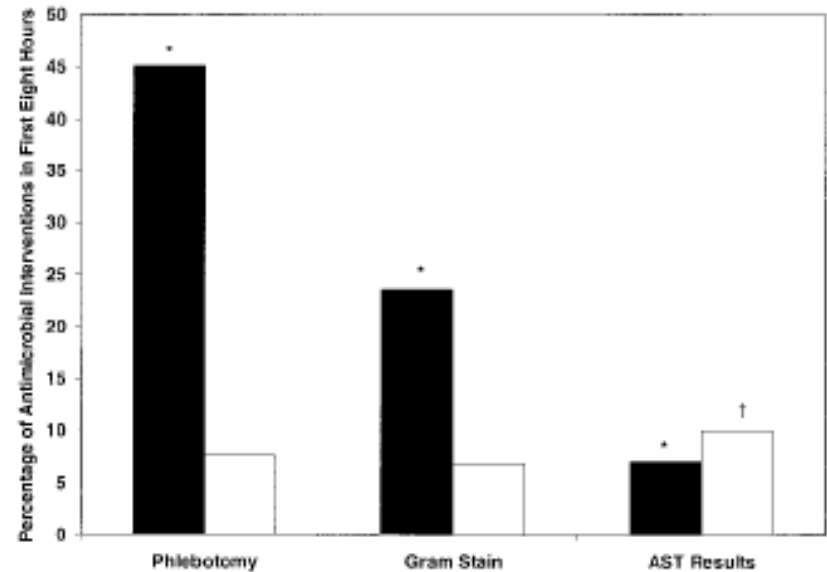


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