Blood Specimens

A discussion put together by

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Outline

- Sepsis Background
- Blood culture process
  - Blood culture collection
  - Blood culture contamination
  - Blood culture incubation
  - Gram Stains and Rapid Identification
  - Traditional identification and susceptibility testing
  - Genotypic/Phenotypic discrepancies
- Relevant Metrics
- Cases and unique situations

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Sepsis Background

- Sepsis = dysregulation of immune system caused by infection
- Leading cause of death in non-cardiac ICUs
- Mortality rates in septic patients as high a 60%
- In 2008, sepsis led to 1.7 million admissions in USA[1]
- Annual costs can exceed $24 billion
- Timely initiation of empiric and targeted therapy can significantly decrease mortality rate in septic patients
- Overuse of antibiotics can:
  - Have adverse host effects (e.g. acute kidney injury)
  - Lead to opportunistic infection (e.g. C. difficile)
  - Increase rate of antibiotic resistant bacteria

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Sepsis Background

- Skin antisepsis decreases risk of contamination
  - Studies show contaminated blood cultures cost hospital >$4K[2]
  - Rapid ID of contaminants can decrease additional costs
- Faster blood culture turnaround yields better outcomes
  - Studies demonstrate mortality rates can increase by 8% for every hour a septic shock patients are not treated[3]
- Provider Intervention Steps:
  - Interventions include, Abx escalation, de-escalation
  - Interventions occur following:
    - Positive gram stain result
    - Identification of offending organism
    - Completion of susceptibility testing

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Sepsis Background

- Patients suspected for sepsis:
  - Started on empiric broad spectrum coverage
    - Vancomycin (gram pos)
    - Piperacillin/tazobactam or cefepime (gram neg)
    - Goal is to tailor coverage as quickly as possible
- How can micro labs play a role
  - Decrease times from collection to loading in incubator
    - Micro guidelines indicate bottles loaded < 2-4 hours[4-6] (most guidelines say < 2 hrs.)
  - Decrease time from collection to appropriate therapy
    - Rapid identification methods
    - Prompt susceptibility information/testing
    - Active antimicrobial stewardship acting on results

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Sepsis Background

- Results that can directly impact therapeutic change
  - Positive identification of *S. aureus* or MRSA
    - MRSA will often receive additional antibiotic
    - MSSA often de-escalated from vancomycin
  - Identification of *Enterococcus* (and VRE)
    - VRE therapy immediately changed to daptomycin/linezolid
  - Identification of *Enterobacterales* resistance mechanism can lead to change
    - Organisms positive for ESBLs, escalated to meropenem
    - Organisms positive for carbapenemases, escalated further
    - Organisms negative for ESBLs/carbapenemases may be de-escalated to ceftriaxone

There are many different approaches to blood cultures!!

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Blood Culture Considerations

- Collection process
  - Who is collecting, Skin prep, diversion devices, bottle type
- Incubation location
  - Centralized vs. decentralized (on-site vs. off-site)
- Processing of positive bottles
  - Where does it occur, rapid identification
- Definitive identification
  - Methods, full identification?
- Susceptibility testing
  - Which isolates, how often, how do your rectify discrepancies
- Relevant metrics
  - Contamination rate, gram stain errors, positivity rate

Blood Culture Collection

- Who is collecting?
  - Why does it matter?
    - Studies demonstrate that trained phlebotomists have decreased contamination rates vs. clinical staff [7,8]
    - Studies demonstrate that gaps in knowledge and technique are common causes of contamination [9,10]
    - Education/training programs have demonstrated significant reduction in blood culture contamination [11]
  - If clinical staff are collecting blood cultures, ensure that:
    - There is an effective training program in place
    - There is a mechanism to follow up and re-educate
    - That the educational program is repetitive

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Blood Culture Collection

- Collection recommendation
  - Blood culture sets can be collected simultaneously rather than over intervals
  - Adults w/ suspected BSI should have 2-3 sets collected over 24 hours
  - Recommendation is a paired aerobic and anaerobic bottle w/ 10 mLs of blood per bottle
- Disinfection recommendations
  - Cleanse venipuncture site for 30 seconds w/ alcohol
  - Allow to air dry
  - Cleanse w/ second disinfectant (iodine or chlorhexidine)
  - Allow to stand for recommended time (30 seconds to 2 minutes)
  - Don’t palpitate vein following disinfection

Blood Culture Collection

- Contaminated cultures lead to:
  - Increased cost, length of stay, work on lab staff

- Definitions of blood culture contaminant
  - Single bottle or single set positive for:
    - Coagulase negative *Staphylococci*
    - *Cutibacterium acnes*
    - *Micrococcus* sp.
    - Viridans group *Streptococcus* sp.
    - *Corynebacterium* sp.
    - *Aerococcus* sp.
    - *Bacillus* sp.

Do you divert/discard blood when collecting blood cultures? If so, which diversion method/device do you use?

A. Kurin Jet
B. Magnolia Steripath devices
C. An extra vacutainer
D. We focus on skin disinfection and do not use a diversion device
E. What are you talking about?

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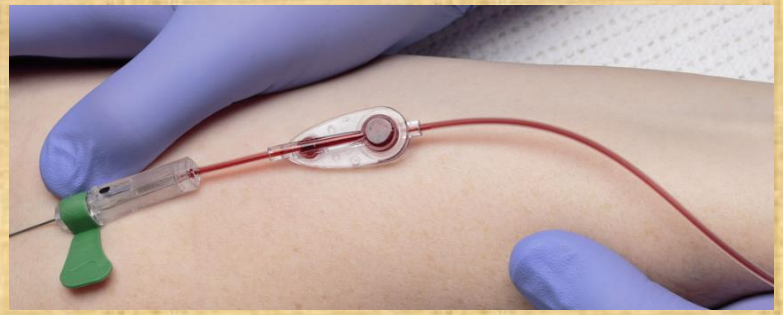
Blood Culture Collection

- Diversion devices
  - Kurin Jet
  - Steripath
  - Steripath micro



- Divert initial blood sample
  - Designed to trap skin plug
  - May decrease contamination (may not)
  - Expensive – Can cost upwards of $15 each

Blood Culture Collection

- Studies demonstrate significant decreases in contamination rates with blood culture diversion devices Bottle Type [2,12,13]
  - Kurin Jet, Steripath, Steripath micro
  - At VMH, use the **second or third syringe from IV starts** to fill blood cultures, diverts blood with **NO** additional cost.
- Internal studies at ACL demonstrated initial decreases w/ diversion device that reverted after several months
  - Implementation included retraining proper disinfection techniques
  - After several months as disinfection practices became more lax contamination rates returned to baseline even with diversion device
  - Our sites with lowest contamination rates don't use diversion
  - Disinfection technique appears to be key to sustaining low rates

Strategies, Physical and Social used at VMH to Reduce Contamination

Physical:

- Insist upon 2 step cleaning
  - Alcohol/iodine or alcohol/Chloroprep
  - If the initial alcohol wipe is brown, start over
- Utilize pressure during cleaning
- Spin the disinfectant out in concentric circles
- Minimum of 30 seconds of "drying time". If you are observing the draw, you are also responsible for ensuring dwell times!!
- Avoid palpitation after cleaning if possible
- Blood cultures should be filled with the second and third syringes from IV starts

Strategies, Physical and Social used at VMH to Reduce Contamination

Social:

- Monitor and verify success with a robust QA/QI program
  - Monitor total contamination rates
  - Individual contamination rates
    - If phlebotomist has > 2 contaminated cultures/quarter, have a focused conversation on technique.
- Send PDSA (**P**lan, **D**o, **S**tudy, **A**ct) emails to nursing and phlebotomy staff drawing blood cultures
  - Include commentary of contamination rates with analysis of venipuncture vs IV start rates if necessary
  - Include technique reminders when rates are high
  - **lavishing praise when rates are low!!**
- Allow use of 2-12 mL instead of 20 mL syringes

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Blood Culture Incubation

- As hospitals and microbiology laboratories consolidate they face decisions on which testing will remain on site
- Considerations on centralization vs. decentralization
  - Micro guidelines indicate blood culture bottles should be loaded into incubators within 2-4 hours [4-6]
    - Centralization requires frequent or STAT courier routes
    - Decentralization requires investment in instrumentation with positive plates and bottles being sent out
  - Mix of skill level
    - Centralization ensures gram stains and cultures are read by dedicated microbiologists
    - Decentralization requires an effective Gram Stain QA/QI program
    - Rapid identification methods on-site??

Blood Culture Incubation

- Incubation protocols??
- 35 – 37°C
- Current recommendation is 5 days with automated systems[14]
  - Recommendation includes slower growing organisms like *Brucella*, HACEK organisms and nutritionally variant *Streptococci*
  - Prolonged incubation unnecessary for suspected endocarditis
  - Studies show you might be able to consider decreasing incubation time to 4 or even 3 days[15-18]

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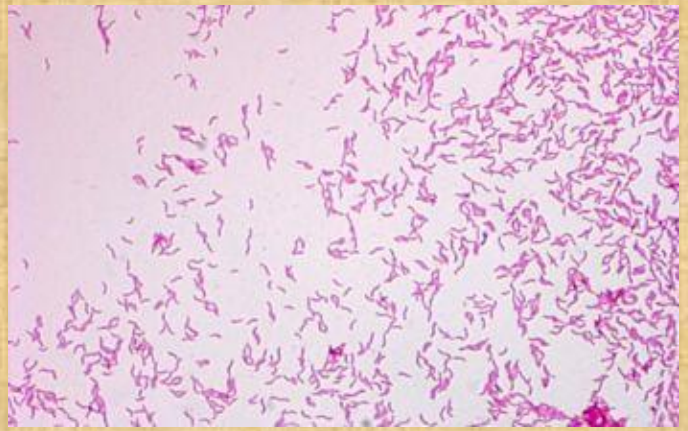
Gram Stains

- Gram stains should be performed promptly on all positive blood culture bottles
  - Can guide additional workup
    - Rapid molecular testing?
  - Can dictate additional media or subculture conditions
    - e.g. Gull wing gram negative bacilli
  - May provide therapeutic guidance
  - May have infection prevention ramifications
    - e.g. Gram-negative diplococci



Gram Stains

- Positive bottles should be processed with biosafety in mind
  - Process in a biosafety cabinet
    - Venting process can aerosolize bacteria
  - Time to positivity and/or morphology can elevate laboratory exposure risk
    - Slow growing gram-negative coccobacilli
    - Gram negative diplococci
    - Do you have criteria in place?
    - How do you ensure others are aware of biosafety risks?
    - At ACL we place stickers on GNRs that go positive > 36 hours and tape plates
      - Alerts others to risk

Which method does your laboratory use for rapid identification methods of positive blood cultures?

A. We don’t use a rapid identification method
B. MALDI (slime/scum method or sepsityper)
C. MRSA/MSSA identification method (e.g. Cepheid MRSA/SA BC)
D. Multiplex molecular panel (> 5 targets)
E. Rapid identification/AST system (e.g. Accelerate Pheno or Biomerieux Vitek Reveal)
F. None of the above (there is no F button so raise your hand)

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If you are using a rapid multiplex molecular panel, which one are you using?

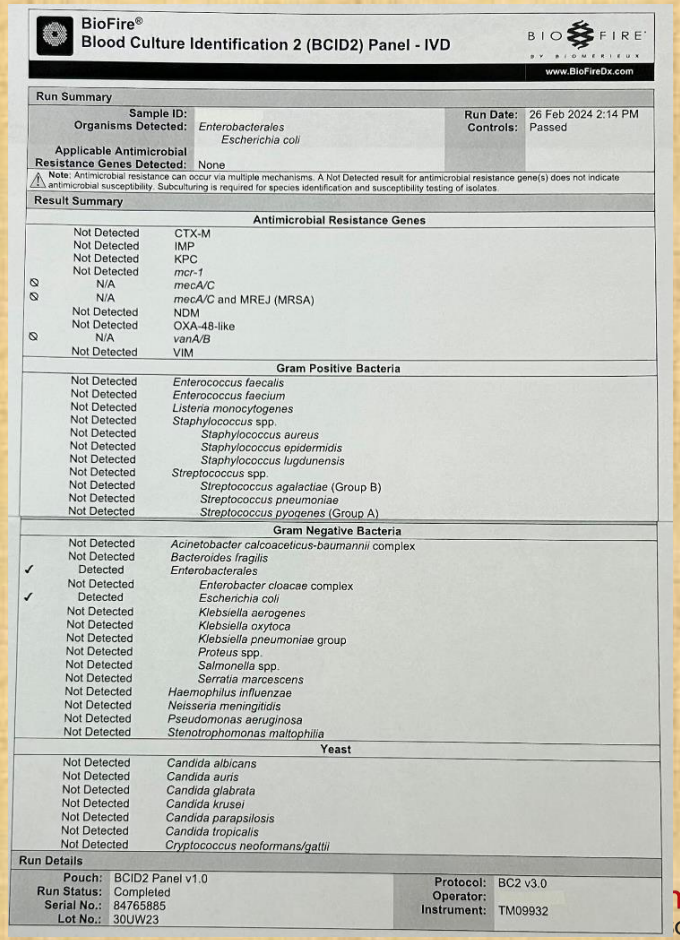
A. BioFire FilmArray BCID2 Panel
B. Nanosphere/Luminex/Diasorin Verigene System
C. Genmark/Roche ePlex
D. Other molecular panel
E. We do not use molecular panels for rapid identification

Rapid Identification Methods

- Molecular
  - Several Types of Panels
    - Comprehensive Panels
    - Smaller panels (separated by morphology)
    - Directed Tests (MSSA/MRSA tests)
  - Rapid
    - Less than an hour
  - Include antibiotic resistance markers
  - Require specialized equipment
  - Expensive
  - Identify offending organism in >80% of positive blood cultures

Rapid Identification

- Large molecular panels have long lists of organisms
- How do you report
  - Line list everything?
  - Only report positive targets?
    - If so, do you list what targets were tested so providers know which targets they can rule out
  - Report all antibiotic resistance markers if no organism is present?
  - Provide some interpretation of results?



BioFire®
Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE by BIOMÉRIEUX
www.BioFireDx.com

Run Summary

Sample ID:		Run Date:	26 Feb 2024 2:14 PM
Organisms Detected:	*Enterobacterales* *Escherichia coli*	Controls:	Passed
Applicable Antimicrobial Resistance Genes Detected:	None		

Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

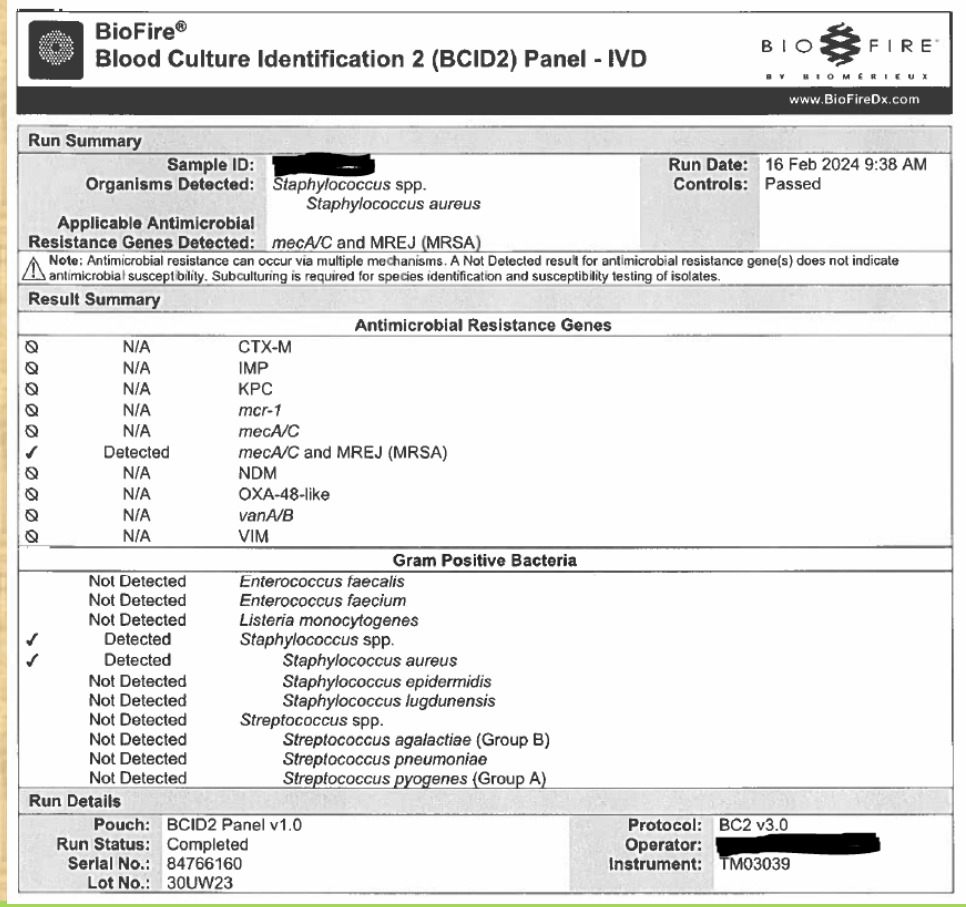
Antimicrobial Resistance Genes		
	Not Detected	CTX-M
	Not Detected	IMP
	Not Detected	KPC
	Not Detected	*mcr-1*
⊘	N/A	*mecA/C*
⊘	N/A	*mecA/C* and MREJ (MRSA)
	Not Detected	NDM
	Not Detected	OXA-48-like
⊘	N/A	*vanA/B*
	Not Detected	VIM
Gram Positive Bacteria		
	Not Detected	*Enterococcus faecalis*
	Not Detected	*Enterococcus faecium*
	Not Detected	*Listeria monocytogenes*
	Not Detected	*Staphylococcus* spp.
	Not Detected	*Staphylococcus aureus*
	Not Detected	*Staphylococcus epidermidis*
	Not Detected	*Staphylococcus lugdunensis*
	Not Detected	*Streptococcus* spp.
	Not Detected	*Streptococcus agalactiae* (Group B)
	Not Detected	*Streptococcus pneumoniae*
	Not Detected	*Streptococcus pyogenes* (Group A)
Gram Negative Bacteria		
	Not Detected	*Acinetobacter calcoaceticus-baumannii* complex
	Not Detected	*Bacteroides fragilis*
✓	Detected	*Enterobacterales*
	Not Detected	*Enterobacter cloacae* complex
✓	Detected	*Escherichia coli*
	Not Detected	*Klebsiella aerogenes*
	Not Detected	*Klebsiella oxytoca*
	Not Detected	*Klebsiella pneumoniae* group
	Not Detected	*Proteus* spp.
	Not Detected	*Salmonella* spp.
	Not Detected	*Serratia marcescens*
	Not Detected	*Haemophilus influenzae*
	Not Detected	*Neisseria meningitidis*
	Not Detected	*Pseudomonas aeruginosa*
	Not Detected	*Stenotrophomonas maltophilia*
Yeast		
	Not Detected	*Candida albicans*
	Not Detected	*Candida auris*
	Not Detected	*Candida glabrata*
	Not Detected	*Candida krusei*
	Not Detected	*Candida parapsilosis*
	Not Detected	*Candida tropicalis*
	Not Detected	*Cryptococcus neoformans/gattii*

Run Details

Pouch:	BCID2 Panel v1.0	Protocol:	BC2 v3.0
Run Status:	Completed	Operator:	
Serial No.:	84765885	Instrument:	TM09932
Lot No.:	30UW23		

Rapid Identification Methods

- MRSA from BioFire BCID2
  - BioFire BCID2 test produces 3 positive targets
    - *Staphylococcus* sp.
    - *Staphylococcus aureus*
    - *mecA/C* and MREJ (MRSA)
- If you report all 3 targets, how will it be interpreted?
  - Multiple *Staph* sp?
  - A coag neg *Staph* and MRSA?
  - Will they just get it?
    (Hint: No, they won't)



BioFire®
Blood Culture Identification 2 (BCID2) Panel - IVD
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Run Summary

Sample ID:	[redacted]	**Run Date:**	16 Feb 2024 9:38 AM
Organisms Detected:	*Staphylococcus* spp. *Staphylococcus aureus*	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes Detected:	*mecA/C* and MREJ (MRSA)		

⚠ **Note:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

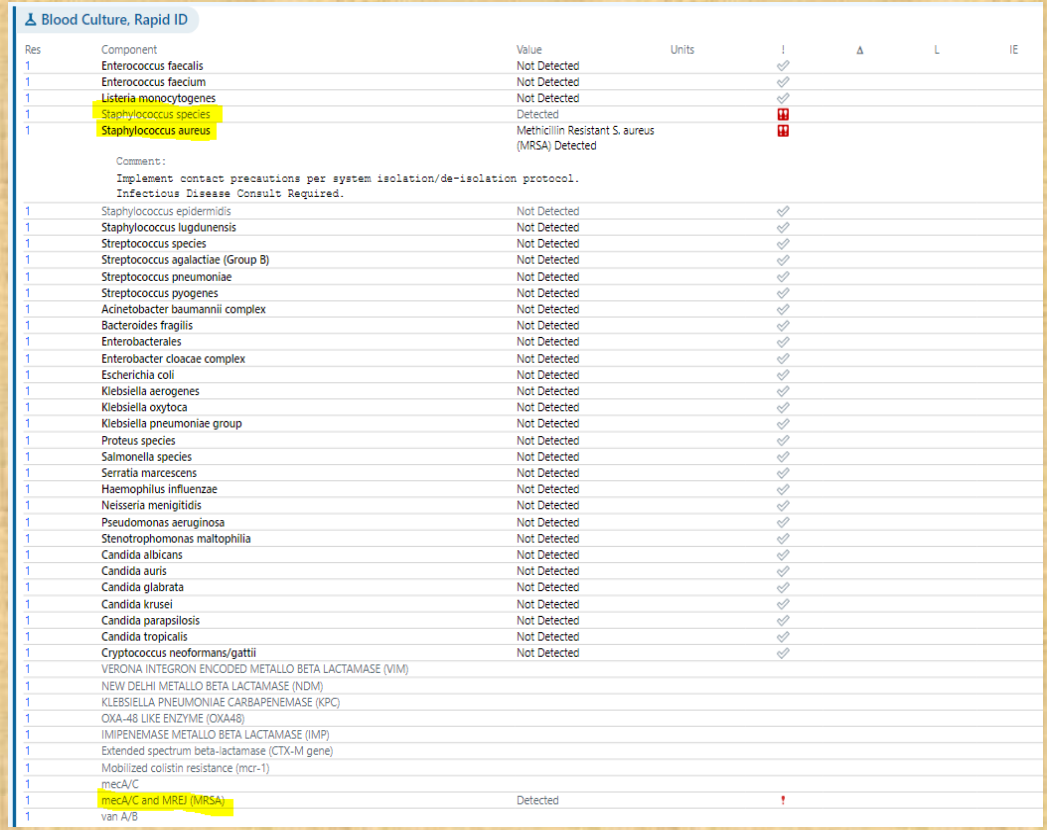
	Antimicrobial Resistance Genes	
⊘	N/A	CTX-M
⊘	N/A	IMP
⊘	N/A	KPC
⊘	N/A	*mcr-1*
⊘	N/A	*mecA/C*
✓	Detected	*mecA/C* and MREJ (MRSA)
⊘	N/A	NDM
⊘	N/A	OXA-48-like
⊘	N/A	*vanA/B*
⊘	N/A	VIM
	Gram Positive Bacteria	
	Not Detected	*Enterococcus faecalis*
	Not Detected	*Enterococcus faecium*
	Not Detected	*Listeria monocytogenes*
✓	Detected	*Staphylococcus* spp.
✓	Detected	*Staphylococcus aureus*
	Not Detected	*Staphylococcus epidermidis*
	Not Detected	*Staphylococcus lugdunensis*
	Not Detected	*Streptococcus* spp.
	Not Detected	*Streptococcus agalactiae* (Group B)
	Not Detected	*Streptococcus pneumoniae*
	Not Detected	*Streptococcus pyogenes* (Group A)

Run Details

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	[redacted]
Serial No.:	84766160	**Instrument:**	TM03039
Lot No.:	30UW23		

Rapid Identification Methods

- At ACL:
  - All BCID2 targets are built in EPIC Beaker
  - All results reported on BioFire instrument cross interface
  - Three positive results in our lab system

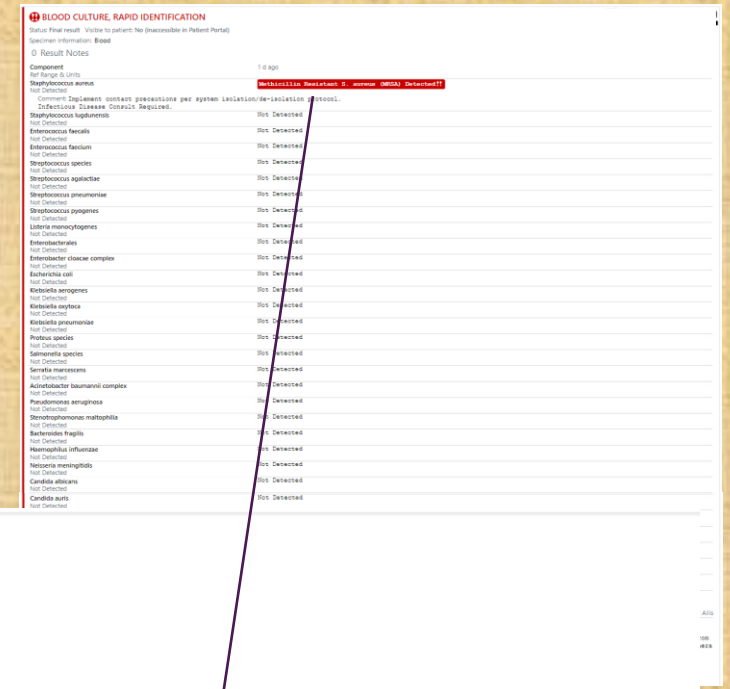


Blood Culture, Rapid ID

Res	Component	Value	Units	!	A	L	IE
1	Enterococcus faecalis	Not Detected					
1	Enterococcus faecium	Not Detected					
1	Listeria monocytogenes	Not Detected					
1	Staphylococcus species	Detected					
1	Staphylococcus aureus	Methicillin Resistant S. aureus (MRSA) Detected					
	Comment: Implement contact precautions per system isolation/de-isolation protocol. Infectious Disease Consult Required.						
1	Staphylococcus epidermidis	Not Detected					
1	Staphylococcus lugdunensis	Not Detected					
1	Streptococcus species	Not Detected					
1	Streptococcus agalactiae (Group B)	Not Detected					
1	Streptococcus pneumoniae	Not Detected					
1	Streptococcus pyogenes	Not Detected					
1	Acinetobacter baumannii complex	Not Detected					
1	Bacteroides fragilis	Not Detected					
1	Enterobacterales	Not Detected					
1	Enterobacter cloacae complex	Not Detected					
1	Escherichia coli	Not Detected					
1	Klebsiella aerogenes	Not Detected					
1	Klebsiella oxytoca	Not Detected					
1	Klebsiella pneumoniae group	Not Detected					
1	Proteus species	Not Detected					
1	Salmonella species	Not Detected					
1	Serratia marcescens	Not Detected					
1	Haemophilus influenzae	Not Detected					
1	Neisseria meningitidis	Not Detected					
1	Pseudomonas aeruginosa	Not Detected					
1	Stenotrophomonas maltophilia	Not Detected					
1	Candida albicans	Not Detected					
1	Candida auris	Not Detected					
1	Candida glabrata	Not Detected					
1	Candida krusei	Not Detected					
1	Candida parapsilosis	Not Detected					
1	Candida tropicalis	Not Detected					
1	Cryptococcus neoformans/gattii	Not Detected					
1	VERONA INTEGRON ENCODED METALLO BETA LACTAMASE (VIM)						
1	NEW DELHI METALLO BETA LACTAMASE (NDM)						
1	KLEBSIELLA PNEUMONIAE CARBAPENEMASE (KPC)						
1	OXA-48 LIKE ENZYME (OXA48)						
1	IMIPENEMASE METALLO BETA LACTAMASE (IMP)						
1	Extended spectrum beta-lactamase (CTX-M gene)						
1	Mobilized colistin resistance (mcr-1)						
1	mecA/C						
1	mecA/C and MREJ (MRSA)	Detected		!			
1	van A/B						

Rapid Identification Methods

- At ACL:
  - Only one positive result goes to chart
  - *Staphylococcus* genus target is hidden
  - *MecA/C* and MREJ target hidden
  - *Staphylococcus aureus* result released with MRSA interpretation



BLOOD CULTURE, RAPID IDENTIFICATION

Status: Final result Visible to patient: No (inaccessible in Patient Portal)

Specimen Information: Blood

0 Result Notes

Component	1 d ago
Ref Range & Units	
Staphylococcus aureus Not Detected	Methicillin Resistant S. aureus (MRSA) Detected!!

Comment: Implement contact precautions per system isolation/de-isolation protocol.
Infectious Disease Consult Required.

Rapid Identification Methods

- MALDI-TOF
  - Can identify organism in nearly all positive BLCs
  - Requires expensive instruments, testing is cheap
  - No sensitivity information
  - Sepsityper (or similar method)
    - Rapid, 15-20 minutes
    - Requires special sample processing kit
  - Rapid/Slime/Scum Method
    - Few drops to blood plate
    - Incubate 5-6 hours
    - Perform MALDI from scant growth
    - Works best for gram negs

Rapid ID of GNRs at ACL

- Prior to 2022, ACL utilized MALDI slime method
  - Cheap; < $1 to perform
  - Relatively fast; ~ 6 hours after positive bottle
  - Accurate for GNRs; > 75% success rate
  - No susceptibility information
- Advocate Health ranges from Green Bay to Chicago
- In Chicago (and Milwaukee), high levels of GNR resistance with ESBLs and CREs
  - Identification of *Enterobacterales* often insufficient to act
  - Providers don't change therapy without susceptibilities
  - For *E. coli, K. pneumoniae,* and *K. oxytoca* average time from collection to susceptibility result was 58.4 hours

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Rapid ID of GNRs at ACL

- In 2022, ACL switched to use of BioFire BCID2
  - 25% of cultures positive for *E. coli, K. pneumoniae,* or *K. oxytoca*
  - Review of 1 year of data (2906 isolates)
  - BCID2 predicted presence/absence of ESBL or CRE phenotype correctly in 98.9% of cases
  - De-escalation based on BCID2 result would have led to ineffective therapy in < 0.6% of cases
- Now
  - Rapid test sufficient to escalate or de-escalate therapy for GNRs; no need to wait for susceptibility results
  - For *E. coli, K. pneumoniae,* and *K. oxytoca* average time from collection to BCID2 result was 30.7 hours
  - 47.4% decrease in time to actionable result

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How do you identify and report organisms from positive blood cultures?

A. We provide (or attempt) a species level identification of all isolates

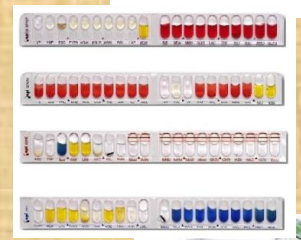
B. Fully identify all pathogens; genus level ID (or other minimal ID) for skin flora bugs regardless of the number of bottles it is found in

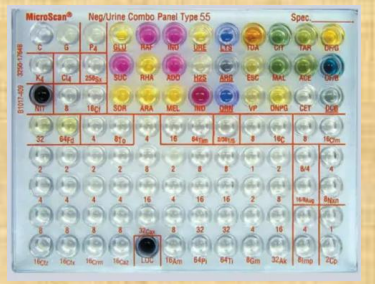
C. Fully identify all pathogens, genus level ID (or other minimal ID) for skin flora bugs in one set, full ID if skin flora bugs found in multiple sets

D. Our policy is so hard to follow I can't even tell you

Traditional Identification Methods

- Traditional MALDI-TOF Method
  - Requires overnight subculture
  - Large database, cheap
  - No sensitivity information
- Biochemical Method
  - Automated Panels on Vitek, Microscan, Phoenix
  - API Strips
  - Larger database than molecular
  - Smaller data base than MALDI
  - Up to 24 hours to get results
  - No sensitivity information

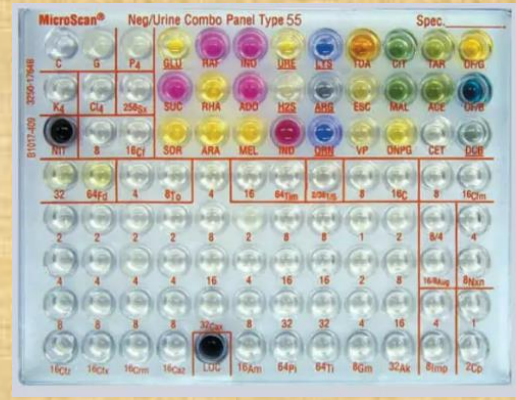


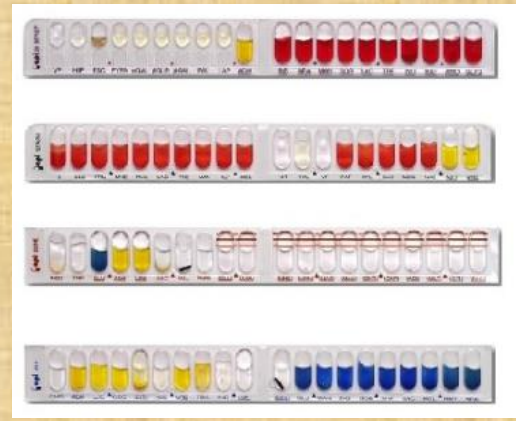


Traditional Identification

- When is a traditional identification required
  - All positive bottles?
  - First positive bottle in each set?
  - Once every few days?
  - Is it required if you have a rapid molecular ID?

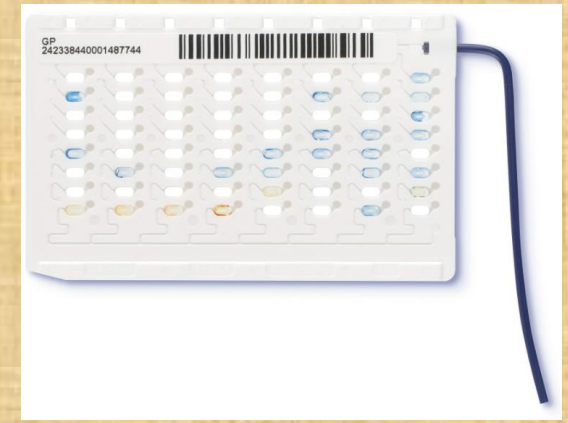
- At ACL, we do a formal ID on the first bottle that is positive from any blood culture
  - ID even if morphology matches rapid test
  - Subsequent positive bottles
    - Minimal confirmatory biochemical ID
  - If multiple sets positive in one day will still do formal ID on first positive bottle from the set





Susceptibility Testing

- Which organisms?
  - May not perform susceptibility testing on common blood culture contaminants, including:
    - Coagulase negative *Staphylococci*
    - *Cutibacterium acnes*
    - *Micrococcus* sp.
    - Viridans group *Streptococcus* sp.
    - *Corynebacterium* sp.
    - *Aerococcus* sp.
    - *Bacillus* sp.
  - May set up susceptibility testing on these organisms if positive in multiple sets of cultures



Susceptibility Testing

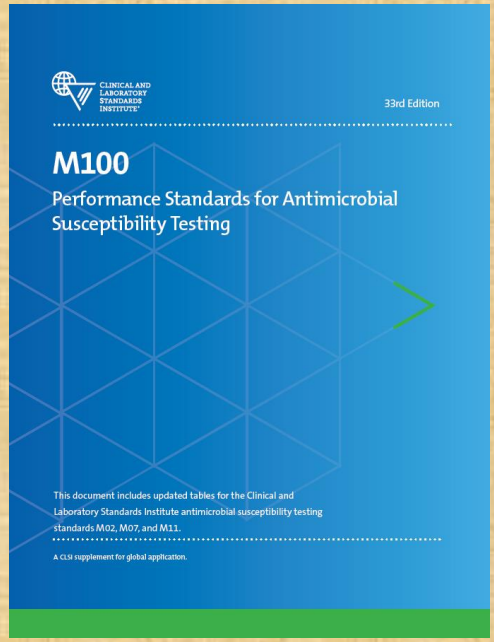
- How often?
  - CLSI M47 ED2, 2022 – Susceptibility testing only needs to be repeated once every five days
  - Some isolates may be tested more frequently
    - *S. aureus* from patients receiving prolonged therapy
    - *P. aeruginosa* due to rapid development of resistance
    - Organisms containing inducible AmpC beta-lactamases

Susceptibility Testing

- Genotypic vs. phenotypic discrepancy
  - CAP MIC.21835; If organism identification and/or antimicrobial susceptibility testing (genotypic or phenotypic) is performed directly from positive blood culture bottles, the broth from the bottle is inoculated onto solid media to assess for consistency with direct results

Genotypic/Phenotypic Discrepancies

- Genotypic vs. phenotypic discrepancy
  - So what do you do if you get disagreement?
  - CLSI M100 Appendix H describes how to resolve discrepancies between rapid testing and traditional testing



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Genotypic/Phenotypic Discrepancies

- MSSA/MRSA or VRE/VSE
- If molecular and susceptibility testing agree, report as tested
- If there is disagreement:
  - Confirm ID
  - Repeat molecular test
  - Repeat AST
  - If still in disagreement err on the side of resistance:
    - Report as MRSA or VRE

Table H1. Strategies for Reporting Methicillin (Oxacillin) Results When Using Molecular and Phenotypic AST Methods for *S. aureus*

Indication	Targets	Methods	Specimen Types	Results: Genotype or Predicted Phenotype	Results: Observed Colony Phenotype (if tested)	Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
Detecting methicillin (oxacillin) resistance in *S. aureus*	PBP2a	Latex agglutination, immuno-chromatography	Colony	PBP2a positive	Cefoxitin R	N/A	Methicillin (oxacillin) R	1
				PBP2a negative	Cefoxitin S	N/A	Methicillin (oxacillin) S	1
				PBP2a positive	Cefoxitin S	Confirm isolate identification, repeat latex agglutination and AST, and consider *mecA* colony NAAT, if available.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	1-2
				PBP2a negative	Cefoxitin R	Confirm isolate identification, repeat latex agglutination and AST, and consider *mecA* colony NAAT, if available.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	1
	mecA	NAAT, microarray hybridization, ISH	Colony, blood culture broth, surveillance specimen	*mecA* detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3-6
				mecA not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3-6
				mecA detected	Cefoxitin S	Confirm isolate identification, repeat AST, and repeat or perform *mecA* colony NAAT, if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2-5, 8-9
				mecA not detected	Cefoxitin R	Confirm isolate identification, repeat AST, and repeat or perform *mecA* colony NAAT, if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	3, 7

Genotypic/Phenotypic Discrepancies

- Detection of ESBLs
- Compare molecular ESBL detection to 3rd and 4th gen. cephalosporins
  - If discrepancies, may consider repeating AST or molecular testing
  - Report phenotypic result; genotypic result may or may not be reported

Table H3. Reporting Results From Extended-Spectrum β-Lactamase Resistance and Carbapenemase Molecular Tests for Enterobacterales

Indication	Targets	Methods	Specimen Types	Results: Molecular Target Results	Results: Observed Phenotype (if tested)	Suggestions for Resolution	Report as:	Comments[a]
Detection of ESBL resistance in Enterobacterales (in an isolate susceptible to all carbapenems)	ESBL type CTX-M, SHV, TEM	NAAT, microarray	Colony, blood culture	Detection of any ESBL target	R to all 3rd- and 4th-generation cephalosporins tested (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R)	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol.	1-12
				Detection of any ESBL target	S to all 3rd- and 4th-generation cephalosporins tested (eg, ceftriaxone S, cefotaxime S, ceftazidime S, cefepime S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported.	1-12
				Detection of *CTX-M* ESBL target	Variable resistance to 3rd- and 4th-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R or S, cefepime R or S)	Expected phenotype for some *CTX-M* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1-12
				Detection of *TEM* or *SHV* ESBL target	Variable resistance to 3rd- and 4th-generation cephalosporins (eg, ceftriaxone R or S, cefotaxime R or S, ceftazidime R or S, cefepime R or S).	Expected phenotype for some *TEM*/*SHV* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1-12
Detection of ESBL resistance in Enterobacterales (in an isolate susceptible to all carbapenems) (Continued)				No detection of ESBL targets	Resistance to 3rd-generation cephalosporins and variable resistance to 4th-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R or S)	Likely non-tested broad spectrum β-lactamase (eg, AmpC, carbapenemase, or other ESBL); consider repeating molecular tests and checking cefepime using reference method if S.	Report phenotypic results as found, including reference cefepime result if tested.	1-12

Genotypic/Phenotypic Discrepancies

- Detection of CREs
- Compare molecular carbapenemase marker result to carbapenem susceptibility
  - If discrepancies, may consider repeating AST or molecular testing
  - If discrepancies cannot be resolved report genotypic and phenotypic results with comments about the discrepancy

Table H3. (Continued)

Indication	Targets	Methods	Specimen Types	Results: Molecular Target Results	Results: Observed Phenotype (if tested)	Suggestions for Resolution	Report as:	Comments[a]
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target or phenotypic detection of carbapenemase production	Susceptibility (S or SDD) to 3rd- and/or 4th-generation cephalosporins but intermediate or resistant to at least one carbapenem tested	Repeat molecular and phenotypic tests.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution: "Current clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy of carbapenemase-carrying strains with an MIC in the S/SDD range will be effective."	1-4, 12-14
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)	NAAT, microarray	Colony, blood culture	No detection of tested carbapenemase targets	Resistance to any carbapenems except ertapenem (eg, meropenem R, imipenem R, doripenem R, ertapenem R or S)	Possible other carbapenemase. If blood culture, check for mixed culture. If mixed, test isolates individually and report as found; consider repeating molecular and AST and performing a phenotypic test for carbapenemase activity (eg, CarbaNP or mCIM).	If carbapenemase activity is detected, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range will be effective or whether the molecular assays are completely accurate. Otherwise report phenotypic results as found.	1-4, 12-16

Relevant Metrics

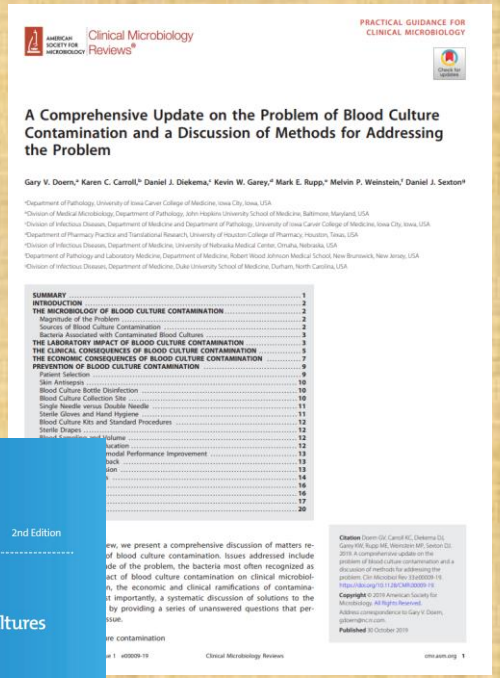
- Blood culture contamination
  - Increases cost to patient and hospital
  - Longer stays
  - More work on lab
  - CAP MIC.22635. The laboratory monitors blood culture contamination rates and has established an acceptable threshold.
    - Current national recommendation is 3.0%
    - There has been discussion of lowering it 1.0%
  - It is important to collect the data, but it is also important to analyze and share the data!!
    - Determine if there are frequent offenders
    - Consider re-training

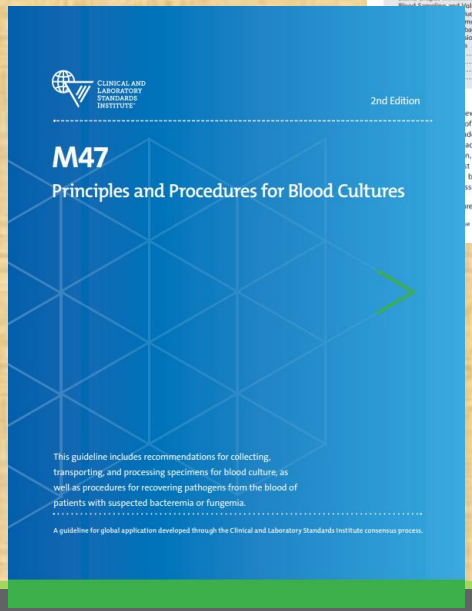
Relevant Metrics

- Low volume blood culture draws
  - How do you measure?
  - Do you make a note in your culture
  - CAP MIC.22640 The laboratory monitors blood cultures from adults for adequate volume and provides feedback on unacceptable volumes to blood collectors
- Gram stain accuracy
  - > 95% gram stain accuracy
- Other metrics
  - % Rapid Id/Sensitivity results matching traditional ID/Sensitivity

Important Resources

- CLSI. Principles and Procedures for Blood Cultures. 2nd ed. CLSI Guideline M47. Clinical and Laboratory Standards Institute: 2022.
- Doern GV, et al. 2020. Practical guidance for clinical microbiology laboratories: A comprehensive update on the problem of blood culture contamination and a discussion of methods for addressing the problem. Clin Microbiolo Rev. **33(1):** e00009-19.





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Summary

- There are many different ways to perform blood cultures
- Laboratories need to consider:
  - Best practices for collection
  - Whether they will use centralized or decentralized model
  - If they will use rapid identification, and if so, which one
  - How will final identification be performed
  - When will susceptibility testing be done
- Appropriate metrics and feedback can be helpful for collection and testing accuracy

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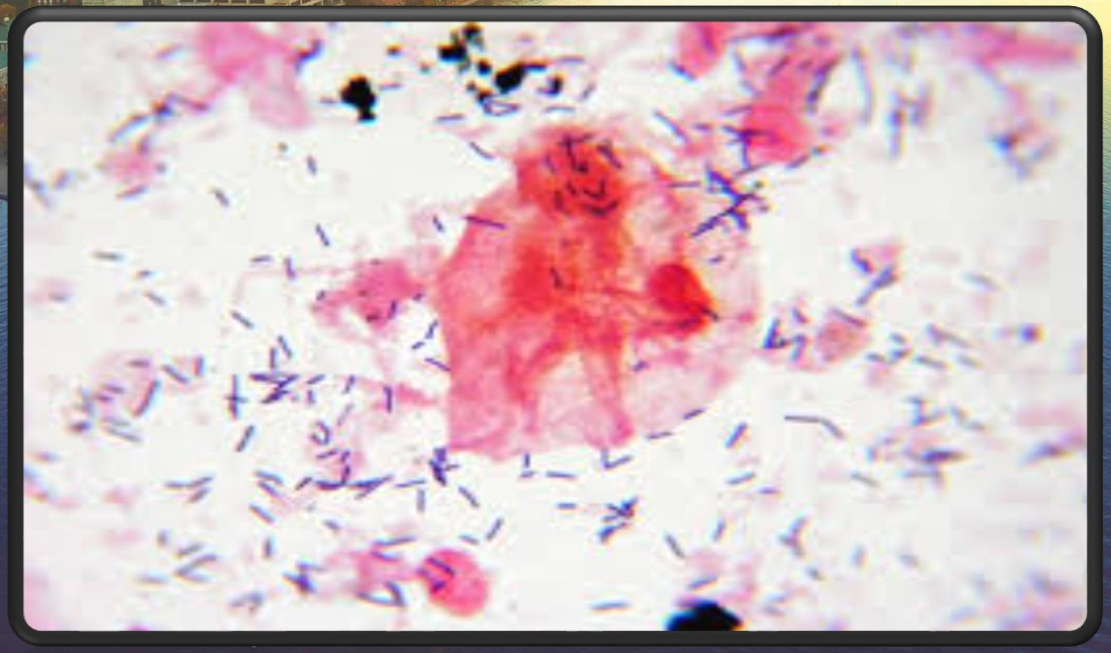
Bugs Bugs Bugs: All You Need is More, but Where (not in blood)?



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Case # 1

- Physician requests already in use umbilical cord IV be used to draw a blood culture on a very sick newborn.
- 1 mL is drawn
- It is not hospital protocol to draw off existing IVs or umbilical cords
- What grew?

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Bacillus Cereus???

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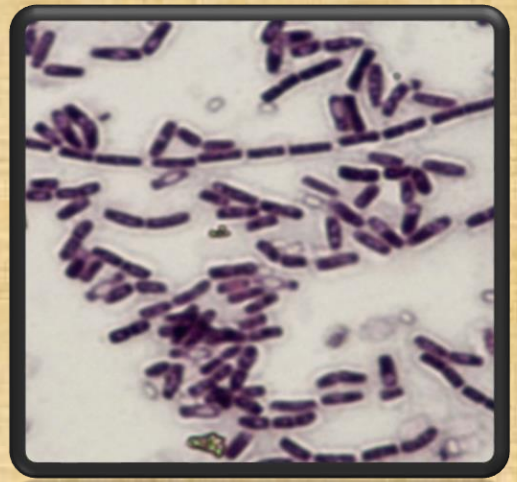
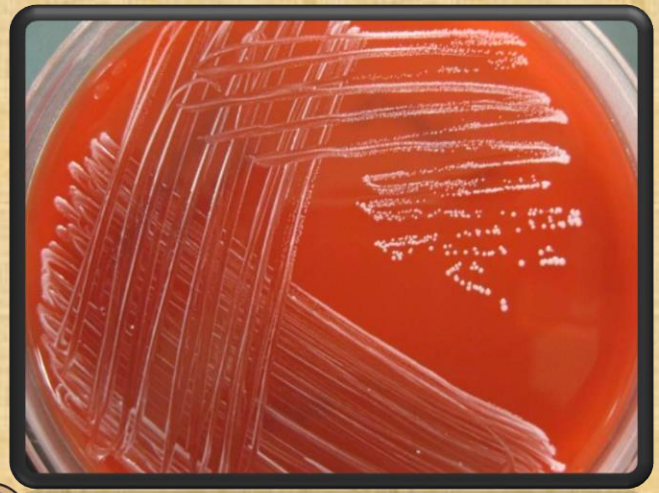
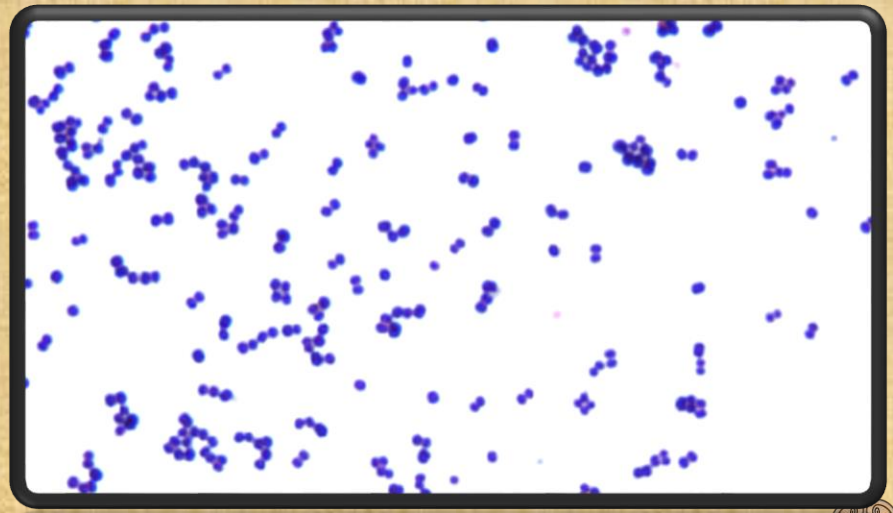
What? *Bacillus*?

- Epidemics of *Bacillus* positive blood cultures at a particular hospital or clinic usually point toward improper blood culture bottle top cleaning, or use of non-sterile equipment.
- Most *Bacillus cereus* infections are intestinal and symptoms are due to toxin and clear on their own in 24 hours.
- But extraintestinal infections occur in immunocompromised patients including newborns.
- Treatment options include Clindamycin, Vancomycin, Gentamycin, Chloramphenicol, and Erythromycin.
- Let's hear from our audience, step up to the microphone



Case # 2: The One in Four Quandary





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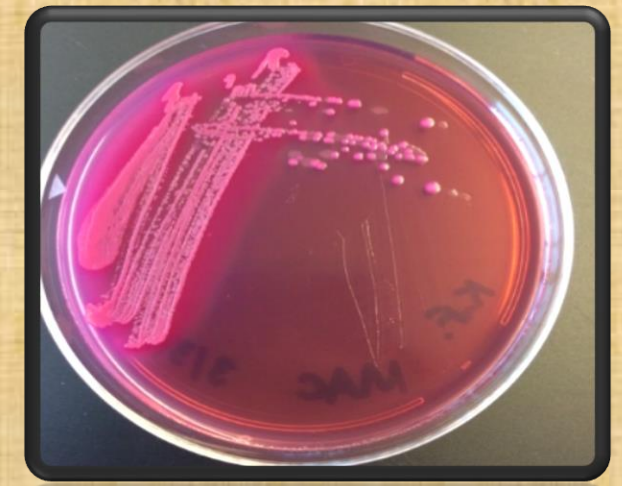
Staph epidermidis

- Everyone has *S. epidermidis*: arm pits, skin, mouth, etc.: it is a facultative anaerobe.
- Coagulase negative Staph are the most common bloodstream infection related to the colonization of indwelling medical devices.
- The most common isolate and contaminant of blood cultures.
- How do you interpret the 1 in 4 quandary:
- Step up to the microphone, What do you do.

Case # 3: Another Wrinkle

- 1 in 4 bottles positive for *E. coli*.
- Not a normal skin flora organism.
- This case correlated with the patient's *E. coli* UTI and a long history of UTIs.



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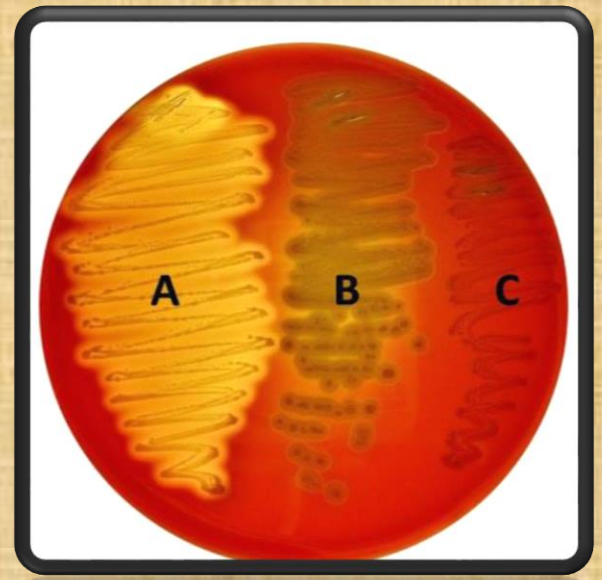
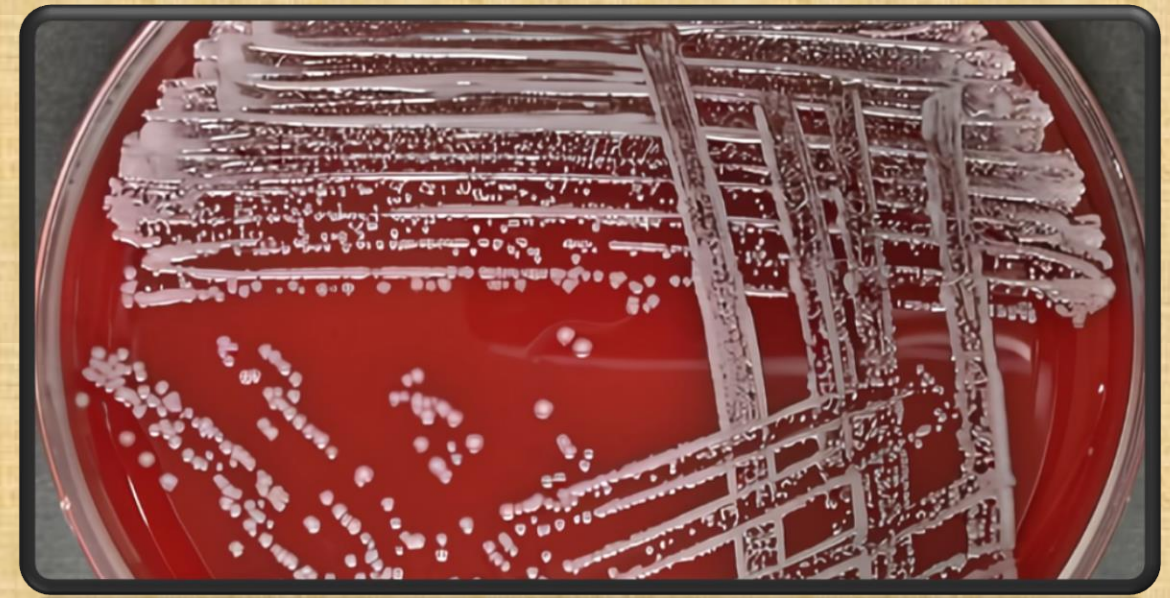
Case # 4: 1 Pediatric Bottle and 1 Full Set Positive

- Multiple organisms in the mix
- Almost like normal flora…..but in the blood?
- *S. aureus*, *Strep anginosus*, gamma *Strep*
- How do you interpret this culture? Tell us!
- Would you work it up?
- It looks like dermal flora colonized patient
- This patient passed due to pancreatic cancer



All Together Now: All 4 Bottles *Staph hominis*



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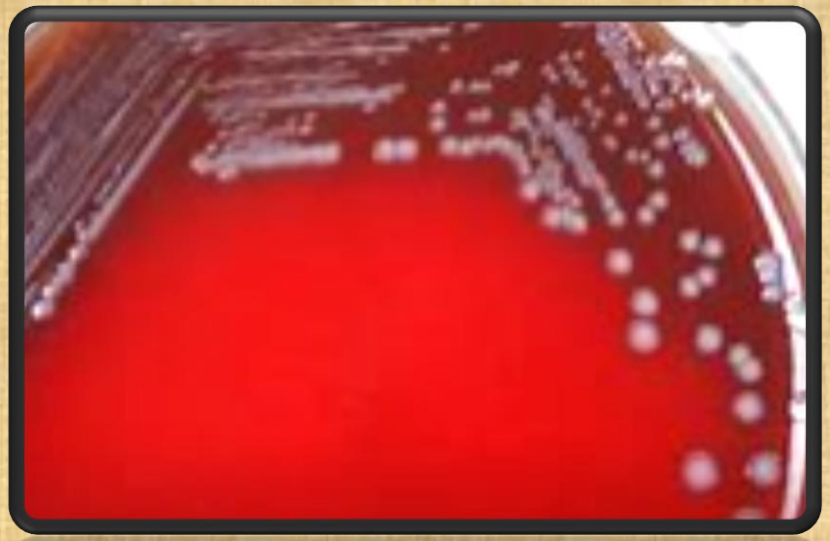
What Could This Mean?

- *Staph hominis* is a normal flora organism
- Would you work *Staph hominis*, the normal of normal flora up?
- Could we be dealing with endocarditis?
- Endocarditis is sometimes a mixture of organisms: *Strep mitis*, *Staph epidermidis*, etc.

Case #5: Strep dysgalactiae ssp. *equisimilis* in 3 of 4 bottles positive.

- How did a normal flora organism do this?
- Cellulitis to sepsis!

Case #6: Pasteurella canis

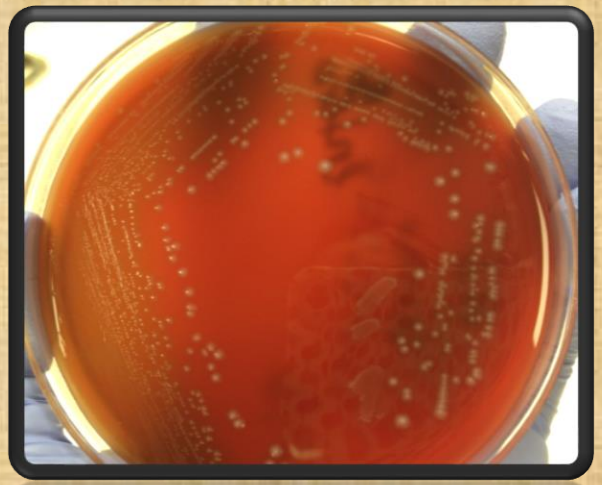


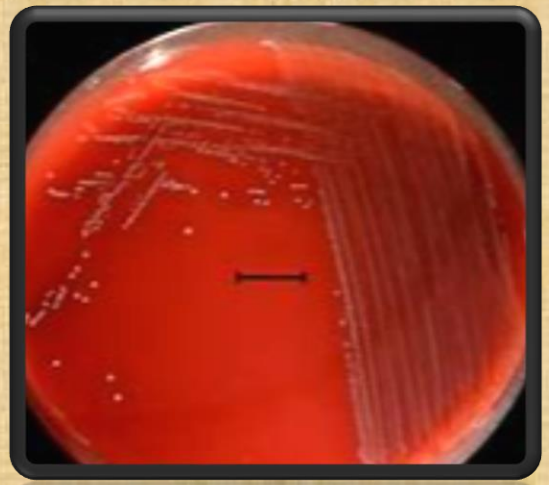
- Dog bites on arms can cause trouble

Case #7: Strep Group B and *Enterococcus*

- In Blood Cultures is sometimes an indication of GI cancer!
- Step up to the microphone: What unique bacteria/sites of infection have you seen in blood cultures?

Thank You





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