Wound, Tissue & Fluid Cultures

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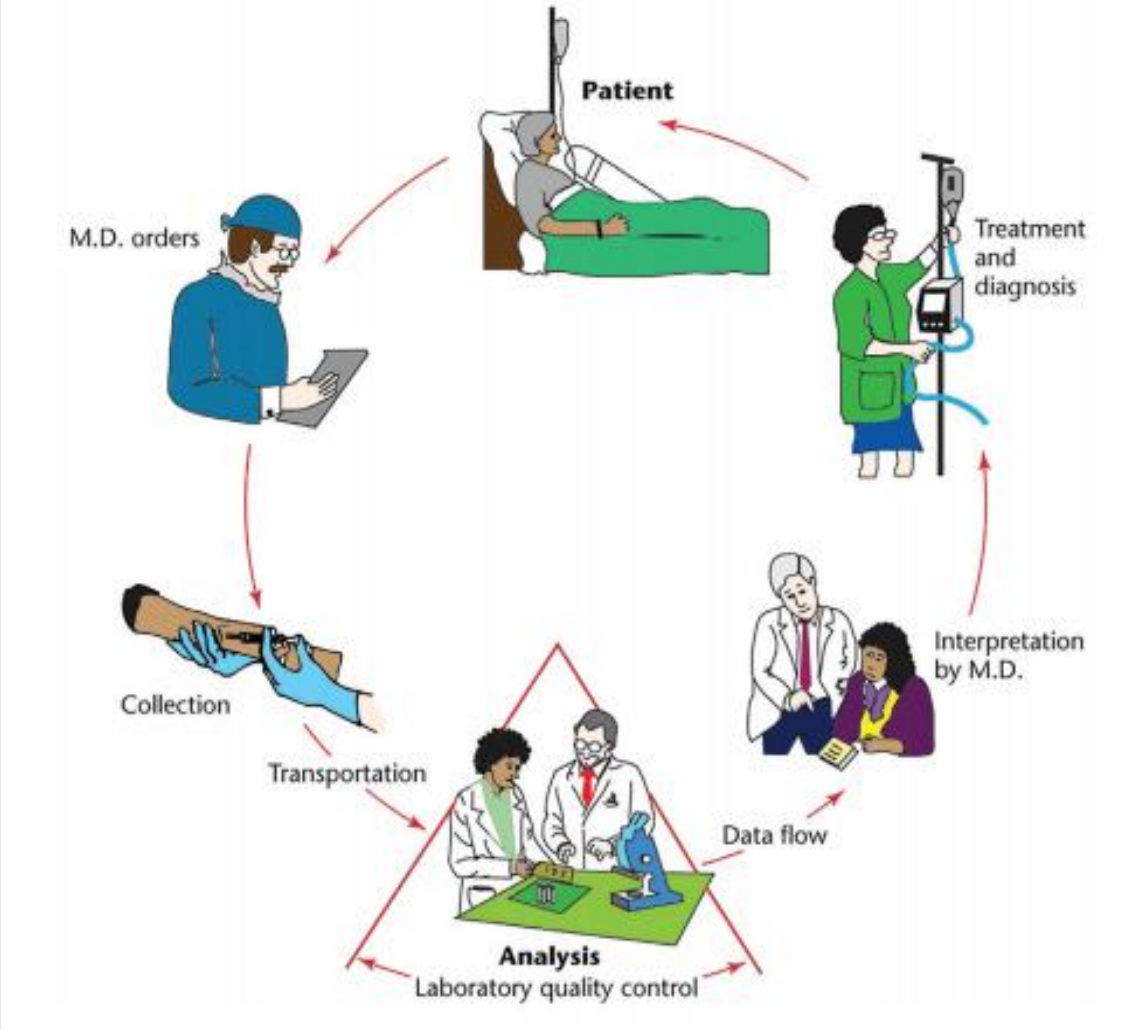
Key Points

- Proper specimen collection and transport is crucial to performing a wound, tissue or fluid culture
- The Gram stain provides rapid, useful information to the clinician, and can guide the workup of the culture
- Culture workup and reporting should aim to focus on the likely pathogens, not to excessively work up incidental commensal microbes
- Use antimicrobial susceptibility testing (AST) selectively

Keys to success

- Preanalytical
  - Ordering, collection, transport
- Analytical
  - Processing
  - Testing
- Postanalytical
  - Report formatting
  - Interpretive comments
  - Timeliness
  - Delivery

- ***All*** phases are ***equally*** important for optimal patient care.



Pre-analytical Keys

- Detailed description
  - Specific anatomic body site, body location, nature of site (e.g. abscess)
  - Specimen type (tissue, aspirate, fluid, swab)

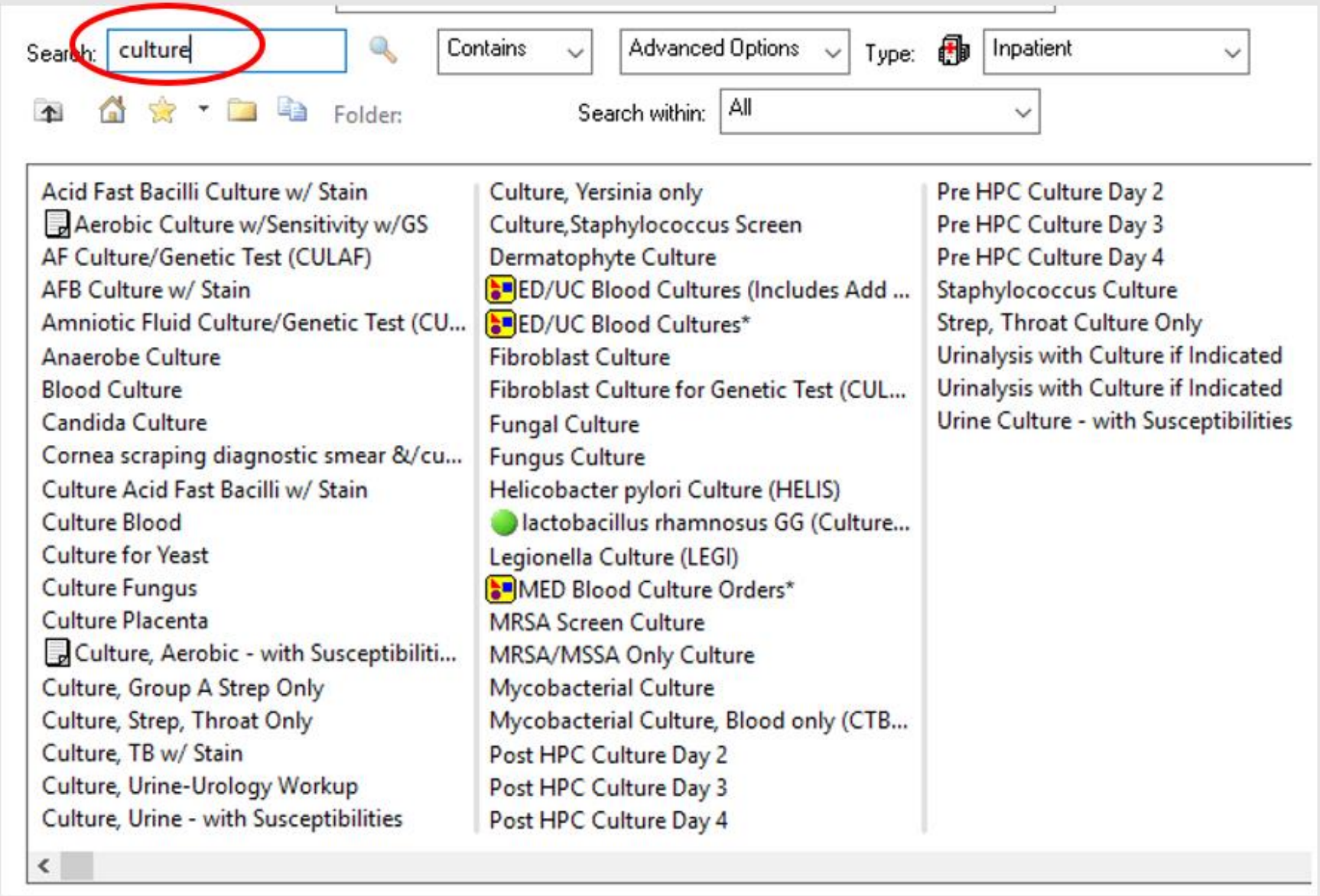
- Material
  - Source material is best (tissue, fluid, aspirate)
  - Dedicated AnO_2 transport media e.g. Remel ACT 1®
  - Syringe, air expelled, needle replaced with sterile cap
  - Swabs?

- Prep the area
  - Blood cultures, sub-dermal abscesses

- Minimize collection to plating time: ≤4hrs best practice (≤0.5hrs for fastidious anaerobes)

What do your Providers See?

- Duplicates
- Non-existent tests
- Non-Micro tests
- Notepad notes not used



Specimen Collection Manual

- May be called something else e.g. test reference manual

- Online and/or printed
  - System to replace old print versions.
  - Online is accessible to all users.

- Policy and Procedure Manual
  - Do you have these for ***every*** process in your lab?
  - Are they up to date?
  - Do techs follow them?

- The CAP requires both of these items

Rejection Criteria

- Communicate what you intend, then, do as you communicate
- GIGO principle
- General Causes for Rejection
  - Leaking containers
  - Improper collection device
  - Sample aged out
  - Improper transport
- That said, be open to exceptions
  - Consider ease of recollection
  - Communicate with ordering provider, explaining the risks of proceeding
  - If accepted, add disclaimer

Analytical Phase

Question

Does your lab perform a direct (i.e. specimen) Gram stain on all wound and tissue samples?

A. Yes

B. No

C. Depends

D. Don’t know

Question

Does your laboratory use the Gram stain to assess the quality of the specimen and guide the culture workup?

A. Yes

B. No

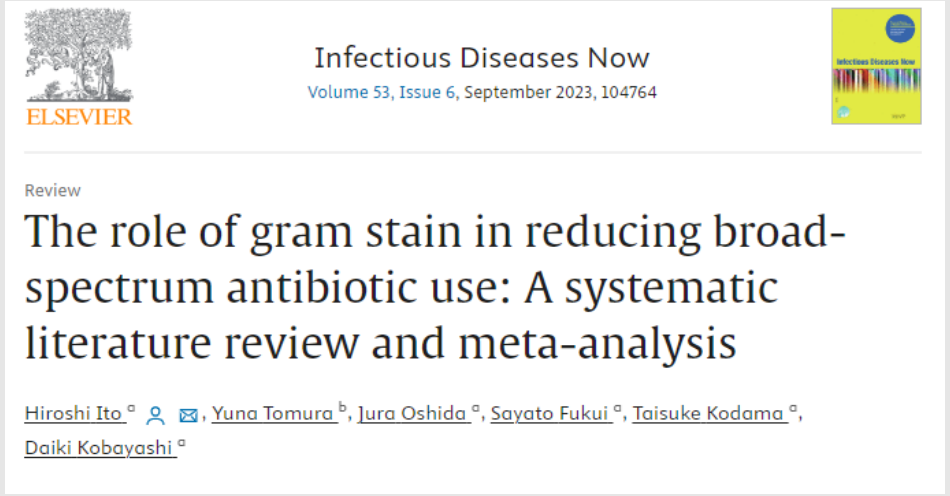
C. Don’t perform Gram stains on wounds

D. Something else

E. Don’t know

Gram Stain Best Practices

- Gram stain: should we be doing them?
  - Best practice is Yes, for both clinician and lab tech
  - Review the direct Gram stain when working up cultures
  - PMNs?
  - Squamous epithelial cells ? (body site - dependent)
  - Correlation with culture findings – lack thereof suggests anaerobes
- If still using conventional swabs consider Eswab™ or similar flocked swab liquid transport device
  - Facilitates direct GS on all specimens
  - Flocked swabs release most of the specimen, spun swabs not so much
  - Enables automatic plating systems



ELSEVIER

Infectious Diseases Now

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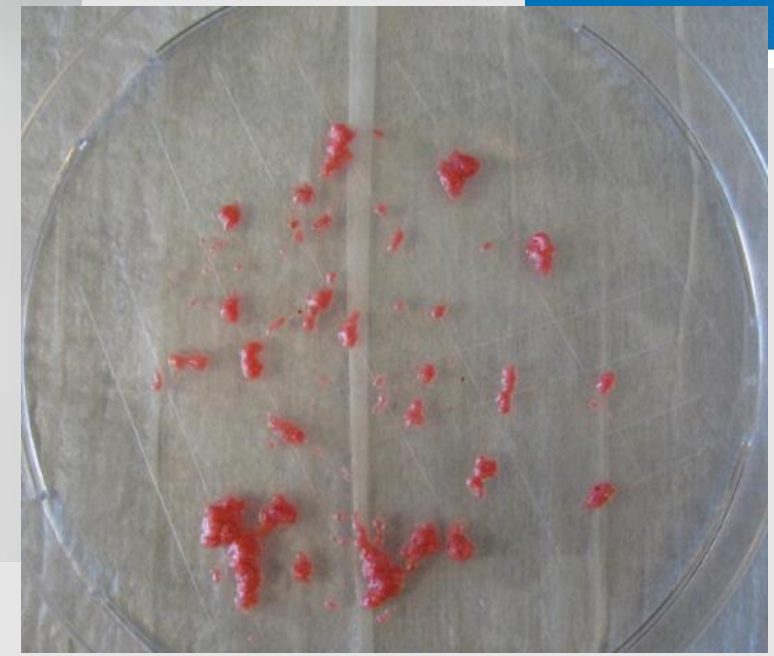
Review

The role of gram stain in reducing broad-spectrum antibiotic use: A systematic literature review and meta-analysis

Hiroshi Ito [a], Yuna Tomura [b], Jura Oshida [a], Sayato Fukui [a], Taisuke Kodama [a], Daiki Kobayashi [a]

Tissue Processing

- Grind, mince or homogenize???



Aerobic Culture Media

- Nutrient agars
  - Chocolate agar – fastidious orgs e.g. *Haemophilus, Neisseria*
  - Sheep blood agar – hemolytic pattern
- Selective/differential agars
  - MacConkey or EMB – GNB, lactose fermentation
  - CNA or PEA – Gram positive orgs
- Broth
  - Routine use? No: broth only orgs = contamination[1]
  - When to use? Hardware, vitreous fluid
  - Eugonic broth: recovers *Haemophilus, Neisseria* & anaerobes
- Blood culture bottles

[1] AJ Morris et al. J. Clin. Microbiol. 1995. 33:161

Anaerobic Culture Media

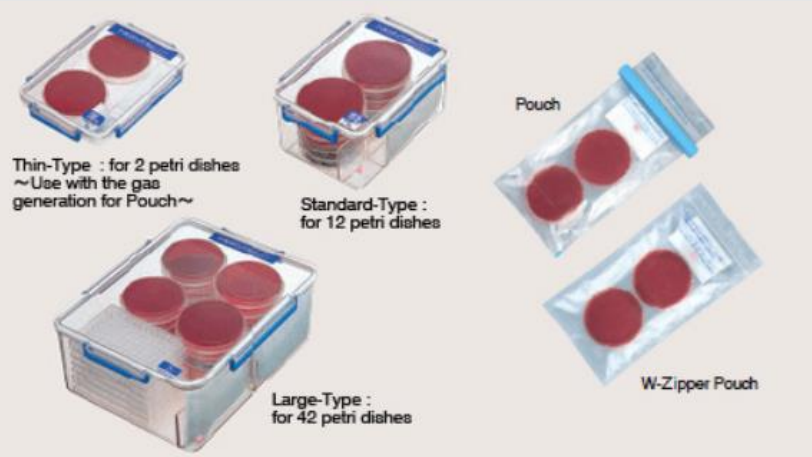
- Nutrient agars (pick one)*
  - CDC Anaerobe
  - BHI w/ sheep blood
  - Fastidious anaerobe agar
  - Columbia w/ sheep blood
- Selective media*
  - *Bacteroides*: BBE, LKV
  - Obligate anaerobes: PEA
- Blood culture bottles
- Pre-Reduced Anaerobically Sterilized (PRAS) media?

*Use anaerobic formulations: vitamin K and hemin are needed

Incubation

- Aerobic: 35°C, 5% CO_2

- Anaerobic: 35°C in an anaerobic atmosphere
  - $$$$$ Anaerobic chamber
  - $$$ Anoxomat III®
  - $$ Gas pack with indicator/sealed bags
  - $ Gas pack with indicator/anaerobic jars



Question

Does your lab

1. Always work up everything?
2. Always select the most prominent 1-2 organisms to work on?
3. Base the level of work up on the site sampled (i.e. deep sterile site vs superficial wound), presence of PMNs, collection method (swab vs tissue/fluid/aspirate) etc.?
4. Some other approach?

Culture Work Up – Best Practices

- By focusing on…
  - ID and AST of clinically significant isolates
  - Providing information on commensal flora also present in the culture

- Culture information reported then leads to…
  - Giving focused information for our providers
  - Better antimicrobial stewardship
  - More efficient use of lab resources
  - Better patient outcomes

- “Always culture everything”?

Workup Conundrums

- Sterile body fluids vs wounds & abscesses
  - Not “one size fits all” workup-wise
- Tissue is not all the same
  - Debridement vs deep organ
  - “Surgically collected”: not always a deep, normally sterile site
- Which orgs are clinically relevant?
  - Challenging to know in the vacuum of the lab
  - EMR specimen description limitations
  - Infection acuity e.g. superficial boil vs deep, loculated carbuncle

How then to Choose the Level of Workup?

- Work with what we get
  - Swab vs the good stuff
  - PMNs present on Gram Stain
  - Read the tea leaves of specimen type and body site
- EMR – the game changer
- If in doubt
  - Consult your manager or doctoral level microbiologist
  - Call the ordering provider

Our Approach

Specimen Type	1-2 ORGS	≥ 3 ORGS, 1-2 ORGS Predominate	≥ 3 ORGS, No Predominance
Non-sterile Sources: • Wounds (including OR wound debridement, Wound Clinic tissue) • Abscesses • Lower GI samples	All ORGS: ID & AST	Predominate ORGS: ID & AST Subordinate ORGS: ✓ Work up *P. aeruginosa, S. aureus,* BHS ✓ Rule out *C. auris* ✓ **All others: Comment "Commensal GI / GU / RESP / SKIN microbes present with no predominance"**	✓ Work up *P. aeruginosa, S. aureus,* BHS ✓ Rule out *C. auris* ✓ **All others: Comment "Commensal GI / GU / RESP / SKIN microbes present with no predominance"**
Normally Sterile Sources: • Body fluids • Deep tissues • Removed hardware	All ORGS: ID & AST	Predominate ORGS: ID & AST Subordinate ORGS: ✓ Work up *P. aeruginosa, S. aureus,* BHS ✓ Rule out *C. auris* ✓ **All others: List by GS morphology up to 3 morphs, then use commensal flora comments**	✓ Work up *P. aeruginosa, S. aureus,* BHS ✓ Rule out *C. auris* ✓ **All others: List by GS morphology up to 5 morphs, then use commensal flora comments**

Our Approach: Footnotes

- We surveille for *Candida auris:* your lab may have other interests
- No heroics to pull out *P. aeruginosa, S. aureus* or BHS in heavy growth, just make the attempt
- Techs use the GS and EMR as a “tie-breaker” in unclear or ambiguous situations
- Techs consult providers
- We round daily to help interpret cultures

Fluids

Fluid	Location
Synovial	The intra-articular joint space
Pleural	Space between the lungs and the chest wall
CAPD*	Within the abdominal peritoneal cavity bounded by the peritoneum
Pericardial	Within space between the pericardium and the heart
Amniotic	Cavity between the amniotic membrane and the fetus

* CAPD, continuous ambulatory peritoneal dialysis

Fluid	Drainage Tubes
Bile	Biliary tube (T-tube)
Pleural Fluid	Chest Tube
Gastric Fluid	G-Tube
Peritoneal (intra-abdominal fluid or pus)	Jackson-Pratt

Let's Talk Anaerobes

- From a seasoned tech

  "I don't know about your lab, but in my lab this is everyone's least favorite time of day. Anaerobes are stinky, they are more often than not messy and complicated, and the process is SLOOOW as molasses."

- Depending on body site, are rare or the predominate flora
  - e.g. the GI tract is composed of >99% anaerobic bacteria

- Some are aerotolerant while others die upon O_2 exposure

- As with aerobes, are opportunistic pathogens

Definitions

- Obligate anaerobe: Won't grow in O_2 but may tolerate it. aka "anaerobe. e.g. *Bacteroides*
- Aerobe: Requires O_2 for growth e.g. *Bacillus*
- Facultative anaerobe aka "aerobe". Grows in normal, reduced or zero O_2 conditions e.g. *E. coli*
- Microaerophilic: Prefers a reduced O_2 level e.g. *Actinomyces.* May grow better anaerobically or aerobically
- Capnophilic: CO_2 loving. *Capnocytophaga*

Anaerobes and infection

- Most anaerobe infections also involve aerobic bacteria.
  - Anaerobes are dependent on aerobes (symbiotic)

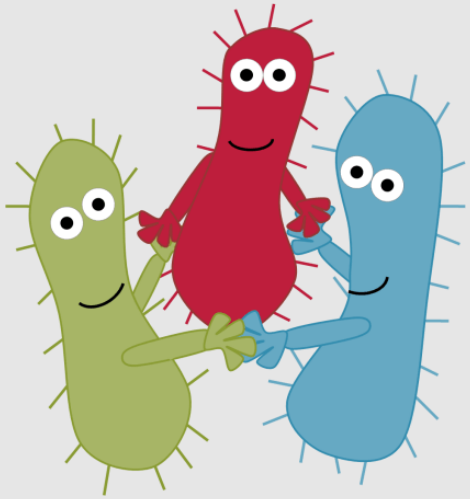
- Common sites
  - Orthodontic and sinus abscesses
  - Dental tissue
  - Intraabdominal or pelvic abscess formation
  - Post surgery at the above sites
  - Traumatic injuries

Question

How do you work up aerobic and anaerobic cultures on the same specimen?

1. One bench works both up together
2. One bench works up both, but at different times
3. Separate aerobe and anaerobe benches
4. Something else
5. Do not perform anaerobic cultures

Aerobic/Anaerobic Workup, Holistic Approach



- Pros
  - Enables assessment of the ***entire culture.***
  - Eliminates extra work e.g. a Staph is isolated only on the AnO_2 culture aerotolerance plate
  - Significance may become non-significance e.g. a leg wound culture with
    - Gram stain: 3+ PMNs, 3+ GNB, 2+ GPB
    - Aerobic: 3+LF GNB, 3+ coryneform GPB
    - Anaerobic culture: 3+ AGNB, 3+ AGPB x2, 1+ AGPC
- Cons
  - Staffing and training: anaerobes is a specialty bench while aerobes a core bench

Practically Speaking…

- Convert anaerobe bench to aerobe/anaerobe cultures bench
  - i.e. segregate all specimens with aerobe & anaerobe components to this bench, staffed by specialist techs
  - Majority of aerobe-only cultures stays as a core bench

- Use the aerobic results to guide anaerobic culture management
  - Day 1, Aerobic: 3+ *E. coli*, 3+ *C. jeikeium*, 1+ mixed skin flora
  - Day 2, Anaerobic culture: 3+ GNB, 3+ GPB
    - Do no aerotolerance testing
    - Report: “This is a mix of bacterial species with no predominance.”
    - Consider withdrawing the *E. coli* AST results

- MALDI TOF MS means anaerobes are now less of a specialty

Suggested Abbreviated Work up for Non-sterile Sites

Gram Stain	If Cocci	If Bacilli
Negative	Report as "Anaerobic Gram negative coccus"	If BBE has growth report as "Presumptive *Bacteroides/Parabacteroides/Phocaeicola* sp."
		If BBE is negative, report as "Anaerobic Gram negative bacillus, NOT *Bacteroides/Parabacteroides/Phocaeicola* sp."
Positive	Report as "Anaerobic Gram positive coccus"	Gram stain shows large boxy bacilli with spready, colonies with a double zone of hemolysis, report as "consistent with *Clostridium perfringens*"
		If spores are present, report as "Spore-forming anaerobic Gram positive bacillus, NOT *Clostridium perfringens*"
		If spores are not present, report as "Anaerobic Gram-positive non-spore-forming bacillus"

SK Shannon and MA Pence. Section 4.8, A Practical Guide to the Workup of Anaerobic Cultures. In *Clin. Microbiol. Proc. Handbook.* 5th Ed. AL Leber & CD Burnham eds in chief. 2023. ASM Press. Washington DC

AST Pearls

- Only perform AST on significant isolates

- Follow guidelines to:
  - Choose the best drug panels
  - Escalate reporting from narrow- to broad-spectrum antimicrobials
  - Perform and interpret manual AST

- Work with fellow stakeholders (e.g. pharmacist, ID physicians,& antimicrobial stewardship committee) to develop the best AST panels and reporting algorithms ***for your institution***

CLSI M100 Table 1 Tables

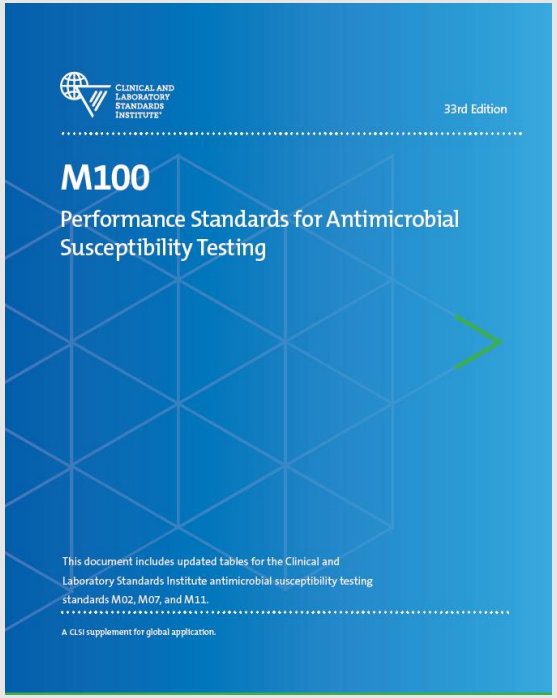


Table 1G. Other Non-Enterobacterales[a,b]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ceftazidime	Cefepime		
	Imipenem Meropenem		
Gentamicin Tobramycin	Amikacin		
Piperacillin-tazobactam			
Trimethoprim-sulfamethoxazole			
	Aztreonam		
	Ciprofloxacin Levofloxacin		
	Minocycline		
			Cefotaxime Ceftriaxone
Urine Only			
Tetracycline[c]			

Abbreviations: MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Special Topics: Wound Healing Cultures

- Non-healing wounds (NH wounds) ≠ Acute wounds

- NH wounds
  - Hypoxia due to poor vascularization
  - Little inflammatory response
  - Local biofilm that inhibits
    - local immunity
    - fibroblast deposition (“cicatrization”) ie scar formation

- Healing is inversely correlated with microbial burden
  - Quantitative cultures (clinical lab specialty bench)
  - Quantitative NGS (research lab now)

Special Topics: Wound Healing Cultures

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Journal of Wound Care, Vol. 3, No. 4 · Discussions

Criteria for identifying wound infection

K.F. Cutting, K.G. Harding

Published Online: 6 Dec 2016 | https://doi.org/10.12968/jowc.1994.3.4.198

Correlates NH wound quantitative bioburden with standard 4-quandrant semi-quantitative culture

Special Topics: Multiple Surgical Samples

Surgeons often submit multiple intra-operatively collected samples…

Is this practice legit?

Can we combine them?

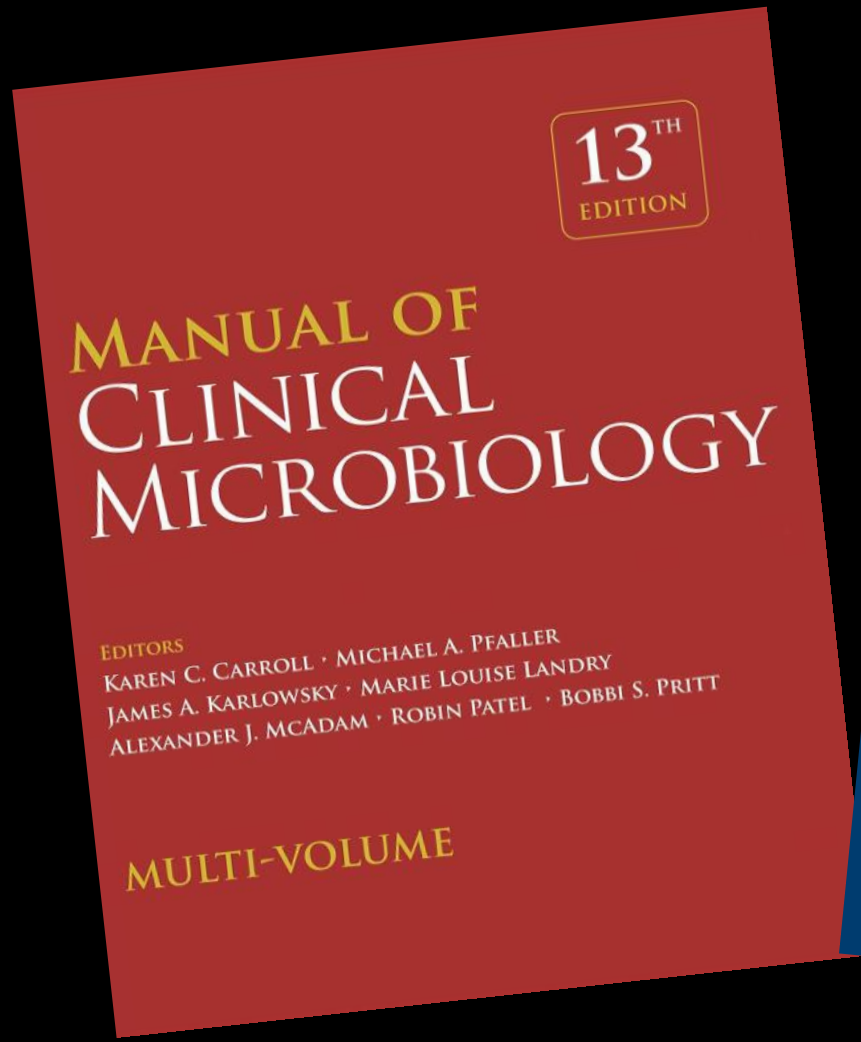
Special Topics: Multiple Surgical Samples

Short Answer: No

- Why?
  - Most often orthopedic surgeries for prosthetic joint infection (PJI)
  - PJI often caused by CoNS, *C. acnes* & other skin flora, so finding the same species in ≥2 samples is diagnostic
  - 5-6 cultures = maximal sensitivity

DR Osmon, EF Berbari, AR Berendt *et al*. 2013. Clin Infect Dis 56:e1
SJ Kim, YJ Cho. 2020. Hip Pelvis 33:11

ASM Press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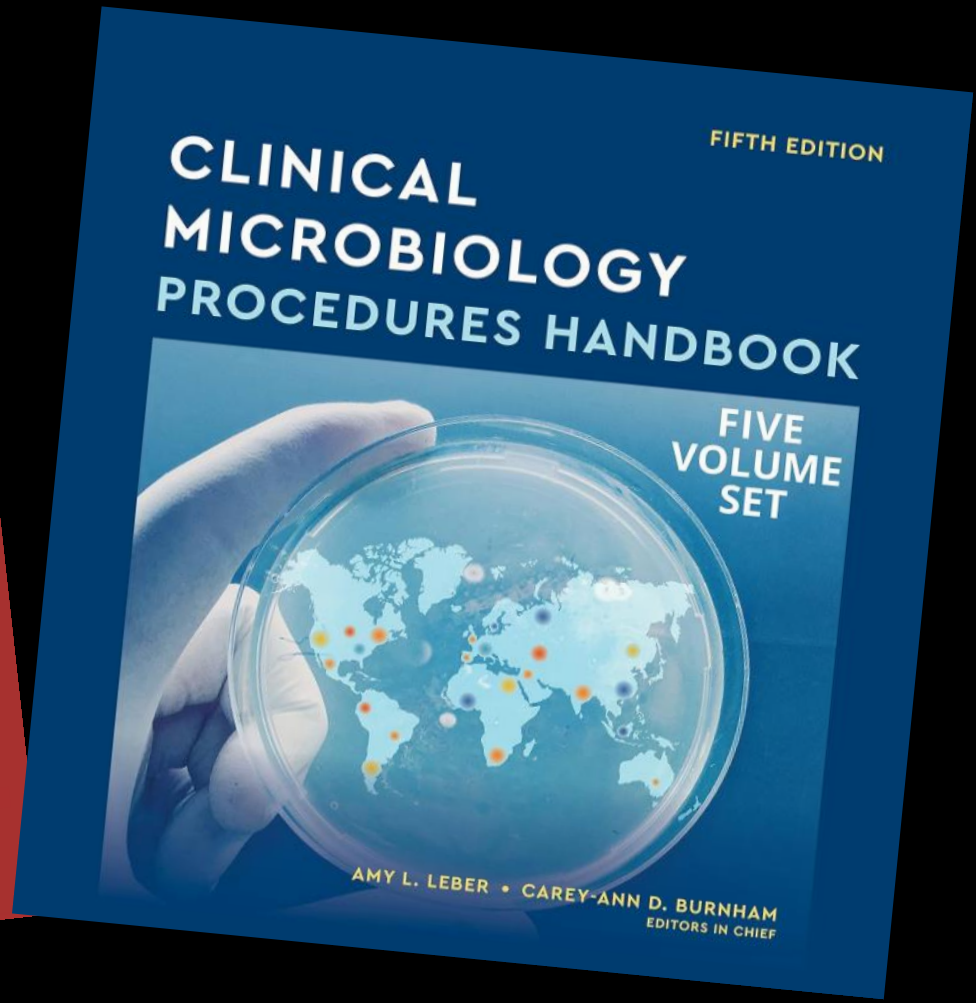
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