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(if it works)

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Weirdobacterium confusii

Identification and Reporting of Uncommonly Encountered Microbes

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Introduction

MALDI-TOF-MS

First FDA cleared in 2013

Two approved systems:

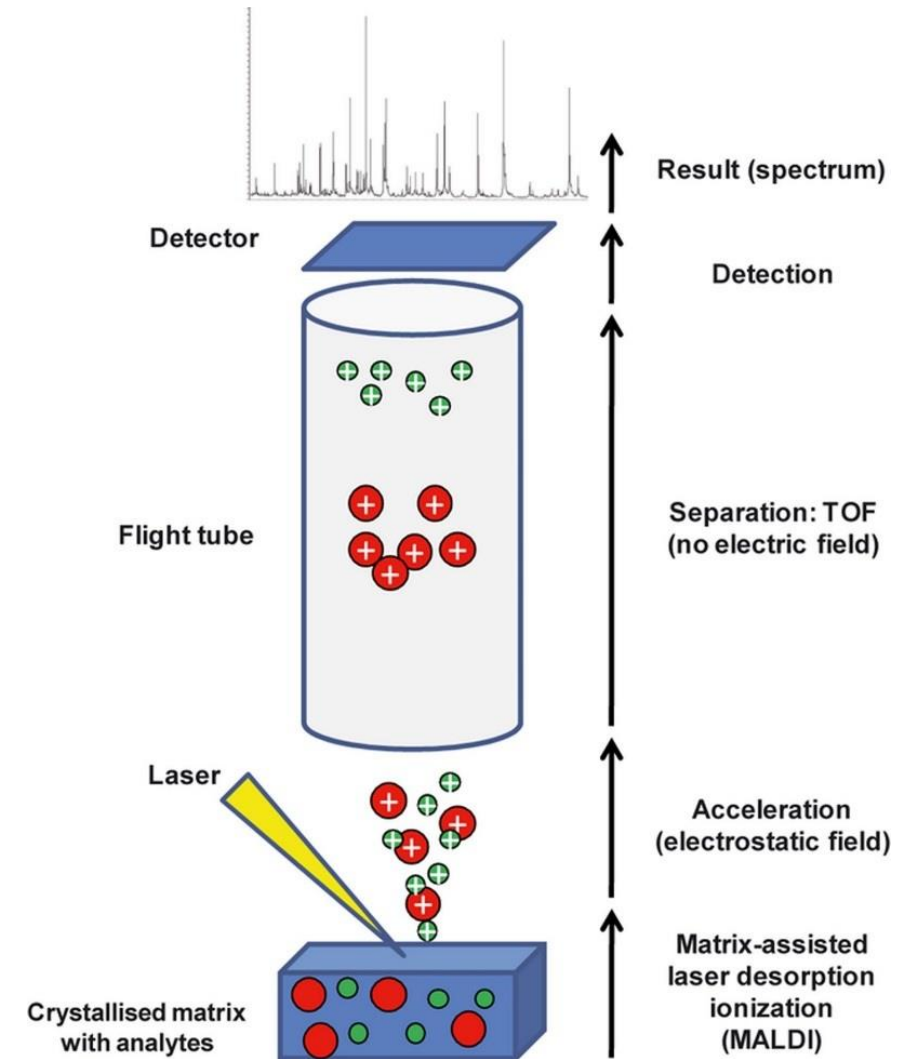
Bruker Biotyper

BioMerieux Vitek MS

Organism ID is fast, (relatively) cheap, accurate

FDA-cleared library: 400-550 organisms

RUO library: >1500 organisms



Identification Conundrum:

How do you report organisms you've never heard of?

Is it clinically significant or a colonizer/flora?

Is it related to something that is clinically significant?

What name should we use when reporting?

Should we preform AST? How?

CASE 1

Case History

48yo old man presents to the Emergency Department with wound infection

Septic: confused, elevated BP, tachycardic
respiratory rate normal, afebrile at presentation
2 weeks history of fever, chills



About that wound...

Paraplegic secondary to trauma ~20 years prior
Chronic sacral wound managed in wound care clinic
At presentation: foul smelling, necrotic, exposed bone, no purulence
Other chronic wounds on feet and heels
Maggots seen in heel wound

CT demonstrated penetration of wound to vertebral body (osteomyelitis)
Surgical debridement performed

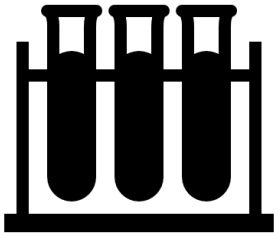
Case History



Empiric antimicrobial therapy

Vancomycin

Cefepime



Labs/Imaging

Basic labs

CT

Blood cultures

Aerobic, anaerobic, fungal cultures of wound during debridement

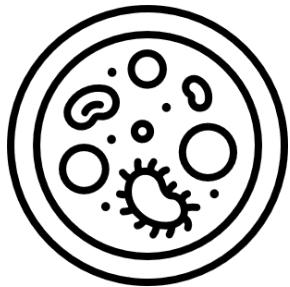
Gram Stain / Culture

Gram stain



Few Gram-negative rods
Few Gram-positive rods
Moderate Gram-positive cocci
No PMNs

Culture



3+ *Pseudomonas aeruginosa*
3+ *Staphylococcus aureus*
3+ *Staphylococcus epidermidis*
2+ *Corynebacterium striatum*
3+ *Wohlfahrtiimonas chitiniclastica*



Gram Stain / Culture

Gram stain

No PMNs, GNR, GPR, GPC

Culture

3+ *P. aeruginosa*

3+ *S. aureus*

3+ *S. epidermidis*

2+ *C. striatum*

3+ *W. chitiniclastica*

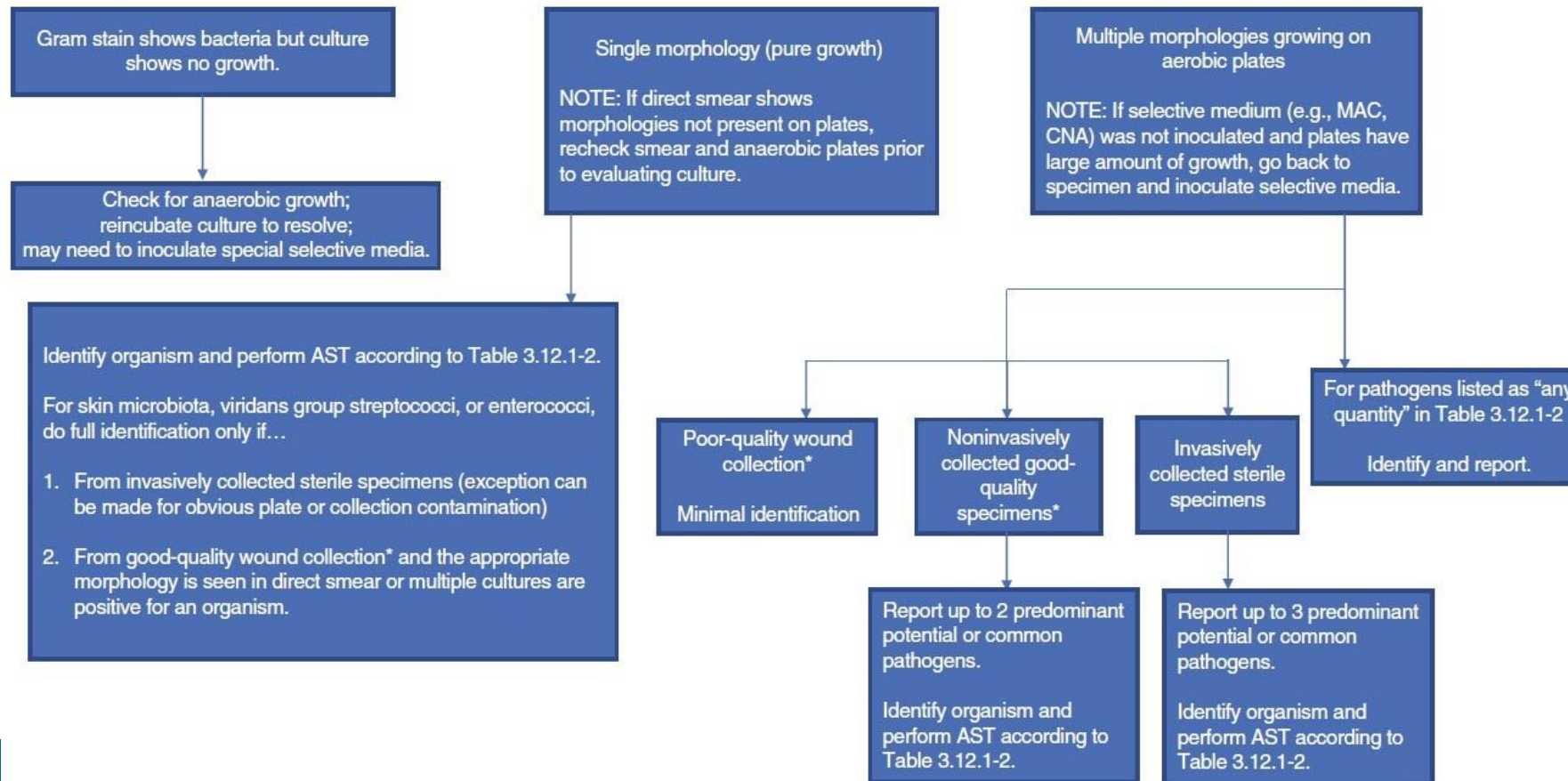
How would you report this culture?

<u>Report</u>	<u>Reason</u>
A 3+ <i>Pseudomonas aeruginosa</i> 3+ <i>Staphylococcus aureus</i> 3+ Normal flora	<i>P. aeruginosa</i> , <i>S. aureus</i> are pathogens Remaining are normal wound colonizers
B 3+ <i>Pseudomonas aeruginosa</i> 3+ <i>Staphylococcus aureus</i> 3+ <i>Wohlfahrtiimonas chitiniclastica</i> 3+ Normal flora	<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>W. chitiniclastica</i> are pathogens Remaining are normal skin flora
C Mixed aerobic bacteria	>3 organisms identified, no predominant pathogens, no PMNs on Gram
D Unsure	What in the world is <i>Wohlfahrtiimonas</i> ???

Wound Culture Workup

New guidance available

Clinical Microbiology Procedures Handbook (CMPH), Chapter 3.12, updated 2023



Wound Culture Workup

Gram stain shows bacteria but culture shows no growth.



Check for anaerobic growth;
reincubate culture to resolve;
may need to inoculate special selective media.

Wound Culture Workup

Single morphology (pure growth)

NOTE: If direct smear shows morphologies not present on plates, recheck smear and anaerobic plates prior to evaluating culture.

Identify organism and perform AST according to Table 3.12.1-2.

For skin microbiota, viridans group streptococci, or enterococci, do full identification only if...

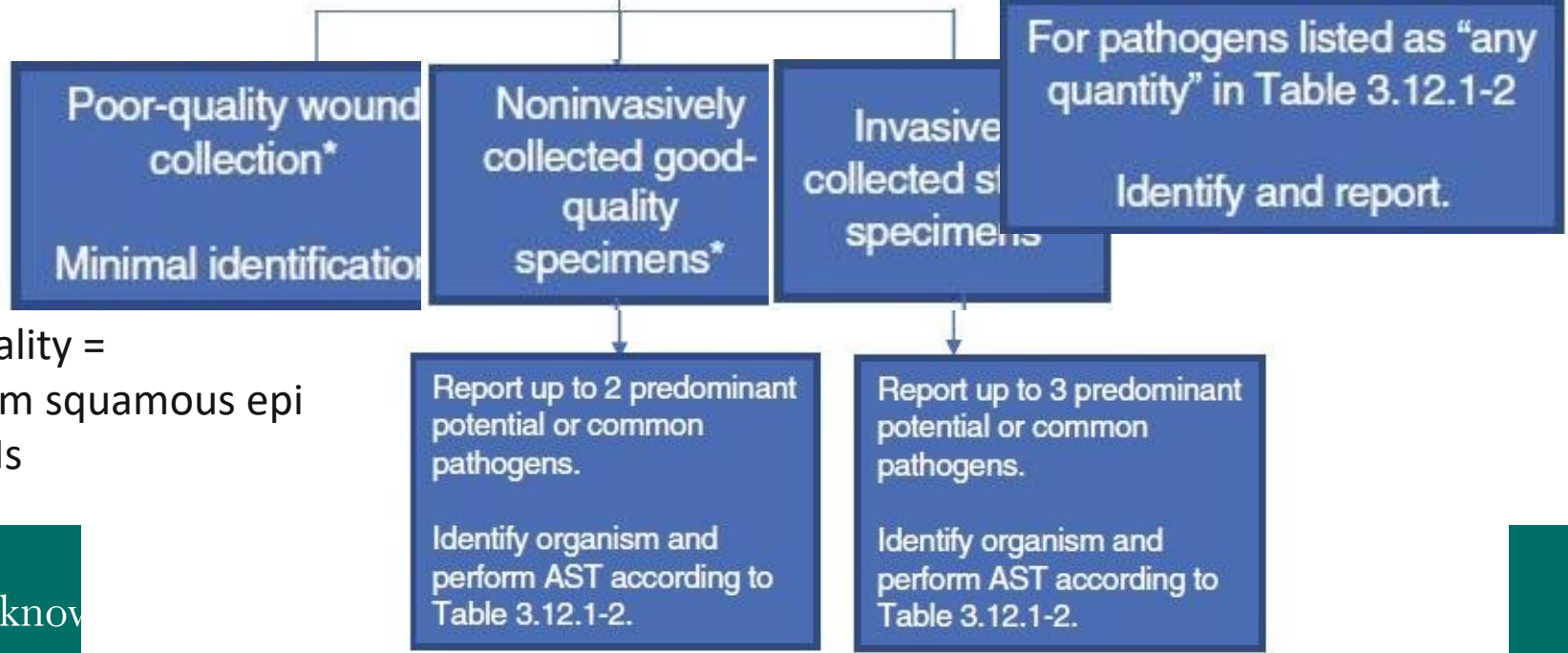
1. From invasively collected sterile specimens (exception can be made for obvious plate or collection contamination)
2. From good-quality wound collection* and the appropriate morphology is seen in direct smear or multiple cultures are positive for an organism.

Wound Culture Workup

Multiple morphologies growing on aerobic plates

NOTE: If selective medium (e.g., MAC, CNA) was not inoculated and plates have large amount of growth, go back to specimen and inoculate selective media.

How was the specimen collected?
Is it a high-quality specimen?



Poor quality =
Mod-num squamous epi
No PMNs

“always report” pathogens

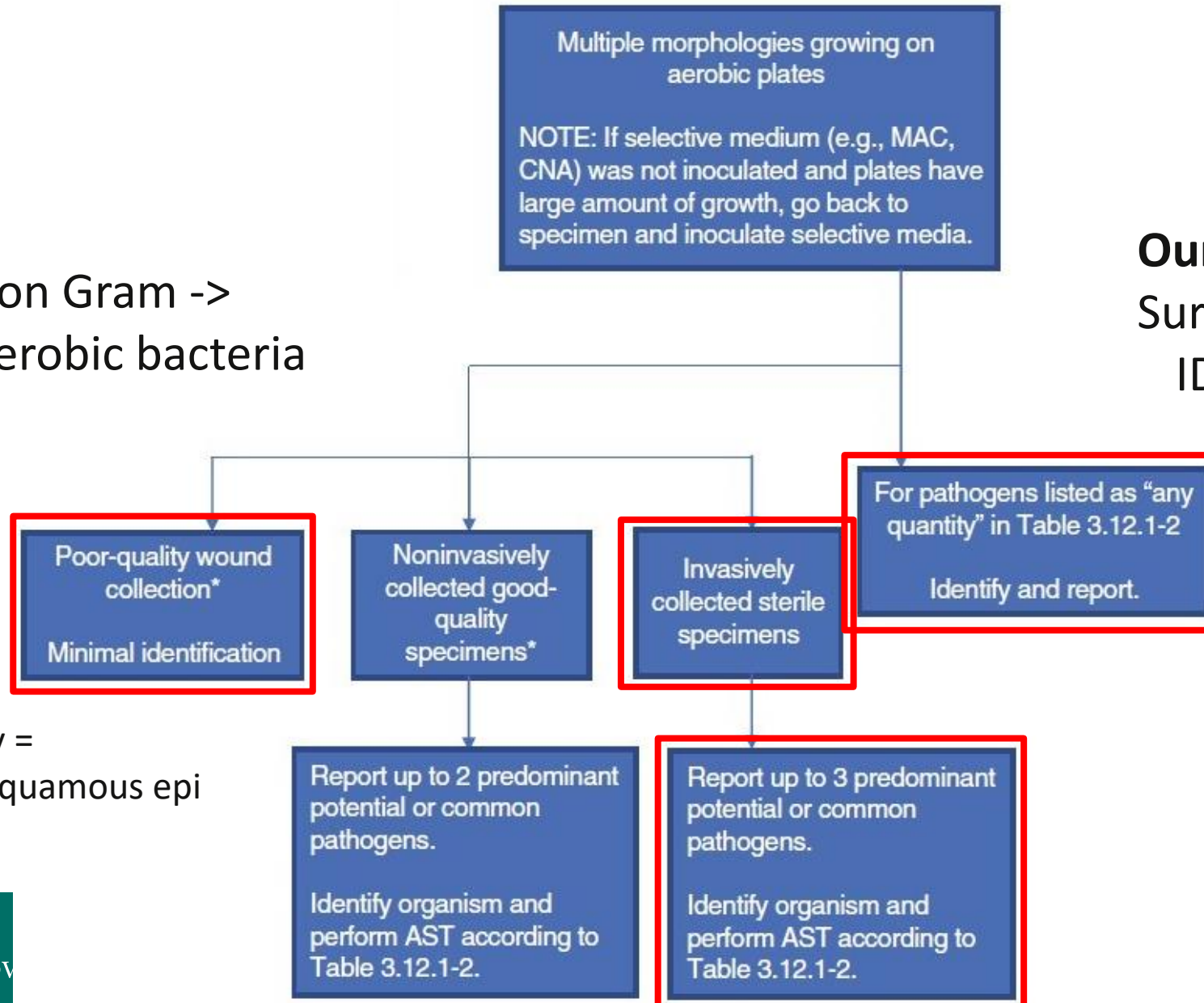
Wound Culture Workup

Our Case

No PMNs on Gram ->
Mixed aerobic bacteria

Our Case

Surgical collection ->
ID up to 3 pathogens



Poor quality =
Mod-num squamous epi
No PMNs

Wound Culture Workup

Guidance on workup of common aerobic/anaerobic isolates (CMPH Table 2)

Wound Pathogenicity

Workup Threshold

Reporting ID/AST

Disease association(s)

Common Pathogen

- *S. aureus*
- *S. pyogenes*
- *P. aeruginosa*
- *C. perfringens*

Common Pathogen / Potential Commensal

- *S. lugdunensis*
- *A. baumannii*

Commensal / Potential Pathogen

- CoNS
- *Enterococcus* spp.
- Non-ferm GNR
- *S. maltophilia*
- *Bacillus* spp (not *anthracis*)

Rare, well-defined pathogen

- *C. kroppenstedtii*
- Select agents
- *V. vulnificus*
- *A. hydrophila*
- *Nocardia* spp.

Wound Culture Workup

Guidance on workup of common aerobic/anaerobic isolates (CMPH Table 2)

Wound
Pathogenicity

Workup
Threshold

Reporting
ID/AST

Disease
association(s)

Any Quantity

- *S. aureus*
- *S. pyogenes*
- *S. agalactiae*
- *P. aeruginosa*
- *C. perfringens*
- *Nocardia* spp.

Pure or predominant

- *S. lugdunensis*
- *S. anginosus*
- *A. baumannii*
- Non-ferm GNR
- *Candida* spp.

Pure

- CoNS
- *Bacillus* spp. (not *anthracis*)

Wound Culture Workup

Guidance on workup of common aerobic/anaerobic isolates (CMPH Table 2)

Wound Pathogenicity

Workup Threshold

Reporting ID/AST

Disease association(s)

ID and AST

- *S. aureus*
- *P. aeruginosa*
- *A. baumannii*
- *Nocardia* spp

ID and AST Mixed flora if not pure/predom

- *S. lugdunensis*
- *Enterococcus* spp
- Viridans group strep

ID, AST on request

- *S. pyogenes* (pen allergic)
- *S. agalactiae* (pen allergic)
- *S. anginosus*
- CoNS

ID, AST for deep tissue infections

- *Eikenella* spp
- *B. fragilis* group
- *Candida* spp

Wound Culture Workup

Guidance on workup of common aerobic/anaerobic isolates (CMPH Table 2)

Wound Pathogenicity	Workup Threshold	Reporting ID/AST	Disease association(s)
Animal bites/exposures <ul style="list-style-type: none">• <i>Pasteurella</i> spp• <i>Capnocytophaga</i> spp• <i>F. tularensis</i>• <i>Staphylococcus schleiferi</i> group• <i>Staphylococcus intermedius</i> group	Gas gangrene <ul style="list-style-type: none">• <i>C. perfringens</i>• <i>C. septicum</i>• <i>C. novyi</i>	Granulomatous mastitis, recurrent breast abscess <ul style="list-style-type: none">• <i>Corynebacterium kroppenstedtii</i>	Leech therapy <ul style="list-style-type: none">• <i>Aeromonas hydrophila</i>

Gram Stain / Culture

Gram stain

No PMNs, GNR, GPR, GPC

Culture

3+ *P. aeruginosa*

3+ *S. aureus*

3+ *S. epidermidis*

2+ *C. striatum*

3+ *W. chitiniclastica*

How would you report this culture?

Report

- A** 3+ *Pseudomonas aeruginosa*
3+ *Staphylococcus aureus*
3+ Normal flora
- B** 3+ *Pseudomonas aeruginosa*
3+ *Staphylococcus aureus*
3+ *Wohlfahrtiimonas chitiniclastica*
3+ Normal flora
- C** Mixed aerobic bacteria
- D** Unsure

Following CMPH guidance:

S. aureus and *P. aeruginosa* are “always report” organisms

S. epidermidis and *C. striatum* report only if pure, otherwise mixed microbiota/normal flora

What in the world is *Wohlfahrtiimonas*??

Wohlfahrtiimonas chitiniclastica

Is the ID correct?

MALDI score was good

No other ID matches with good scores

Automated ID system: *Comamonas testosterone*

Not really reliable for rare non-fermenting GNR

Biochemicals:

Catalase pos

Oxidase pos

Glucose/lactose non-fermenting (MAC)

Gram stain: GNR



Image: PMID 33826527

Wohlfahrtiimonas chitiniclastica

What is *Wohlfahrtiimonas*? When is it a pathogen?

Gram negative rod, non-glucose fermenter

Grows fine on MAC

First identified in 3rd-stage larva of *Wohlfahrtia* flies

Also identified in *Chrysomya*, *Lucilia*, *Hermetia* flies

Normal fly flora

Associated with larval contamination of wounds

Bloodstream infections secondary to wounds reported

At risk patients:

Chronic wounds, poor self-care, homelessness

Maggot debridement therapy

Other maggot-associated bacteria: *Ignatzschineria* spp.



Image: PMID 33826527

Wohlfahrtiimonas chitiniclastica

How do I ID *Wohlfahrtiimonas* and *Ignatzschineria*?

MALDI ID reliable

RUO libraries

Biochemical based ID systems

Likely no ID, sometimes mis-ID (other non-fermenters)

What about AST?

Non-fastidious non-fermenting GNR ->

CLSI M100 Non-Enterobacterales breakpoints

Perform by gradient diffusion (Etest) or broth microdilution

Commercial system won't recognize organism

No disk BPs



Image: PMID 33826527

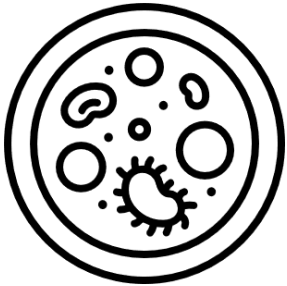
Final Report

Gram stain



Few Gram-negative rods
Few Gram-positive rods
Moderate Gram-positive cocci
No PMNs

Culture



3+ *Pseudomonas aeruginosa*
3+ *Staphylococcus aureus*
3+ *Wohlfahrtiimonas chitiniclastica*
3+ Normal flora

Tips for Workup of Rarely Encountered Organisms

Tip 1

Workup of rarely identified organisms requires assessment of the clinical significance and disease association of the organisms

Helpful Resources

Manual of Clinical Microbiology

Available in print, pdf, online via ClinMicroNow subscription

Journal of Clinical Microbiology, other review articles

The Brief Case

Google (when you're desperate)

CASE 2

Case History



64yo old man presents to the Emergency Department with shortness of breath

Febrile (39.1°C)

Respiratory rate 19 per minute (normal 12-20)

Slightly tachycardic (pulse 104 beats per min)

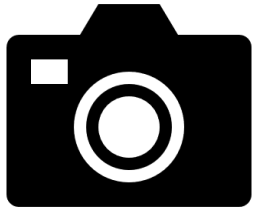
Past medical history: chronic obstructive pulmonary disease (COPD)

Admitting diagnosis: pneumonia

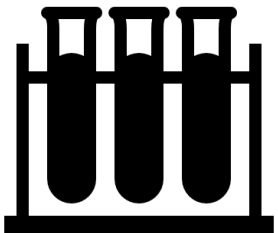
Case History



Empiric antimicrobial therapy
Ceftriaxone



Imaging
Chest radiograph: left lower lobe infiltrate,
consistent with diagnosis of pneumonia



Micro labs
COVID/RSV/Flu NAAT
Sputum culture



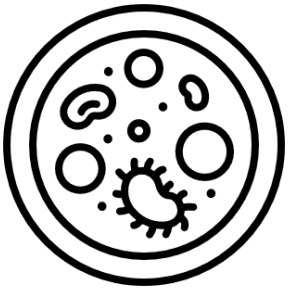
Gram Stain / Culture



Gram stain

>25 PMNs

No organisms seen



Culture

1+ normal respiratory flora

3+ small alpha hemolytic colonies

-> sent to MALDI

How would you report this culture?

- A. 3+ *S. pneumoniae*, 1+ normal flora
- B. 3+ normal flora
- C. More workup needed
- D. Unsure

MALDI ID	Score
<i>Streptococcus pneumoniae</i>	2.10
<i>Streptococcus pseudopneumoniae</i>	2.04
<i>Streptococcus mitis/oralis</i>	1.99

Confirmatory Testing

Optochin Susceptibility Test

Principle

S. pneumoniae is susceptible to optochin

First published in 1915, in common use since 1955

Procedure

BAP with lawn of bacteria + optochin (P) disk

Incubate 18-24h, 35-37°C, 5-10% CO₂

Measure zone diameter

Reporting

Zone	Interp	Report
≥14mm	Susceptible	<i>S. pneumoniae</i>
6mm (no zone)	Resistant	Viridans group <i>Streptococcus</i>
7-14mm	Intermediate	NA



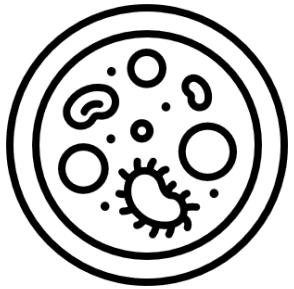
Gram Stain / Culture



Gram stain

>25 PMNs

No organisms seen



Culture

1+ normal respiratory flora

3+ small alpha hemolytic colonies

-> sent to MALDI

Optochin intermediate (9mm)

MALDI ID	Score
<i>Streptococcus pneumoniae</i>	2.10
<i>Streptococcus pseudopneumoniae</i>	2.04
<i>Streptococcus mitis/oralis</i>	1.99

That's weird...

1. Optochin intermediate?
2. **Predominant, patient has pneumonia... it SHOULD be *S. pneumoniae*...**

Confirmatory Testing

Bile Solubility Test

Principle

S. pneumoniae enzyme amidase causes autolysis

Viridans group *Streptococcus* species lack these enzymes

Organisms autolyze when reaching stationary growth phase ('dimple' in colony)

Sodium deoxycholate (bile salt) activates autolytic enzymes

Procedure (Direct Plate Method)

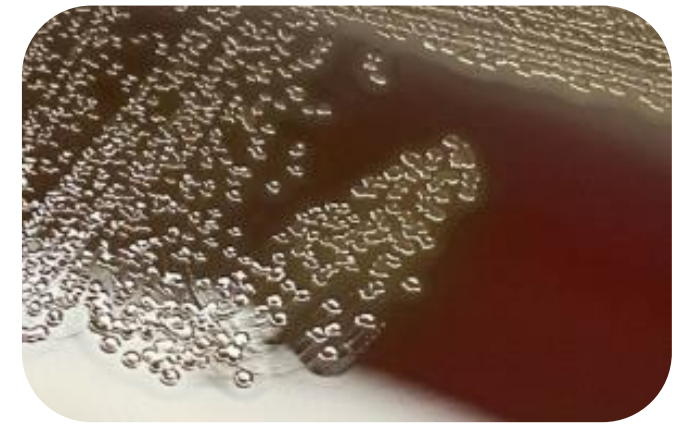
Drop 10% bile salt solution near 18-24h colonies

Incubate 35-37°C 15-30min until drop has evaporated

Observe for colony flattening / disintegration

Reporting

Observation	Report
Flattened / disintegrated colony	<i>S. pneumoniae</i>
Insoluble colony	Viridans group <i>Streptococcus</i>



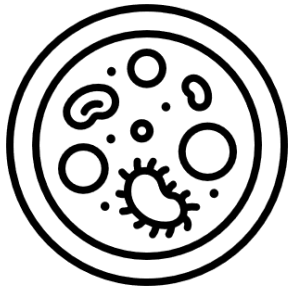
Gram Stain / Culture



Gram stain

>25 PMNs

No organisms seen



Culture

1+ normal respiratory flora

3+ small alpha hemolytic colonies

-> sent to MALDI

Optochin intermediate (9mm)

Bile insoluble

MALDI ID	Score
<i>Streptococcus pneumoniae</i>	2.10
<i>Streptococcus pseudopneumoniae</i>	2.04
<i>Streptococcus mitis/oralis</i>	1.99

That's weird...

Predominant, patient has pneumonia... it SHOULD be *S. pneumoniae*...

Streptococcus pseudopneumoniae

Description

Described in 2004, member of *S. mitis/oralis* group

Clinical Significance

Associated with lower respiratory tract disease in CF, COPD patients

Clinical relevance remains somewhat unclear

Lack of guidance on when to report / consider significant

Reasonable: Normal flora if equal to other NF, ID/AST when predominant

Identification

MALDI: does not reliably differentiate *S. mitis/oralis*, *S. pneumoniae*, *S. pseudopneumoniae*

Bile insoluble

Optochin intermediate/resistant at 5% CO₂, susceptible in ambient air

AST

CLSI M100 viridians group *Streptococcus* species breakpoints

Confirmatory Testing

Organism	Bile Soluble	Optochin 5% CO ₂	Optochin Ambient
<i>S. pneumoniae</i>	Yes	Susceptible (≥14mm)	Susceptible (≥14mm)
<i>S. pseudopneumoniae</i>	No	Resistant (6mm) / Intermediate (<14mm)	Susceptible (≥14mm)
<i>S. mitis</i> group	No	Resistant (6mm)	Resistant (6mm)

CMPH recommends consistent performance of **both** optochin and bile solubility

Neither test is 100% sensitive / specific

Perform optochin in ambient air if *S. pseudopneumoniae* is suspected

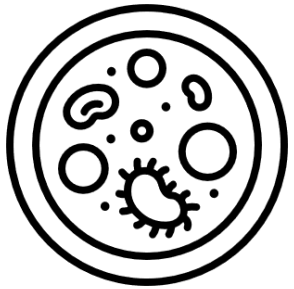
Gram Stain / Culture



Gram stain

>25 PMNs

No organisms seen



Culture

1+ normal respiratory flora

3+ small alpha hemolytic colonies

-> sent to MALDI

Optochin 5% CO₂ intermediate

Optochin ambient susceptible

Bile insoluble

MALDI ID	Score
<i>Streptococcus pneumoniae</i>	2.10
<i>Streptococcus pseudopneumoniae</i>	2.04
<i>Streptococcus mitis/oralis</i>	1.99

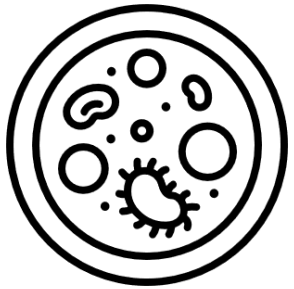
Final Report



Gram stain

>25 PMNs

No organisms seen



Culture

3+ *Streptococcus pseudopneumoniae*

1+ normal respiratory flora

Tips for Workup of Rarely Encountered Organisms

Tip 1

Workup of rarely identified organisms requires assessment of the clinical significance and disease association of the organisms

Tip 2

Understand the limitations of your MALDI database and establish procedures to identify organisms that cannot be adequately differentiated

Be vigilant for IDs that don't make sense

CASE 3

Case History

68yo old man presents to the ID clinic due to persistent foot pain/drainage



History of present illness

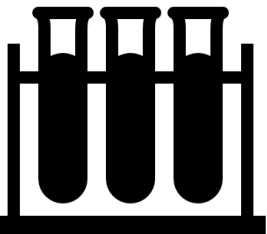
Stepped on “something” bare footed several months prior

X-ray did not demonstrate any foreign objects

Empirically treated with amoxicillin/clavulanic acid with no changes

Labs

Aerobic culture of wound drainage (swab)



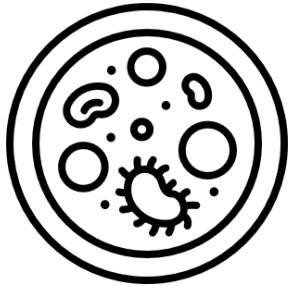
Gram Stain / Culture



Gram stain

2+ Gram-positive cocci

No PMNs



Culture

1+ *Streptococcus agalactiae*

1+ *Staphylococcus argenteus*

1+ *Staphylococcus haemolyticus*



Gram Stain / Culture

Gram stain

No PMNs, 2+ GPC

Culture

1+ *S. agalactiae*

1+ *S. argenteus*

1+ *S. haemolyticus*

How would you report this culture?

<u>Report</u>	<u>Reason</u>
A 1+ <i>S. agalactiae</i> 1+ normal skin flora	Beta-streps are pathogens Coagulase-negative staphylococci are skin flora
B 1+ <i>S. agalactiae</i> 1+ <i>S. argenteus</i> 1+ normal skin flora	Beta-streps and <i>S. argenteus</i> are pathogens Coagulase-negative staphylococci are skin flora
C 1+ <i>Streptococcus agalactiae</i> 1+ <i>Staphylococcus argenteus</i> 1+ <i>Staphylococcus haemolyticus</i>	Report up to 3 potential pathogens
D 1+ Normal skin flora	No predominant organism, no PMNs on Gram



Gram Stain / Culture

Gram stain

No PMNs, 2+ GPC

Culture

1+ *S. agalactiae*

1+ *S. argenteus*

1+ *S. haemolyticus*

How would you report this culture?

Report

- A 1+ *S. agalactiae*
1+ normal skin flora
- B 1+ *S. agalactiae*
1+ *S. argenteus*
1+ normal skin flora
- C 1+ *Streptococcus agalactiae*
1+ *Staphylococcus argenteus*
1+ *Staphylococcus haemolyticus*
- D 1+ Normal skin flora

Following CMPH guidance:

S. agalactiae: “always report” organisms

S. haemolyticus: report only if pure, otherwise consider mixed microbiota/normal flora

Who is *Staphylococcus argenteus*?

Staphylococcus argenteus

Description

Described in 2015

Member of the *S. aureus* complex (w/ *S. schweitzeri*, *S. roterodami*, and *S. singaporensis*)

Clinical Significance

Clinical presentation similar to *S. aureus*

Carries many of the same virulence genes as *S. aureus*

Identification

Beta-hemolytic, white

MALDI: reliable, may only be on RUO database

Coagulase positive (tube, slide, latex agglutination)

“always report” organism



Image: PMID 39386113

Staphylococcus argenteus

Reporting

Staphylococcus argenteus

Clinical team ALSO doesn't know what *S. argenteus* is

Best Case: clinician googles the organism, figures out it's important, treats accordingly

Worse Case: clinicians thinks "we only treat *S. aureus*", assumes org is CoNS, consider contaminant and does not treat

Recommended reporting:

S. aureus complex (*S. argenteus*)

S. argenteus (*S. aureus* complex)

Provides context; *S. aureus* is an indication to treat



Image: PMID 39386113

Related Reporting Guidance

Taxonomic Changes

Updated nomenclature often included in MALDI library updates

Review the updates before implementation!

Address nomenclature changes prior to installation of library updates

Provide previous name in report if reporting updated nomenclature

Example: *Candida krusei* -> revised name *Pichia kudriavzevii*

NO *Pichia kudriavzevii*

YES *Pichia kudriavzevii* (formerly known as *Candida krusei*)

Pichia kudriavzevii (aka *Candida krusei*)

Pichia kudriavzevii (*Candida krusei*)

Candida krusei (*Pichia kudriavzevii*)

Staphylococcus argenteus

Antimicrobial susceptibility testing

CLSI M100 *Staphylococcus* species breakpoints

S. aureus oxacillin/cefoxitin breakpoints

Species	Antimicrobial Agent	Interpretive Categories, Zone Diameter (mm)		Interpretive Categories, MIC (ug/mL)	
		S	R	S	R
★ <i>S. aureus</i> (including members of the <i>S. aureus</i> complex), <i>S. lugdunensis</i>	Oxacillin	NA	NA	≤2	≥4
	Cefoxitin	≥22	≤21	≤4	≥8
<i>S. epidermidis</i>	Oxacillin	≥18	≤17	≤0.5	≥1
	Cefoxitin	≥25	≤24	NA	NA
<i>S. pseudintermedius</i> , <i>S. schleiferi</i>	Oxacillin	≥18	≤17	≤0.5	≥1
	Cefoxitin	NA	NA	NA	NA
✗ <i>Staphylococcus</i> species, not otherwise listed	Oxacillin	NA	NA	≤0.5	≥1
	Cefoxitin	≥25	≤24	NA	NA

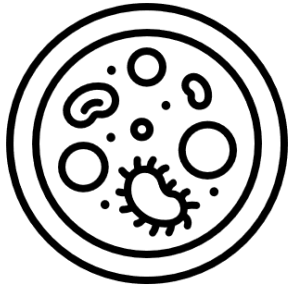
Final Report



Gram stain

2+ Gram-positive cocci

No PMNs



Culture

1+ *Streptococcus agalactiae*

1+ *Staphylococcus aureus* complex (*S. argenteus*)

1+ Normal skin flora

Tips for Workup of Rarely Encountered Organisms

Tip 1

Workup of rarely identified organisms requires assessment of the clinical significance and disease association of the organisms

Tip 2

Understand the limitations of your MALDI database and establish procedures to identify organisms that cannot be adequately differentiated

Tip 3

Report rarely encountered organisms with important clinical context

Highlight group/complex as applicable, relatedness to recognizable organisms

For taxonomy changes, report previous, recognizable name

Summary

Maintain up-to-date resources to understand the pathogenic potential and disease states associated with uncommonly encountered organisms

ClinMicroNow, Manual of Clinical Microbiology, CAP responses

Understand MALDI-TOF MS constraints and incorporate additional confirmatory testing required to discriminate closely related species when clinically relevant

MALDI package insert

Review taxonomic changes associated with MALDI-TOF MS spectral library updates and incorporate these changes into clinical reports

JCM publications, CAP responses, MALDI package insert

Thank you!

