



University
of Manitoba

Wound Microbiology: Specimen Collection and Laboratory Report Interpretation

Dr. Andrew Walkty

Medical Microbiologist, Shared Health, Department of Clinical
Microbiology, Health Sciences Centre

Associate Professor, Department of Medical Microbiology and Infectious
Diseases, Max Rady College of Medicine, University of Manitoba

Winnipeg, Manitoba, Canada
Email: awalkty@sharedhealthmb.ca

Disclosures

- **Faculty:** Dr. Andrew Walkty
- **Relationships with commercial interests:**
 - **Not applicable**

Wound Microbiology

- Learning Objectives:
 - At the end of this session, the attendee will be able to:
 - State the names of common pathogens responsible for skin and soft tissue/wound infections
 - Describe how (and when) to submit a specimen to the microbiology laboratory and list key pieces of information that need to be included on the microbiology laboratory requisition
 - Interpret the data presented in microbiology laboratory reports

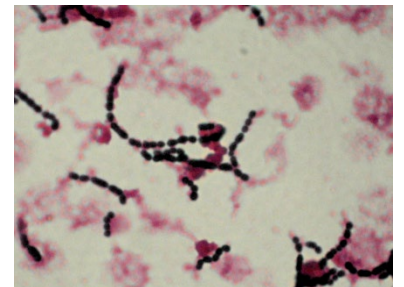
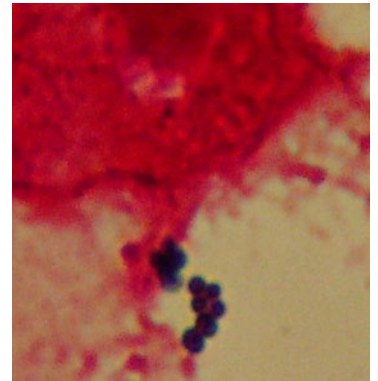
Wound Microbiology

- Outline

1. Skin and soft tissue infection/wound microbiology
2. Specimen collection – practical guidance
3. Microbiology requisition
4. Laboratory protocols
5. Interpreting data in the microbiology laboratory report (with examples)

Wound Infections - Microbiology

- Common skin and soft tissue infection pathogens:
 - *Staphylococcus aureus*
 - Impetigo and ecthyma
 - Furuncles, carbuncles, cutaneous abscesses
 - Cellulitis
 - Necrotizing fasciitis
 - Beta-hemolytic Streptococci (*Streptococcus pyogenes* [Group A *Streptococcus*], others – Group C and G Strep)
 - Impetigo, ecthyma
 - Cellulitis, erysipelas
 - Necrotizing fasciitis



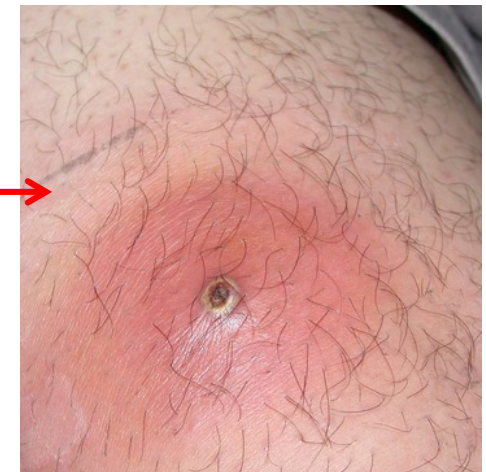
Wound Infections - Microbiology



Erysipelas



Impetigo



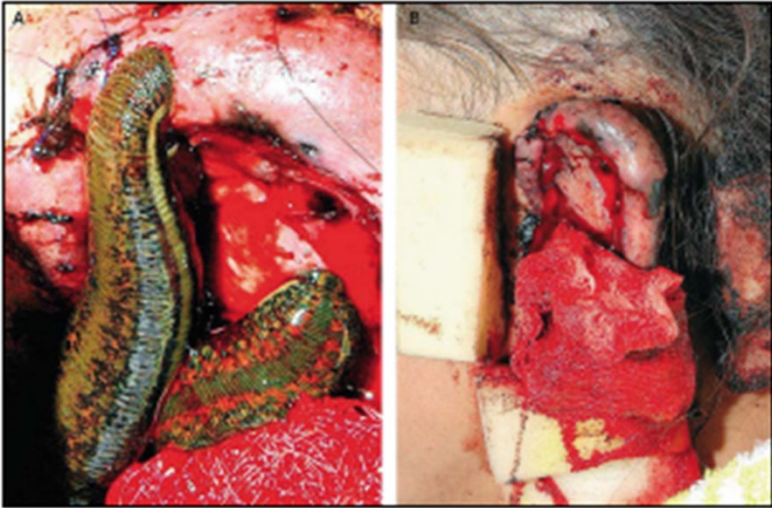
Furuncle

<http://www.skindermatologists.com/bacterial-skin-infection.html>, www.uptodate.com

Wound Infections - Microbiology

- Uncommon pathogens – importance of taking a complete history:
 - *Aeromonas* spp.
 - May cause soft tissue infection after exposure to fresh or brackish water
 - Also associated with soft tissue infection following use of medicinal leaches
 - *Vibrio vulnificus*
 - Soft tissue infections may result from contact with seawater or shellfish
 - *Acinetobacter baumannii*
 - Described as a cause of soft tissue infection following combat trauma wounds

Wound Infections - Microbiology



Lancet Infectious Diseases 2005;5:501-13. *NEJM* 2005;355:15. *Clin Infect Dis* 2008;47:444-9.

Wound Infections - Microbiology

- Bite wounds - polymicrobial:



Dog Bite Wounds

TABLE 1. Common aerobic and anaerobic bacterial genera isolated from 50 infected dog bite wounds^a

Bacterial genus	Frequency (%)
Aerobic organisms	
<i>Pasteurella</i>	50
<i>Streptococcus</i>	46
<i>Staphylococcus</i>	46
<i>Neisseria</i> ^b	32
<i>Corynebacterium</i>	12
<i>Moraxella</i>	10
<i>Enterococcus</i>	10
<i>Bacillus</i>	8
Anaerobic organisms	
<i>Fusobacterium</i>	32
<i>Porphyromonas</i>	28
<i>Prevotella</i>	28
<i>Propionibacterium</i>	20
<i>Bacteroides</i> ^b	18
<i>Peptostreptococcus</i>	16

^a Based on data from reference 206.

^b The frequencies differ from the actual numbers cited in the reference due to the reclassification of some of the isolates since its publication.

Cat Bite Wounds

TABLE 2. Common aerobic and anaerobic bacterial genera isolated from 57 infected cat bite wounds^a

Bacterial genus	Frequency (%)
Aerobic organisms	
<i>Pasteurella</i>	75
<i>Streptococcus</i>	46
<i>Staphylococcus</i>	35
<i>Neisseria</i> ^b	35
<i>Moraxella</i>	35
<i>Corynebacterium</i>	28
<i>Enterococcus</i>	12
<i>Bacillus</i>	11
Anaerobic organisms	
<i>Fusobacterium</i>	33
<i>Porphyromonas</i>	30
<i>Bacteroides</i>	28
<i>Prevotella</i>	19
<i>Propionibacterium</i>	18

^a Based on data from reference 206.

^b The frequency differs from the actual number cited in the reference due to the reclassification of some of the isolates since its publication.

Cat and dog bite wounds: Staphylococci, Streptococci, *Neisseria* spp., *Pasteurella* spp., anaerobes

Wound Infections - Microbiology

- Surgical site infections:
 - Rare in the first 48 hours after surgery
 - If occurring in this timeframe, think *S. pyogenes* or *Clostridium* spp.
 - Beyond 48 hours, if a surgical wound infection occurs, the microbiology is influenced by the site of operation
 - Mixed gram-positive and gram-negative flora, both facultative and anaerobic organisms common for surgical wound infections after an operation on the intestinal tract or female genitalia
 - *S. aureus* and streptococcal species common for surgical wound infections following clean procedures

Wound Infections - Microbiology

- Diabetic foot wounds:
 - Superficial diabetic foot wounds in antimicrobial naïve individuals
 - Most commonly due to gram-positive cocci (*S. aureus*, beta-hemolytic streptococci)
 - Deeper wounds and/or wounds previously treated with antimicrobials are more likely to be polymicrobial
 - Here, may also consider Enterobacterales, *P. aeruginosa*, and anaerobes

Specimen Collection

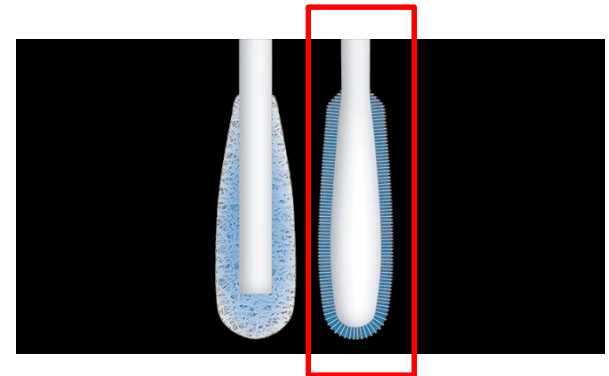
- Do:
 - Obtain an appropriate specimen for culture from almost all infected wounds
 - Collect the specimen prior to initiation of antimicrobial therapy if possible
 - Cleanse and debride the wound before obtaining the specimen
 - Obtain a tissue specimen by scraping with a scalpel or dermal curette, or biopsy from the base of a debrided ulcer
 - Aspirate purulent fluid using a sterile needle and syringe
 - Remove the needle prior to transport to the micro lab
 - Promptly transport samples to the laboratory in a sterile container
 - Be sure to label correctly (patient name AND PHIN required on the specimen container – must match the requisition)

Specimen Collection

- Do not:
 - Culture a clinically uninfected wound
 - Obtain a specimen for culture without first cleansing or debriding the wound
- Use of swabs is discouraged
 - The amount of material collected by a swab is often inadequate
 - Prone to contamination by commensal microbiota
 - May fail to recover deep tissue pathogens

Specimen Collection

- If a swab is going to be used as the collection device, a flocked swab (e.g., E-swab™) is preferable
 - Flocked swabs have nylon fibers perpendicular to the shaft that increase the surface area and improve the amount of specimen that can be collected
 - Used in conjunction with a liquid medium transport system
 - These swabs are superior to fiber swabs for the recovery of bacteria



Submit Specimens ...Not Swabs

Tips for Collecting Quality Surgical Specimens for Microbiology

Do the Math:

An effective culture requires 6 plates
1 thio
AND
1 gram stain



If a swab yields only 3 bacteria,
what are your chances for 'cultural' success?

FOR QUALITY RESULTS, SEND TISSUE
AND FLUIDS TO MICROBIOLOGY



Nancy Cornish MD
www.cap.org

Tips for Collecting Quality Surgical Specimens for Microbiology

Swabs don't do the job...

- Out of every 100 bacteria absorbed on a swab, only 3 make it to culture.
- Anaerobes on swabs die upon exposure to air, but survive in tissues and fluids.
- Swabs hold only 150 microlitres of fluid.



FOR QUALITY RESULTS, SEND TISSUE
AND FLUIDS TO MICROBIOLOGY



Tips for Collecting Quality Surgical Specimens for Microbiology

Make the Right Choice!

Good Specimens

- Tissue (in large mouth sterile container)
- Whole fluid (in original syringe or container, NOT on a swab)

Bad Specimens

- Any specimen collected with a swab
- Tissue or fluid placed into a swab tube/device
- Any surface specimens



FOR QUALITY RESULTS, SEND TISSUE
AND FLUIDS TO MICROBIOLOGY



Microbiology Requisition



CLINICAL MICROBIOLOGY LABORATORY TEST REQUISITION

Health Sciences Centre
204-787-1273

LIS Barcode
Lab use only

PLEASE COMPLETE THE INFORMATION BELOW – PRINT CLEARLY

<input type="checkbox"/> Copy to Last Name _____ First Name _____ Facility name _____ Address _____ Fax # _____ Full name, address & fax number MUST be provided		PHIN/Health Care Number _____	Chart# _____	Visit# _____
		<input type="checkbox"/> M Patient Legal Name (Last) _____ (First) _____ (Initial) _____ <input type="checkbox"/> F _____	Birthdate DD ____ MM ____ YY ____	
		Outpatient Address _____	Outpatient Phone _____	
		Ordering Address/Location _____	Physician Code _____	
Date Specimen Collected DD ____ MM ____ YY ____	Time (24 h) ____	Report Address if Different _____		
Collector _____		Ordering Physician/Practitioner _____	Physician Critical Results Phone Number _____	
Diagnosis/Relevant Clinical Information: <input type="checkbox"/> UTI symptoms (any of; flank pain, frequency, dysuria) <input type="checkbox"/> Pregnant <input type="checkbox"/> Animal bite <input type="checkbox"/> Necrotizing fasciitis <input type="checkbox"/> Immunocompromised <input type="checkbox"/> Penicillin allergy <input type="checkbox"/> Human bite <input type="checkbox"/> MRSA positive				
Diagnostic Information: _____				

Be sure to include the following on the requisition:

Patient name, PHIN number, date of birth, gender, physician name and contact number, patient location, sample collector, specimen type, test requested, relevant clinical information, date and time of collection

Microbiology Requisition

Wounds/Skin/Abscesses/Surgical Specimens/Tissues	
Specify site:	Test:
<input type="checkbox"/> Swab	<input type="checkbox"/> Bacterial culture – aerobic
<input type="checkbox"/> Tissue/Biopsy	<input type="checkbox"/> Bacterial culture – anaerobic
<input type="checkbox"/> IV catheter tips	<input type="checkbox"/> Yeast culture (e.g. <i>Candida</i> , <i>Cryptococcus</i>)
<input type="checkbox"/> Ulcer	<input type="checkbox"/> Moulds & systemic mycoses (e.g. <i>Aspergillus</i> , <i>Blastomyces</i>)
<input type="checkbox"/> Aspirate	<input type="checkbox"/> Mycobacterial culture (AFB)
<input type="checkbox"/> Bone chips	
<input type="checkbox"/> Skin scrapings	
<input type="checkbox"/> Device (specify type) _____	
Facility:	Form #

Swabs are suboptimal. If used, send one swab for each test requested (e.g., if culture for aerobes and anaerobes requested, send 2 swabs)

Only request the required tests (i.e., do not ask for anaerobes, fungi, or mycobacteria unless clearly indicated by the clinical presentation)

Importance of Clinical Details

'B'

	Media	Gram	Test code	Bench
BARTHOLIN CYST (aspirate or swab) *any external female genitalia described as abscess/cyst*	BA, MAC, CA/TM BAK, PEA, LKV	Yes	MSTD MIAN	STD
BILE	BA, MAC Aseptically acquired ADD: BAK, PEA, LKV	Yes	WS WAN	WND
BITE – human or animal	BA, MAC, CA BAK, PEA, LKV (if requested)	Yes	WS WAN	WND
BONE - Large bone fragments	Cover bone with BHI broth add 1 mL LHB (w check plt)	No	TIS	F/T
- Small bone fragments	BA, CA BAK and FAB	Yes	TIS TIAN	F/T
BREAST MILK (suspected mastitis ONLY)	BA	No	FLU	F/T

If the specimen is collected from a bite wound and this is provided in the clinical details, culture for anaerobes is automatically set up by the microbiology laboratory.

Importance of Clinical Details

3.12 Table 12. *Streptococcus* species (Large Colony, β -hemolytic) (only test in penicillin allergic patients or upon request)

Antibiotic ^{1,2}	Antimicrobial to Report	Comments
Clindamycin	(✓)	Report using D-test ^{3,4,5} . Do not report on CSF, urine from males, and urine from non-pregnant females.
Erythromycin ⁶	(✓)	Report using D-test ^{3,4} . Do not report on CSF, urine, and vaginal-rectal swabs (screening in pregnancy).
Levofloxacin	(✓)	Report where erythromycin and clindamycin are resistant, OR if isolate is from urine of a non-pregnant female. Only report on patients ≥ 18 years of age. Do not report on CSF samples. Do not report on pregnant women.
Vancomycin	(✓)	Non-urine isolates: report only where erythromycin and clindamycin are tested by D-test and isolate is resistant to EITHER erythromycin OR clindamycin. Urine isolates: report only if isolate is from a pregnant female or from a non-pregnant female ≥ 18 years of age when their isolate is levofloxacin-resistant.

(✓): Indicates that the antibiotic is only reported under certain conditions as outlined under the comment section. Testing by disk diffusion when required.

Large colony beta-hemolytic streptococci (e.g., Group A *Streptococcus*, Group B *Streptococcus*): Susceptibility testing NOT routinely done, but will be performed if a history of penicillin allergy is provided.

Organism (number tested): January through December 2016	Percent Susceptible												
	Penicillin	Ampicillin	Oxacillin ^b	Vancomycin	High-Level Gentamicin ^c	High-Level Streptomycin ^c	Erythromycin ^d	Clindamycin	Trimethoprim-Sulfamethoxazole	Rifampin ^e	Linezolid	Tetracycline	Nitrofurantoin ^f
<i>Streptococcus pyogenes</i> (n.a.) ^g (Group A <i>Streptococcus</i>)	100												
<i>Streptococcus agalactiae</i> (162) ^h (Group B <i>Streptococcus</i>)	100			100				60					

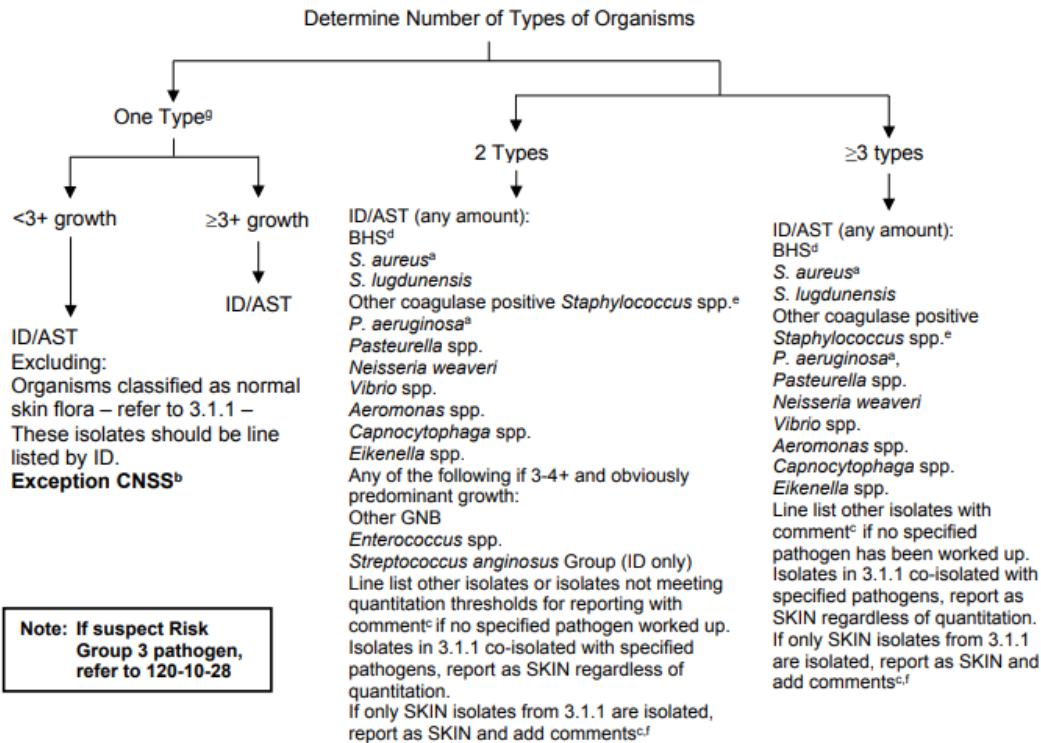
☐ = Not tested, not routinely reported, or not recommended

Laboratory Protocol for Wounds

3.0 Wound Culture Workup

3.1 Routine Wound (Excludes IV/IA tips and bile cultures from drains (NOTE: for Pathology cultures, stock isolate, AST testing not required))

Plates are held for 72 hours prior to being reported as no growth. If culture plates are negative at 72 hours and direct Gram showed $\geq 3+$ PMNs, incubate plates a further 48 hours.



The laboratory protocol emphasizes work-up of organisms likely to be pathogens. Tissues and sterile fluids will be worked up to a greater extent than wound swabs.

NB. the lab is NOT seeing the patient – call if further work-up required.

Laboratory Protocol for Wounds

3.1.1 Organisms Classified as Normal Skin Flora or Non-Pathogenic Skin Contaminants (reported as SKIN)

This only includes swabs taken from skin surface areas and not swabs of abscess fluids, invasive samples, or samples taken from sites other than skin. If the sample is not from a skin surface area (e.g., deep swab, swab of abscess, invasively collected swabs etc.), line list organisms by name.

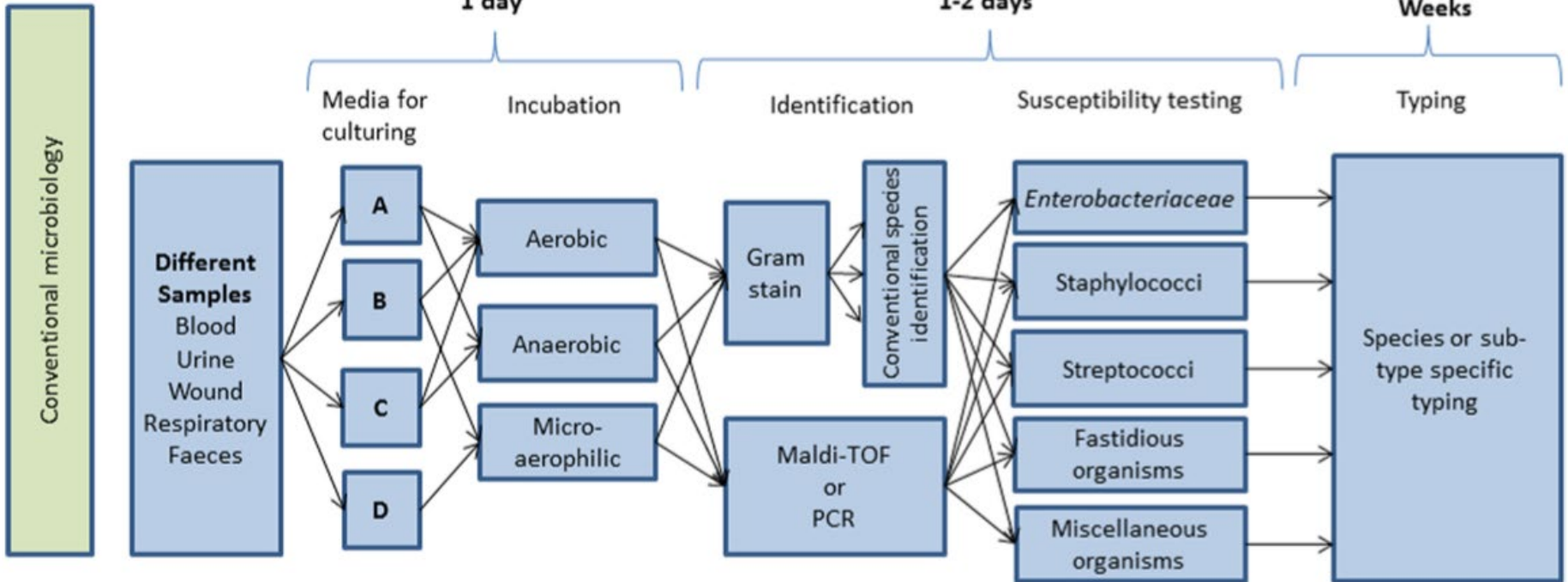
If MALDI-ToF does not provide an adequate ID, line list using spot tests, i.e., Gram, catalase, etc.

Abiotrophia spp.
Actinobaculum spp.
Actinomyces spp.
Aerococcus spp.
Alloiococcus otitis
Arthrobacter spp.
Bacillus spp.
Bifidobacterium spp.
Brachybacterium spp.
Brevibacterium spp.
Cellulomonas spp.
Cellulosimicrobium spp.
Collinsella spp.
Commensal oral microbiota (e.g., oral
Neisseria spp.) Consult if in question
Corynebacterium spp. (except for
potentially toxigenic strains
C. diphtheria, *C. ulcerans* and
C. pseudotuberculosis)
Corynebacterium otitidis
Curtobacterium spp.
Cutibacterium spp.
Dermabacter hominis
Dermacoccus spp.
Dolosicoccus spp.
Dolosigranulum spp.
Eggertella spp.
Enterococcus spp. (except when
requiring identification by wound
protocol)
Eubacterium spp.
Exiguobacterium spp.
Facklamia spp.
Filifactor spp.
Gemella spp.
Geobacillus spp.
Globicatella spp.
Glutamicibacter spp.
Granulicatella spp.

Helcobacillus massiliensis
Helcococcus spp.
Ignavigranum spp.
Janibacter spp.
Knoellia spp.
Kocuria spp.
Kytococcus spp.
Lactobacillus spp.
Lactococcus spp.
Leuconostoc spp.
Macrococcus spp.
Microbacterium spp.
Micrococcus spp.
Mogibacterium spp.
Nesterenkonia spp.
Oerkskovia spp.
Paenibacillus spp.
Paenarthrobacter spp.
Paeniglutamicibacter spp.
Pediococcus spp.
Propionimicrobium spp.
Pseudoarthrobacter spp.
Pseudoclavibacter spp.
Pseudoramibacter spp.
Pseudoglutamicibacter spp.
Rothia spp.
Staphylococcus spp. (except *S. aureus*,
S. intermedius, *S. pseudintermedius*,
S. hyiacus, *S. lugdunensis*, *S. delphini*,
S. schleiferi, and *S. lutrae*)
Streptococcus spp. (except *S. pyogenes*,
S. agalactiae, *S. dysgalactiae*, *S. equi*,
S. canis, *S. pseudoporcinus*, *S. iniae*, and
members of the anginosus group
streptococci)
Vagococcus spp.
Weissella spp.

Not all bacteria recovered from a wound are significant – bacteria found as part of the normal flora of the skin may simple be reported as “Normal skin flora”

Turn-around Time



Typical turn-around-time:

Gram stain – same day, preliminary organism identification 1 to 2 days, susceptibility testing results 2 to 3 days

Reading the Microbiology Laboratory Report

- Gram stain report:
 - Quantitation of Gram stained organism (1+, 2+, 3+, 4+) and inflammatory cells seen
- Culture report:
 - Preliminary
 - Organism identification with quantitation (1+, 2+, 3+, 4+)
 - Report will indicate “Further report to follow.”
 - NB. may be additional organisms recovered and reported before the report is finalized
 - Final
 - Clinically relevant organisms are reported (with quantitation) and susceptibility results
 - NB. normal skin flora may simply be identified as “Normal skin flora”
 - NB. the lab is NOT seeing the patient – call if further work-up required
- Comments added as appropriate
 - Comments pertaining to organism susceptibility or adequacy of the specimen may be provided
 - Report copied to public health and infection prevention and control as required

Reading the Microbiology Laboratory Report

- 47 y.o. male with a knife puncture wound to the hand
 - The hand is now swollen, red, and painful, and purulent material is draining from the puncture site
- Culture result:

===== WOUND SWAB/DRAIN =====

Site L HAND DEEP PUNCTURE WOUND

GRAM SMEAR : 4+ PMN
 2+ Gram positive cocci

Gram stain result

CULTURE

1) 4+ Streptococcus pyogenes (Group A Streptococcus)
 This organism is intrinsically susceptible to penicillin and susceptibility testing is not routinely performed. Please contact the Clinical Microbiology Laboratory if susceptibility testing for other agents is required (e.g., penicillin allergy).

Bacteria recovered with comments provided by the lab

2) 1+ Staphylococcus aureus
 Resistance or sensitivity to oxacillin is predictive of resistance or sensitivity to cefazolin and cloxacillin.

	(1)	(2)
Oxacillin.....		S
Erythromycin		R
Clindamycin		R
Tetracycline		S
Trimethoprim-sulfamethoxazole		S
	(1)	(2)

Susceptibility information

S = Susceptible I = Intermediate R = Resistant

Reading the Microbiology Laboratory Report - Examples

- 47 y.o. male with left arm skin lesions (?ecthyma) and associated cellulitis
- Culture result:

===== WOUND SWAB/DRAIN =====

Site L ARM SWAB

GRAM SMEAR : 3+ PMN
 3+ Gram positive cocci

CULTURE

1) 4+ Streptococcus pyogenes (Group A Streptococcus)
This organism is intrinsically susceptible to penicillin and susceptibility testing is not routinely performed. Please contact the Clinical Microbiology Laboratory if susceptibility testing for other agents is required (e.g., penicillin allergy).

2) 2+ Normal skin flora

S. epidermidis was also isolated from this specimen – likely not significant, so the lab has simply reported this as Normal skin flora

Reading the Microbiology Laboratory Report - Examples

===== WOUND SWAB/DRAIN =====

Site L ARM SWAB

GRAM SMEAR : 3+ PMN
 3+ Gram positive cocci

CULTURE

1) 4+ Streptococcus pyogenes (Group A Streptococcus)
This organism is intrinsically susceptible to penicillin and susceptibility testing is not routinely performed. Please contact the Clinical Microbiology Laboratory if susceptibility testing for other agents is required (e.g., penicillin allergy).

Further report to follow.

NB. if the report says “Further report to follow.” it has NOT been finalized. Additional pathogens and/or other important information (e.g., further susceptibility results) may be added. Be sure to follow-up the finalized microbiology result.

Reading the Microbiology Laboratory Report - Examples

- 46 y.o. female with infection of a dialysis fistula site
- Culture result:

```
===== TISSUE FOR ROUTINE CULTURE =====  
  
Site LT INFECTED ARM VEIN  
  
CRITICAL RESULT PHONED/FAXED at 19:23 on 3 Apr 18 to BRIDGETTE VIC by L8AC8  
:STERILE GRAM STAIN  
  
GRAM SMEAR :      No PMN  
              3+ Gram positive cocci  
  
CULTURE  
This culture has been revised.  See details below.  
  
1) 4+ Staphylococcus aureus  
Resistance or sensitivity to oxacillin is predictive of resistance or  
sensitivity to cefazolin and cloxacillin.  
Multiple morphotypes of this organism were isolated.  All morphotypes  
demonstrated identical antimicrobial susceptibility testing results.  
Therefore, antimicrobial susceptibility testing results are only reported for  
one of the morphotypes identified.
```

```
2) 4+ Enterococcus faecalis  
PLEASE DISREGARD PREVIOUS RESULTS. REPORT REVISED ON  
April 7, 2018.  Previously reported as a VRE (resistant [R] to Vancomycin).
```

```
3) 2+ Streptococcus mitis group  
  
Oxacillin.....          (1) (2) (3)  
S  
Erythromycin .....      S  
Clindamycin .....       S  
Tetracycline .....       S  
Trimethoprim-sulfamethoxazole  
S  
Ampicillin .....         S  
Linezolid .....          S  
Vancomycin .....         S  
  
          (1) (2) (3)  
  
S = Susceptible  I = Intermediate  R = Resistant
```

Further report to follow.

```
COMMENTS  
A copy of this report has been generated for Infection Control.
```

The lab occasionally makes mistakes – when a microbiology report is amended, this will be clearly indicated.

Additional comments may be provided at the bottom of the report. If the report is copied to infection control or public health, this is also indicated at the bottom of the report.

Reading the Microbiology Laboratory Report - Questions

1. Which of the following organisms is not typically responsible for acute skin and soft tissue infections in otherwise healthy hosts?
 - a) Methicillin-susceptible *Staphylococcus aureus*
 - b) Methicillin-resistant *Staphylococcus aureus*
 - c) *Staphylococcus epidermidis*
 - d) Group A *Streptococcus*
 - e) Group C or G *Streptococcus*

Reading the Microbiology Laboratory Report - Questions

- 2) Which of the following information must be included on the requisition that accompanies specimens submitted to the microbiology laboratory for culture?
- a) Patient's name, date of birth, and personal health information number
 - b) Relevant clinical information
 - c) Practitioner's name and contact information
 - d) The test that is requested
 - e) All of the above

Reading the Microbiology Laboratory Report - Questions

- 3) Which of the following statements regarding specimen collection from a wound for culture is false?
- a) Use of a swab to obtain the specimen is preferred over sending tissue or fluid to the laboratory for culture
 - b) A specimen for culture should not be obtained from a clinically uninfected wound
 - c) The wound should be cleansed and debrided before obtaining a specimen for culture
 - d) A specimen for culture should be promptly transported to the microbiology laboratory in a sterile container
 - e) A specimen for culture should be obtained prior to the initiation of antimicrobial therapy