

Understanding the Microbiology Report: Wound Microbiology

Dr. Andrew Walkty

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

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

> Winnipeg, Manitoba, Canada Email: <u>awalkty@sharedhealthmb.ca</u>

Disclosures

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

Understanding the Microbiology Report: 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
 - List key pieces of information that need to be included on the microbiology laboratory requisition
 - Interpret the data presented in microbiology laboratory reports

Understanding the Microbiology Report: Wound Microbiology

- Outline
 - 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)

- 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







- 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

Principles and Practice of Infectious Diseases, 8th ed., Clin Infect Dis 2014;59:e10-52.







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

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	
Pasteurella	
Streptococcus	
Staphylococcus	
Neisseria ^b	
Corynebacterium	
Moraxella	
Enterococcus	
Bacillus	
Anaerobic organisms	
Fusobacterium	
Porphyromonas	

· orphyronna ac
Prevotella
Propionibacterium
Bacteroides ^b
Peptostreptococcus

^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	
Pasteurella	
Streptococcus	46
Staphylococcus	
Neisseria ^b	
Moraxella	
Corynebacterium	
Enterococcus	12
Bacillus	11
Anaerobic organisms	
Fusobacterium	
Porphyromonas	
Bacteroides	
Prevotella	19
Propionibacterium	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

Clin Micro Rev 2011;24:231-46.

- 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

- Diabetic foot wounds:
 - Superficial diabetic foot wounds
 - 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 Enterobacteriaceae, *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
 - 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)

Clin Infect Dis 2012;54:132-73.

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

Submit Specimens ... Not Swabs

Tips for Collecting Quality Surgical Specimens for Microbiology

o 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

METHODIST HOSPITAL PATHOLOGY CENTER

Nancy Cornish MD www.cap.org



Microbiology Requisition

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			114	

DIAGNOSTIC SERVICES SERVICES DIAGNOSTIC MANITOBA MANITOBA

CLINICAL MICROBIOLOGY LABORATORY TEST REQUISITION

Health Sciences Centre 204-787-1273				LIS Barcode	Lab use only
PLEASE COMPLETE	THE INFO	DRMATION BELOW - PRIN	T CLEARLY		
Copy to		PHIN/Health Care Number	Chart#		Visit#
Last Name First Name		Patient Legal Name (Last)	(First)	(Initial)	Birthdate DD MM YY
Address		Outpatient Address		Outpatient Phone	
Fax # Full name, address & fax numb MUST be provided	er	Ordering Address/Location			Physician Code
Date Specimen Collected Ti DD <u>MM YY</u>	ne (24 h)	Report Address if Different			
Collector		Ordering Physician/Practitioner		Physician Critical	Results Phone Number
Diagnosis/Relevant Clinical Information: UTI symptoms (any of; flank pain Necrotizing fasciitis MRSA positive Diagnostic Information:	, frequency,	dysuria)]Immunocompromised	□Pregnant □Penicillin allergy		□Animal bite □Human bite

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/S	urgical Specimens/Tissues
Specify site:	
Swab	Test:
Tissue/Biopsy	Bacterial culture – aerobic
□IV catheter tips	Bacterial culture – anaerobic
Ulcer	Yeast culture
Aspirate	(e.g. Candida, Cryptococcus)
Bone chips	Moulds & systemic mycoses
Skin scrapings	(e.g. Aspergillus, Blastomyces)
Device (specify type)	Mycobacterial culture (AFB)
Eacility: 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

Skin and Soft Tissue – epidermis, dermis, sweat glands, sebaceous glands, muscle, fascia							
Specimen	C&S media	Gram	Test Code	Bench			
Wound							
swabs/ulcers/aspirates/abscess/drainage	BA, MAC	Yes	WS	Wnds			
or pus from wound							
 anaerobes if requested from suitable 	BAK, PEA, LKV		WAN	Wnds			
source							
 from Northern nursing unit; 	Add MRSA Select agar			Wnds			
 if request C&S and MRSA from any 	Add MRSA Select agar						
location							
Tissues, biopsies, aspirates	BA, MAC, CA	Yes	TIS/TIAN	F/T			
	BAK, PEA, LKV FAB		Aspirates-FLU/FLAN				
swabs collected in the OR	BA, MAC, CA		Swabs – MIST/MIAN				
 anaerobes on request 	BAK, PEA, LKV FAB						
Burns	BA. MAC	Yes	WS	Wnds			
Bites – Human or Animal	BA, MAC, CA	Yes	WS	Wnds			
	BAK, PEA, LKV		WAN				

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, beta-hemolytic)

Antibiotic ^{1,2}	Antimicrobial to Report	Comments
Clindamycin	(√)	Report for penicillin allergic patients using D-test ^{3,4,5} . Do not report on CSF, unne from males, and urine from non- pregnant females.
Erythromycin ⁶	(✓)	Report for penicillin allergic patients using D-test ^{3,4} . Do not report on CSF and urine.
Levofloxacin	(√)	Report for Group A, B, C or G β -hemolytic streptococci only in penicillin allergic patients where erythromycin and clindamycin are resistant, or if isolate is from urine (irrespective of erythromycin and clindamycin test results). Only report on patients ≥18 years of age. Do not report on CSF samples. Do not report on pregnant women.
Vancomycin	(√)	Report for β -hemolytic streptococci only in penicillin allergic patients where erythromycin and clindamycin are resistant or if patient is pregnant and specimen is urine.

(\checkmark): Indicates that the antibiotic is only reported under certain conditions as outlined under the comment section.

						Percer	t Susc	eptible	9				
Organism (number tested): January through December 2016 = Not tested, not routinely reported, or not recommended	Penicillin	Ampicillin	Oxacillin ^b	Vancomycin	High-Level Gentamicin⁰	High-Level Streptomycin ^c	Erythromycin ^d	Clindamycin	Trimethoprim- Sulfamethoxazole	Rifampin ^e	Linezolid	Tetracycline	Nitrofurantoin ^t
Streptococcus pyogenes (n.a.) ^g (Group A Streptococcus)	100												
Streptococcus agalactiae (162) ^h (Group B Streptococcus)	100			100				60					

Large colony betahemolytic 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

Laboratory Protocol for Wounds

3.0 Wound Culture Workup

3.1 Routine Wound (wound swabs, swabs non-sterile sites, wound drain fluid, penis swabs, swabs from Pathology and decubitis ulcer swabs) 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 ≥3+ PMNs, incubate plates a further 48 hours.

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



Note: S. lugdunensis is a significant pathogen and susceptibility testing should be performed if this organism is isolated.

The laboratory protocol emphasizes workup 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 workup required.

Laboratory Protocol for Wounds

3.1.1.1 Organisms Classified as Normal Skin Flora (reported as SKIN)

Abiotrophia spp. Actinobaculum spp. Actinomyces spp. Aerococcus spp. Alloiococcus otitis Arthrobacter spp. Bacillus spp. Biffidobacterium spp. Brachybacterium spp. Brevibacterium spp. Cellulomonas spp. Cellulosimicrobium spp. Collinsella spp. Corvnebacterium spp. (except for potentially toxigenic strains C. diphtheria, C. ulerans and C. pseudotuberculosis) Curtobacterium spp. Dermabacter hominis Dermacoccus spp. Dolosicoccus spp. Dolosigranulum spp. Eggertella spp. Enterococcus spp. (except when requiring identification by wound protocol) Eubacterium spp. Exquiobacterium spp. Facklamia spp. Filifactor spp. Gemella spp. Geobacillus spp. Globicatella spp. Granulicatella spp.

Helcobacillus massiliensis Helcococcus spp. Ignavigranum spp. Janibacter spp. Knoellia spp. Kocuria spp. Kytococcus spp. Lactobacillus spp. Lactococcus spp. Leuconostoc spp. Microbacterium spp. Micrococcus spp. Mogibacterium spp. Nesterenkonia spp. Oerkskovia spp. Paenibacillus spp. Pediococcus spp. Propionibacterium spp. Propionimicrobium spp. Pseudoclavibacter spp. Pseudoramibacter spp. Rothia spp. Staphylococcus spp. (except S. aureus, S. intermedius, S. pseudointermedius, S. hviacus, S. lugdunensis, S. delphini, S. schleiferi, and S. lutrae) Streptococcus spp. (except S. pyogenes, S. agalactiae, S. dysgalactiae, S. equi, S. canis, S. porcinus, S. iniae, and members of the anginosus group streptococci) Turicella otitidis Vagococus 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

J Clin Microbiol 2014;52:139-46.

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 co	cci	Gram stain result
 CULTURE 1) 4+ Streptococcus pyogenes (Group A This organism is intrinsically suscept testing is not routinely performed. P Laboratory if susceptibility testing f penicillin allergy). 2) 1+ Staphylococcus aureus Resistance or sensitivity to oxacillin sensitivity to cefazolin and cloxacill 	Streptococcus) ible to penicillin and susceptibility lease contact the Clinical Microbiology or other agents is required (e.g., is predictive of resistance or in.	Bacteria recovered with comments provided by the lab
Oxacillin Erythromycin Clindamycin Tetracycline Trimethoprim-sulfamethoxazole	(1) (2) S R R S S (1) (2)	Susceptibility information

- 45 y.o. female with wound infection post C-Section
 - The wound is noted to have necrotic tissue and a foul odor

• Culture result:

===== TISSUE FOR ROUTINE CULTURE ======

Site ABDOMINAL ABSCESS TISSUE

GRAM SMEAR : 4+ PMN 4+ Gram positive cocci 3+ Gram negative bacilli

CULTURE

 2+ Staphylococcus epidermidis 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) 2+ Streptococcus anginosus group

3) 2+ Staphylococcus epidermidis Resistance or sensitivity to oxacillin is predictive of resistance or sensitivity to cefazolin and cloxacillin.

	(1)	(2)	(3)
Oxacillin	R		R
Erythromycin	R	R	R
Clindamycin	R	R	R
Vancomycin	S	s	S
Tetracycline	S		R
Trimethoprim-sulfamethoxazole	S		R
Penicillin		S	
Ceftriaxone		S	
Levofloxacin		S	
	(1)	(2)	(3)

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S = Susceptible I = Intermediate R = Resistant
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===== TISSUE FOR ANAEROBES ======

Site ABDOMINAL ABSCESS TISSUE

GRAM	SMEAR	:	4+	PMN		
			4+	Gram	positive	cocci
			3+	Gram	negative	bacilli

CULTURE

1) 2+ Prevotella oralis

- 2) 4+ Finegoldia magna
- 3) 4+ Peptoniphilus harei

4) 1+ Enterococcus faecalis Additional growth of aerobic organism detected.

2+ Facklamia hominis
 Additional growth of aerobic organism detected.

	(1)	(2)	(3)	(4)	(5)
Clindamycin	R				
Metronidazole	s				
Penicillin	s				
	(1)	(2)	(3)	(4)	(5)
S = Susceptible	I = Intermediate	R = Resistant			

The key pathogens here are likely the S. *anginosus* group and anaerobes – the *S. epidermidis* isolates are likely skin contaminants

- 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

===== 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

- 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

3+ Gram positive cocci

CULTURE

This culture has been revised. See details below.

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.

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

			1.4.5	(0)	(0)	
			(1)	(2)	(3)	
Oxaci	llin		S			
Eryth:	romycin		S			
Clind	amycin		S			
Tetra	cycline		S			
Trime	thoprim-sulfametho	xazole	S			
Ampic	illin			s		
Linez	olid			s		
Vanco	mycin			s		
			(1)	(2)	(3)	
	S = Susceptible	I = Inte	rmediate	R	= Resist	ant

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

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Reading the Microbiology Laboratory Report - Questions

- 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