Wound cultures…lab decisions and clinical interpretation

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Disclosures

I live in the sweetest place on earth, otherwise…

None related

Learning Objectives

- Describe best practices in clinical microbiology protocols for wound culture
- Select from a list the most common organisms isolated from wound cultures
- Discuss the implications of result reporting and the clinical and therapeutic management of patients

Overview

- Introduction
- You’re on your bench and ready to go…what would you do?
- Review of bugs
- Review of bug protocols
- Challenges / Ideas
  - Our lab
    - Result reporting
  - Others lab
    - Result reporting
  - Help

Introduction

- 2 colonies of Acinetobacter, what does it mean?

- Implications for the way we report results and the way clinicians use those results…infection control, close the flap, etc.

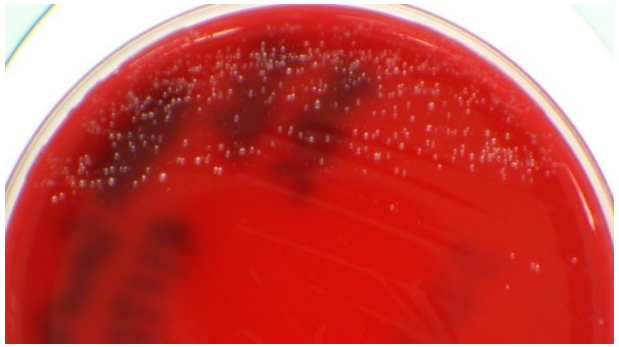
You sit down on your bench and…

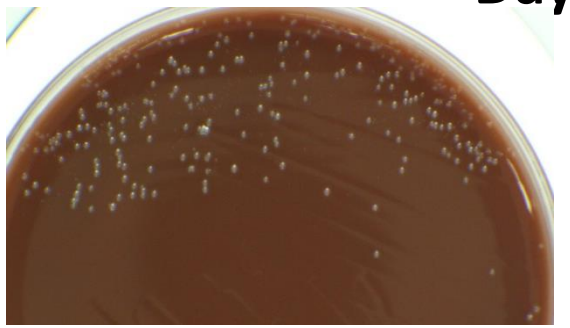
- Wound culture of right forearm
- Direct Gram: 1+ GPCs, 1+ GPRs, few PMNS, 2+ RBCS
- Day 1
  - SBA / CHOC / CNA: 1+ prevalence of an organism, with scant other growth?
  - NG on MAC
- What preliminary report do you enter on Day 1?

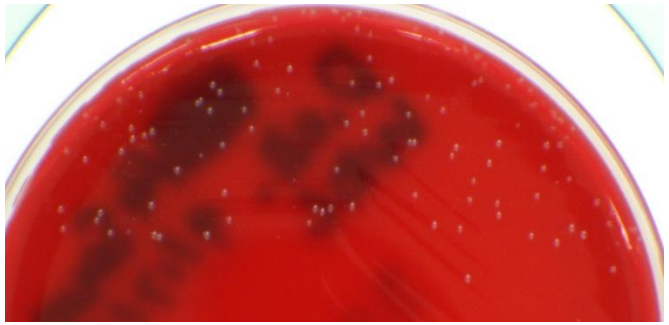
Wound culture of right forearm

Direct Gram: 1+ GPCs, 1+ GPRs, few PMNS, 2+ RBCS

Day 1

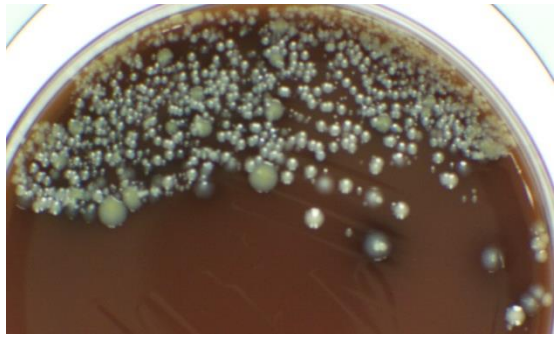


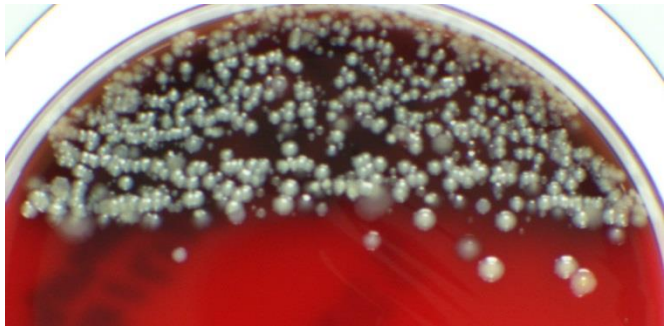




No Growth
MAC

Day 2





No Growth
MAC

What preliminary culture report do you enter on Day 1?

1. No growth to date
2. Growth or mixed growth (“in progress”) with no additional work-up
3. Perform rapid tests and/or MALDI-TOF and report ID only
4. #3. plus set up ASTs
5. Subculture all colony morphologies and move on to the next plates

Review of bugs

Wounds Bench: Path Residents & ID Fellows

- Tissue, not swabs!
- Skin contaminants
- Mixed
- ID and Susceptibility?
- Pathogens
  - *S. aureus*, *S. aureus*
  - Mixed aerobes and anaerobes - polymicrobic
  - Nosocomial organisms
    - MRSA, VRE, ESBL, CRE
    - MDR *Acinetobacter* spp. and *Pseudomonas aeruginosa*

Bites

- Dog bites man
- Cat bites man
- Man bites man

- Cat scratches man
- Rat scratches man
- Dog dies
- All animals die

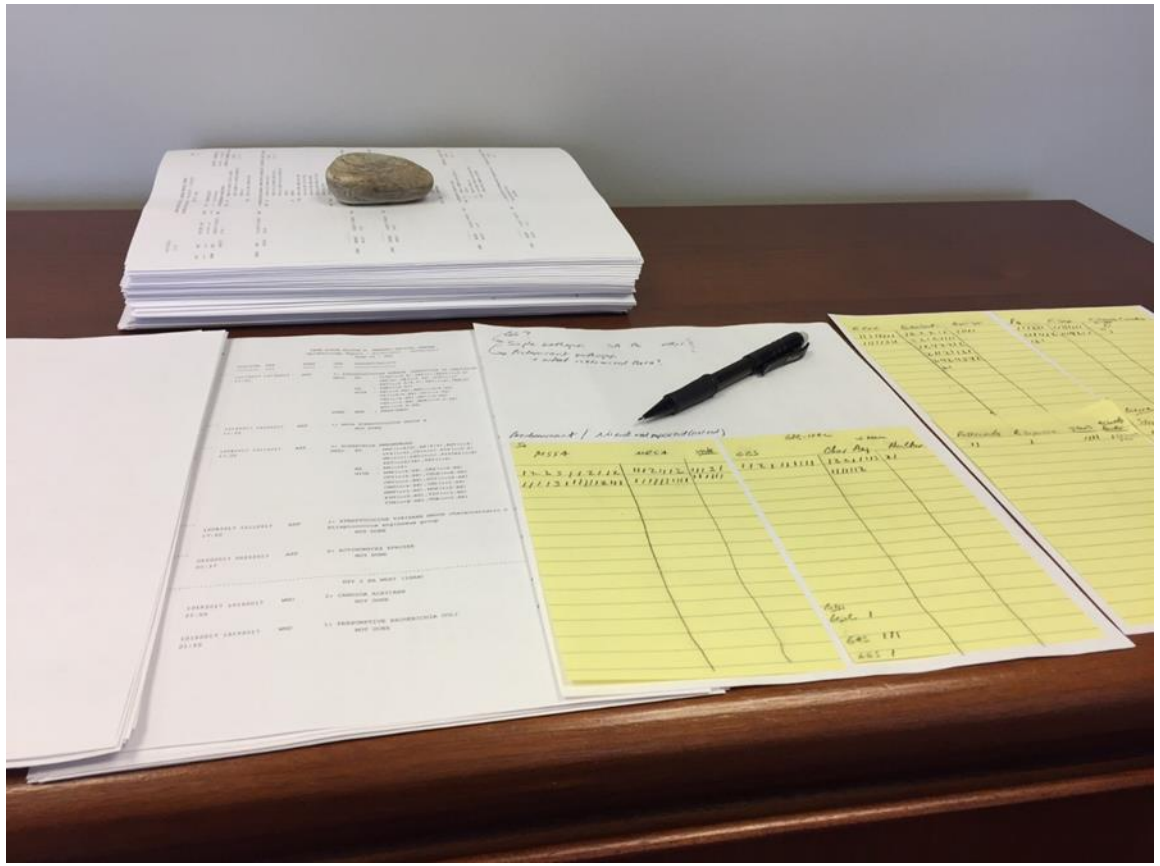
- *Capnocytophaga canimorsus*
- *Pasteurella multocida*
- Mixed anaerobes
- *Staphylococcus/Streptococcus*
- *Bartonella henselae*
- *Streptobacillus monoliformis*
- (Rabies)
- Taxidermists
  - *B. anthracis*
  - *E. rhusiopathiae*
  - *F. tularensis*

Water bugs - trauma

- *Staphylococcus/Streptococcus*
- *Aeromonas hydrophila*
- *Vibrio vulnificus* and other *Vibrio* spp. (salt water)
- *Mycobacterium* spp
  - *marinum*
  - *haemophilum*
  - *ulcerans*

Hershey Medical Center Bugs

- Advanced data analytics

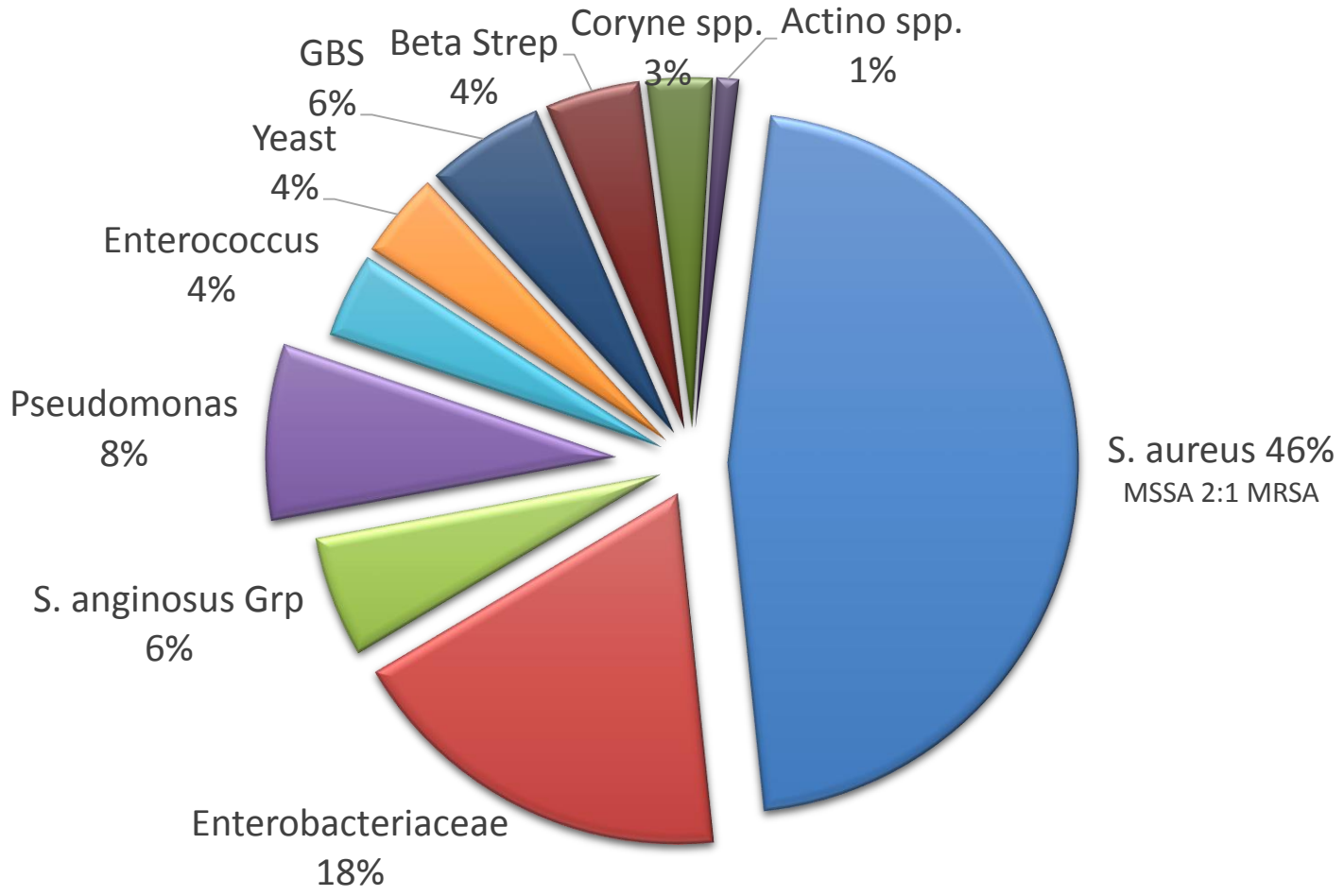


Are common things really that common? What are your top 4 in order of prevalence?

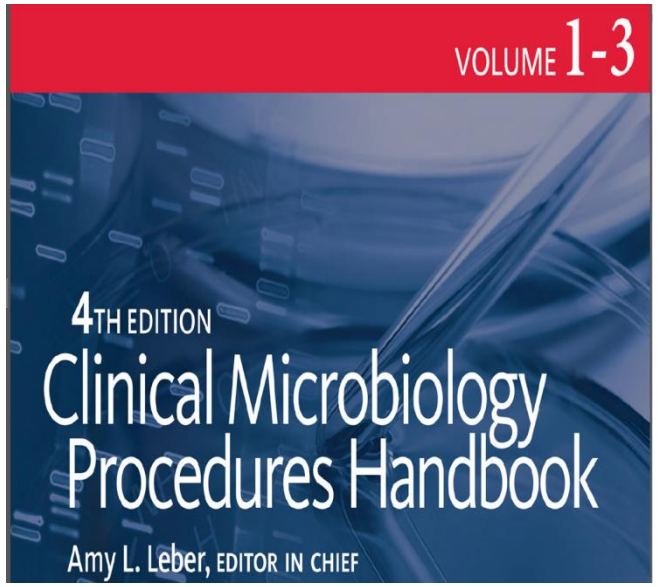
1. *S. aureus*, Enterobacteriaceae, Pseudomonas, GPC- IPAC
2. Enterobacteriaceae, S. aureus, Pseudomonas, GPC- IPAC
3. *S. aureus*, GPC- IPAC, Enterobacteriaceae, Pseudomonas
4. Enterobacteriaceae, Pseudomonas, *S. aureus*, GPC- IPAC
5. How would I know, my Director is never around when I need them (probably at some meeting in West Virginia…)

Wound Culture, first you treat…

Wound Culture



Review of bug protocols



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0095-1137/06/$08.00+0 doi:10.1128/JCM.44.5.1869–1872.2006

Evaluation of the Q Score and Q234 Systems for Cost-Effective and Clinically Relevant Interpretation of Wound Cultures

Carol Matkoski,[1] Susan E. Sharp,[2] and Deanna L. Kiska[1]*

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Received 4 January 2006/Returned for modification 26 January 2006/Accepted 8 March 2006

The Q score and Q234 systems were compared to our current protocol for interpreting wound cultures. The Q score and Q234 systems were more cost effective than our current method, with the Q234 system being considered the most useful protocol for implementation by both the laboratory and our clinicians.

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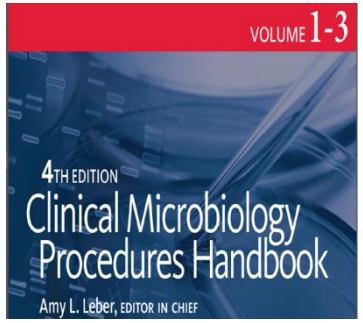
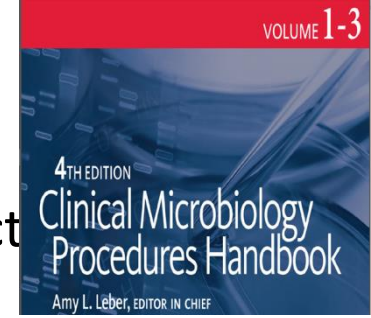
Title:	CG 08 Wound Culture Guidelines
Ou Name:	Microbiology
Code:	CG 08
Version:	1.0
Authors:	Debra Myers
Section:	Microbiology Culture Guidelines
Location Of Copy:	unknown
Authorised By:	Jennifer Vitale
Authorised At Name:	05-Apr-2013
Document Type:	SOP
Date Time Printed:	PREVIEW ONLY - UNCONTROLLED

CG 08 Wound Culture Guidelines, Version: 1.0, CG 08



The microbiologist plays a critical role in the treatment of wound infections because practitioners often consider the report from the laboratory as definitive proof of infection. Providing inappropriate identifications and susceptibility results can prompt unnecessary treatment.

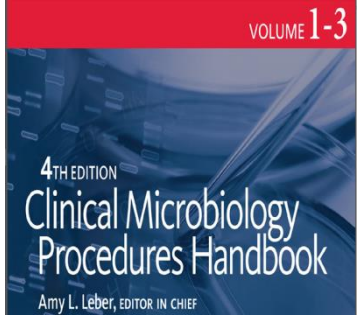


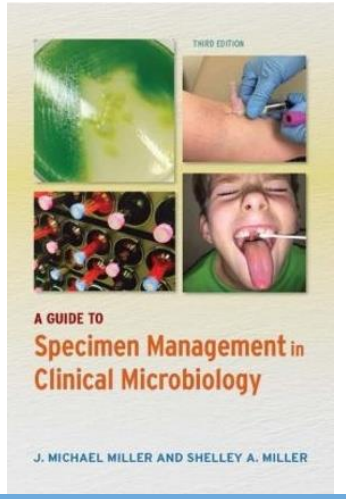
General:

- Superficial wound, abscess/fluid, tissue specimens vs. deep body sites
- Superficial specimens usually grow primary pathogens causing skin and soft tissue infect
- Broader microbial diversity from deep wound and invasively collected specimens.
- Acute wound infections - external damage to intact skin, surgery, trauma, bites.
- Conversely, chronic infections, such as decubiti, complications related to impaired vascular flow or metabolic disease (dm).
- Wound colonization and/or infection is often polymicrobic, with both aerobes and anaerobes.
- The accumulation of inflammatory cells and collection of pus signifies local infection.
- Evidence of this process can be documented by the presence of PMNs in the Gram-stained smear.
- Therefore, quality of wound specimen can be assessed by Gram stain, which can guide culture workup.
- Bacteria found in tissue in significant amounts is associated with delayed healing and correlates to infection.
- When tissue is not readily available, a swab sample may be a convenient substitute for tissue biopsy.

Specimens:

- Swabs - Superficial wounds must often be swabbed to collect a sample for culture because there is not enough pus or fluid to aspirate.
- Abscesses (purulent collections) that are closed off and not yet draining externally should be aspirated.
- Drainages - abdominal, chest tube, and biliary t-tube; drainage tube devices should not be cultured.
- Tissues and biopsy from areas within and adjacent to infection…obtain enough (3- to 4-mm).
- Anaerobes, a separate piece of tissue in a sterile tube containing PRAS media.
- FNA - use a safety device on the needle, do not submit needle to the lab.





Processing:

Figure 3.13.1–2 Illustration of mortar-and-pestle method of homogenization of tissue.

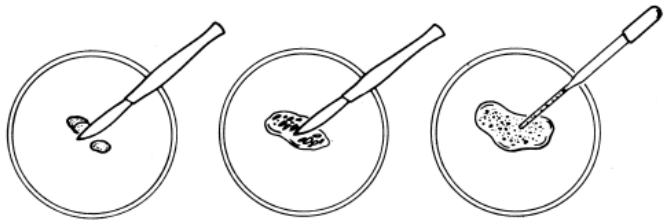


Figure 3.13.1–1 Illustration of sterile-scalpel method of homogenization of tissue.

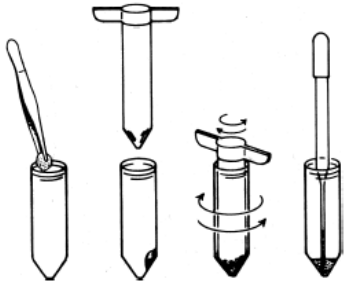


Figure 3.13.1–4 Illustration of tissue-grinding kit method of homogenization of tissue.

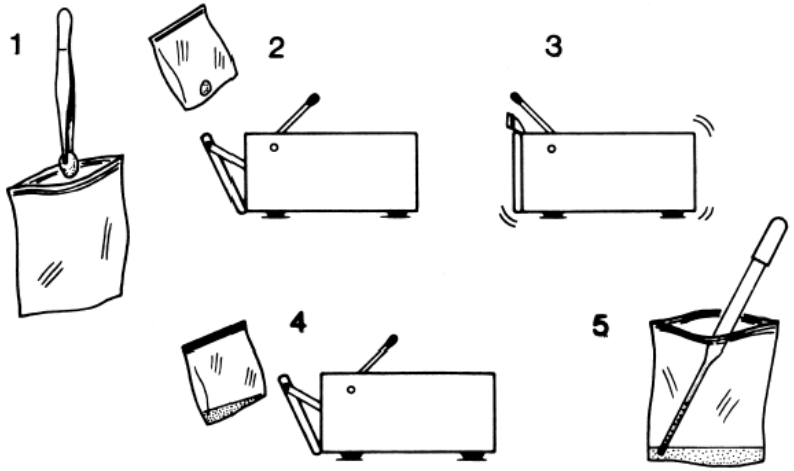
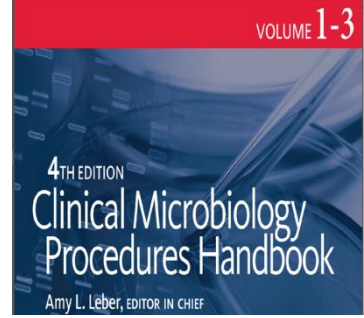
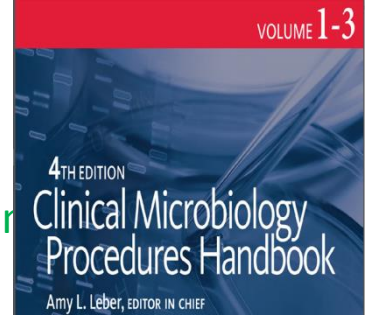


Figure 3.13.1–3 Illustration of stomacher method of homogenization of tissue.



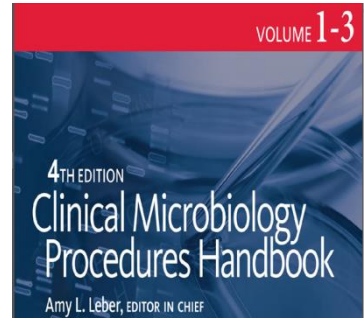
Culture:



- Incubate in humidified incubator at 35 to 37C with 5% CO2
  - minimum of 48 hours and 3 - 4 days for invasively collected specimens with no initial growth.
- Generally identify up to three microorganisms if PMNs on direct smear, or sterile site, or of good quality by sr
- Minimal testing
  - noninvasively collected specimens
  - moderate or numerous epis on smear
  - no PMNs and/or clinical information indicating infection
  - ≥3 organisms growing in the culture.
- Save all culture plates with growth for several days in case further work requested (7 days).
- GPRs
  - sterile site or biopsy
  - Rule out *L monocytogenes*, *E. rhusiopathiae*, *B. cereus*, *B. anthracis*, Arcanobacterium, *C. diphtheriae*, *C. ulcerans*, Nocardia, and Actinomyces.
  - Other GPRs if numerous and with PMNs in smear, or isolated from multiple cultures. Otherwise skin microbiota, including yeast
- Enteric GNRs
  - predominant or moderate to numerous
  - ID/AST if only one or two species are present or predominant and smear suggests infective process.
  - Few in amount or not predominant, or if > 2 species are present with no predominant strain, report as “mixed GI microbiota.”
- ID *P. aeruginosa* and *S. maltophilia*, do AST if pure culture or significant amounts and smear suggests infective process.
- Identify organisms likely to be Aeromonas or Vibrio, examine for pigmented GNRs (*C. violaceum* and *Sphingobacterium*).

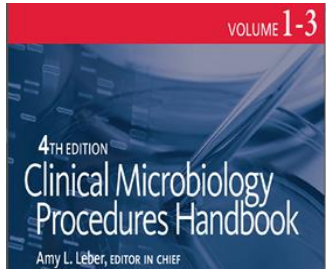
Report:

- Report and quantitate orgs always considered pathogenic, use preliminary identification initially and the genus and species as the final ID.
- Due to their virulence factors, indicate the presence of Beta-hemolytic streptococci, *S. aureus, P. aeruginosa, C. perfringens*, and pigmented anaerobes, *Bacteroides* spp., and mixed anaerobes without further ID.
- Report other pathogens (definitive or minimal), depending on quantitation, number of species present, and smear results.
- For tissues associated with prosthetic material, skin flora can be pathogens…
  - AST should be set up when present in multiple samples
  - there is evidence of an infectious process
  - evidence from sonicated material
- **When multiple morphologies are present, report with minimal identification.**
- Low levels of organisms or fastidious organisms that grow poorly on the direct plates may be missed in culture.
- Many wound infections are polymicrobic, and the isolation of an organism in culture may or may not correlate with infection of the wound.



Quantitative Wound Culture?

3.13.2



Quantitative Cultures of Wound Tissues

PREANALYTICAL CONSIDERATIONS

I. PRINCIPLE

Quantitative culturing is a patient management tool that can be used with a limited variety of specimen types. However, as indicated in the review by Bowler et al. (1), several publications have demonstrated a correlation between quantitative tissue biopsy cultures and the semiquantitative method of enumeration of organism growth (*see* Table 3.3.2–2) in a qualitative swab culture. Tissues from acute wounds, such as those from trauma and burn patients, and duodenal aspirates are the specimen types that may be used for quantitative microbiological analysis. This procedure describes collection and processing of tissue specimens and determination of bacterial counts. The presence of bacteria in tissue in significant amounts is one of a number of factors that have been associated with delayed healing and has also been correlated with infection. When tissue is not readily available, a swab sample may be a convenient substitute for a tissue biopsy sample, and, in a quantitative culture, it may similarly be an indicator of an infectious process. However, semiquantitative swab culture is generally sufficient for patient management (2).

Quantitative cultures for anaerobic bacteria are problematic and thus less meaningful. Anaerobic microorganisms tend to live in microbial synergy with other organisms in the culture and do not grow well when diluted.

Quantitation of bacteria in duodenal aspirates can predict defects in mobility of the intestines. See procedure 3.8.6 for details. For quantitative culture of specimens from bronchoscopy, refer to the respiratory procedure (*see* Appendix 3.11.2–1).

This procedure contains information presented in procedure 3.13.2 by Mary K. York in the third edition of this handbook (3).

The American Journal of Surgery (2010) 200, 489–495

The American Journal of Surgery®

Clinical Science

The majority of US combat casualty soft-tissue wounds are not infected or colonized upon arrival or during treatment at a continental US military medical facility

Forest R. Sheppard, M.D.[a,b,c,*], Paul Keiser, M.D.[d], David W. Craft, Ph.D.[d], Fred Gage, B.S.[a,b], Martin Robson, M.D.[e], Trevor S. Brown, Ph.D.[b], Kyle Petersen, D.O.[f], Stephanie Sincock, Ph.D.[g], Matt Kasper, Ph.D.[g], Jason Hawksworth, M.D.[b,h], Doug Tadaki, Ph.D.[b,c], Thomas A. Davis, Ph.D.[b], Alexander Stojadinovic, M.D.[h], Eric Elster, M.D.[a,b,c]

Q Scores

MIC.21530 Direct Gram Stain Procedures - (Phase I)

- **The laboratory has policies in place to use Gram stain results to provide a preliminary identification of organisms, evaluate specimen quality when appropriate, and to guide work-up of cultures.**

NOTE: The laboratory should have policies for the interpretation of the Gram stain reaction of the organism, morphology of the organism, and the quantification of organisms and cells. ***The policy should address correlation of direct gram stain results with final culture results.***

This does not mean that interpretation of the Gram stain morphology suggesting a specific organism identification (e.g. gram positive diplococci morphologically suggestive of pneumococcus) is required.

Evidence of Compliance:

- ✓ Written procedure for gram stain (laboratories may use the correlation of Gram stain results with the final culture results as a component of QC program).

Work up of Wound Cultures

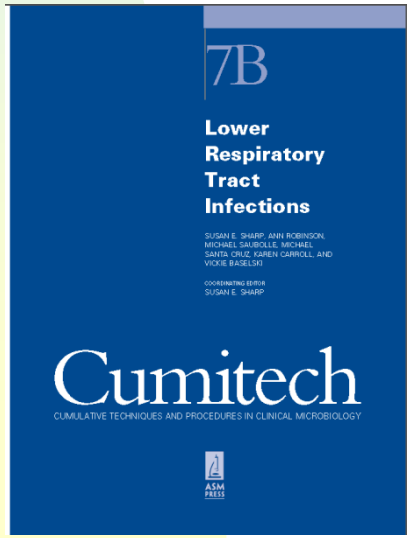
Specimen Quality

- **Premise:**
  - **PMNs are an indication of infection or inflammation**
  - **SECs indicate superficial contamination**
  - **Extensive testing on heavily mixed cultures should not routinely be performed.**

Work up of Wound Cultures:

Two approaches

- Q-Score System
- Q/234 System
- The lower quality of the specimen (e.g., the more SEC present) the fewer the organisms worked up.



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Work up of Respiratory & Wound Cultures:

Q-Score System (RC Bartlett, 1974)

"Q-SCORE" =
# of potential pathogens (PP) to work up

key:
0 = no cells
1 = 1-9/lpf
2 = 10-24/lpf
3 = ≥25/lpf

Neutrophils (+) \ Squamous cells (-)	0	-1	-2	-3
0	3	0	0	0
+1	3	0	0	0
+2	3	1	0	0
+3	3	2	1	0

Q0 = no cult
Q1 = 1PP
Q2 = 2PP
Q3 = 3PP

Hershey Medical Center

Gram Stain shows 3+/4+ PMN's: ID / AST:

- If there are more than 3 potential pathogens present, work-up only those organisms that are 3+ or 4+
- If there are greater than 3 potential pathogens in the 1+ and 2+ range, do not do any work-up.

Gram Stain shows no PMN's, few 1+ or 2+ PMN's. ID / AST:

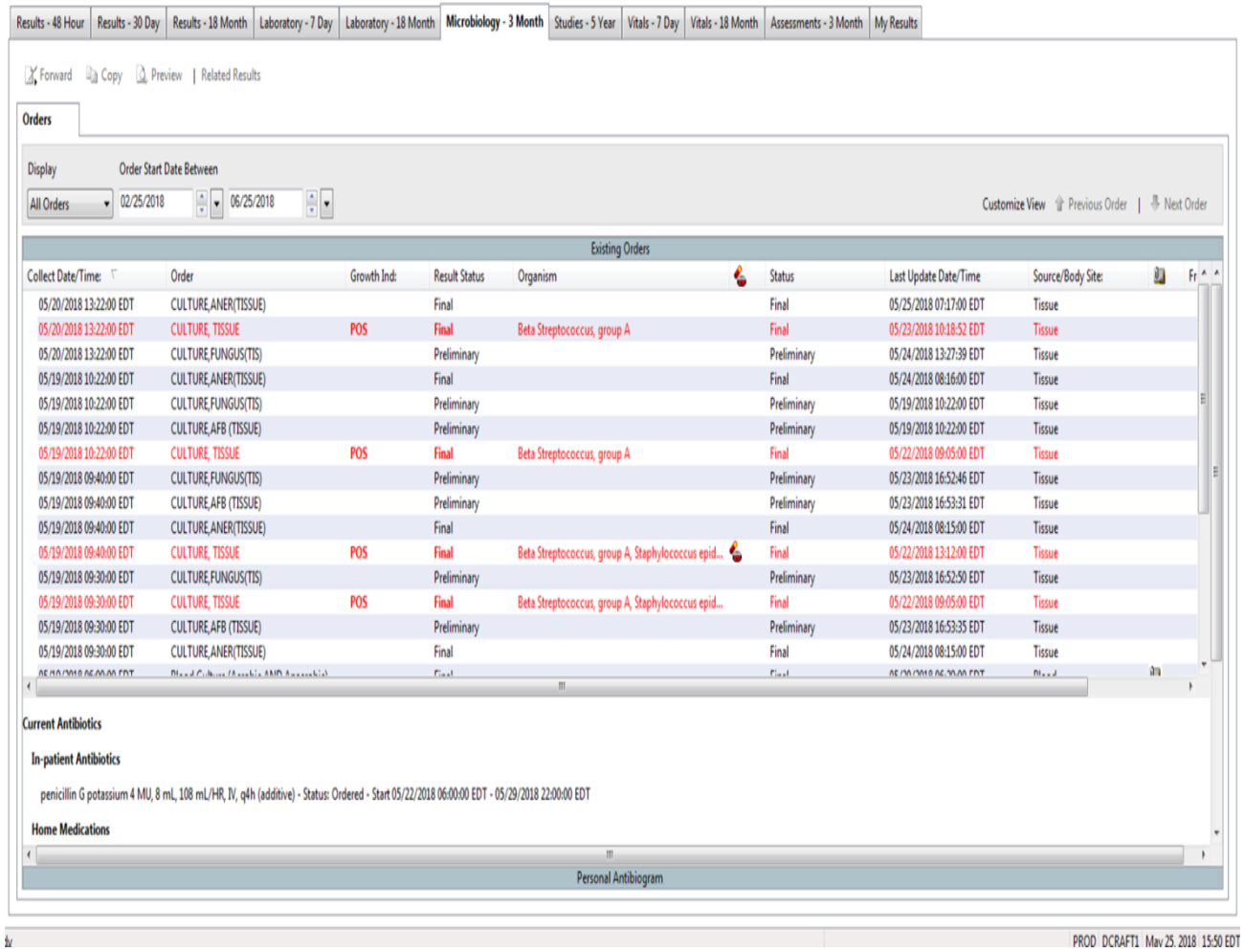
- Suspicious potential pathogens in the 3+ or 4+ (maximum of 3).
- Suspicious potential pathogens in pure culture (except for few amounts).
- Suspicious organism is a recognized pathogen not part of the normal body flora (ex. *P. multocida*, *L. monocytogenes*)

Culture

- Plates are examined at 24 and 48 hours before a negative aerobic report is issued.
- If primary smear is positive and culture is no growth at 48 hours, incubate an additional 24 hours.
- Coagulase negative Staphylococci from orthopedic sources (especially deep hip and knee sites) *may be* worked-up irrespective of amounts.
- Deep *abscesses* from brain, liver, lung, hepatic and sub-hepatic sites are also critical wound specimens and *may be* worked-up irrespective of organism.

Challenges in our lab

- Preliminary reports and implications
- Specimen – aspirate, swab, …
- Mixed flora – define
- “Presumptive”
- Correlate anaerobes to aerobes
- Micro Viewer –
  - Organism ID / AST
  - The red flag of stewardship
  - Mixed flora workup…you gave a name on Day 1
- Do you get add-ons? See next
- Keep plates for further workup…



Add-ons…
Talk given to ID faculty a month ago

- Why am I calling you?
- Why rules?
- What rules?
- Specifics for HMC

Why am I calling you?

- Micro Lab failed to follow protocol
- Micro Lab followed protocol but did not meet the expectations of the clinical staff
  - Patient unique discussion
  - New literature?
  - If NA above, “no”…
  - Educate

Add-ons August 2018

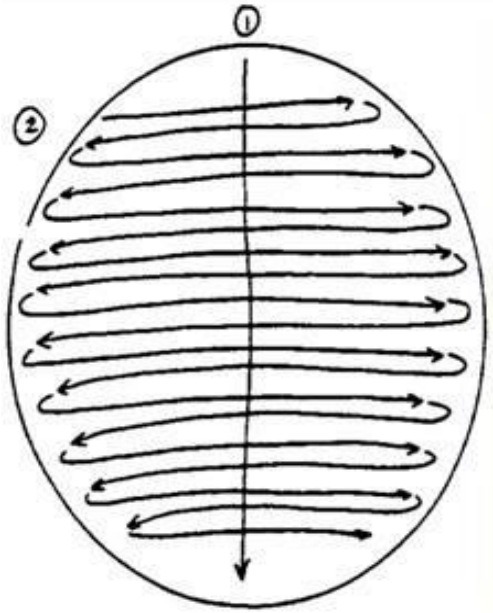
- Total = 36
- Request
  - ID = 1
  - AST = 35 (but may include additional ID work)
    - Viridans Group Strep = 10
    - Yeast = 10
- Requesters
  - ID = 18
  - PA / CRNP = 5
  - Urology = 2

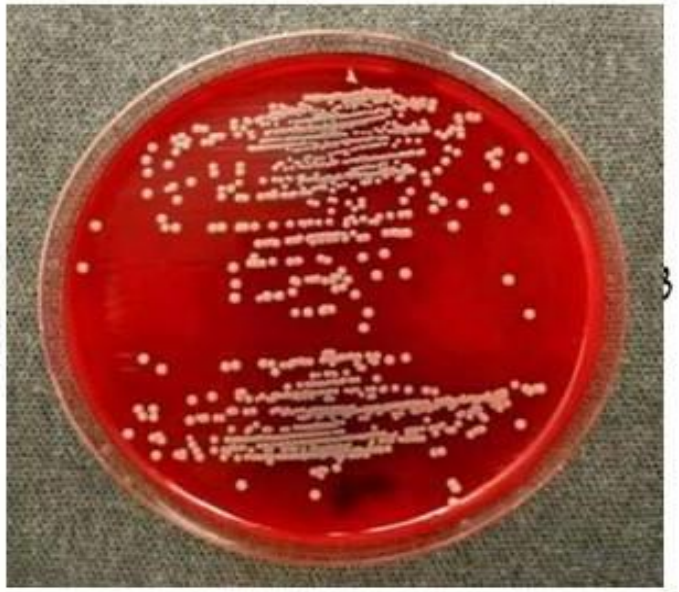
Add-on considerations

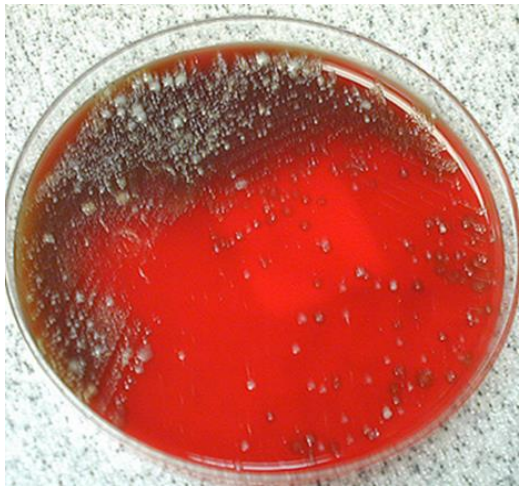
- Compliance
  - Billing
  - Reporting
    - FDA disclaimer (LDT only)
  - Related: Ordering
- Additional ID
  - Mixed flora – urine, others
  - Blood Culture Contamination
  - Growth from broth only
- Additional AST
  - CLSI intrinsic resistance tables
  - CLSI interpretive guidelines

Introduction

- Additional ID
  - Mixed flora – urine, others
  - Blood Culture Contamination
  - Growth from broth only







Discussion w/ ID Fellow…

How long do you keep your positive plates after final report?

1. 1 day
2. 2-5 days
3. 6-10 days
4. Longer
5. Are you kidding me? We don't have time or space to keep plates after final report!

ClinMicroNet: What to do with those GPRs!?

-----Original Message-----
Subject: [clinmicronet] Recovery / Reporting of Actinomyces

We had a recent case of a wound culture (buttocks abscess) that grew 4+ *B. fragilis* and 2+ mixed aerobic and anaerobic Gram positive organisms of 6 varieties. In the evaluation of the mixed anaerobes, *Actinomyces europaeus* was identified by MALDI-TOF (not quite sure why it was IDed, but that is another issue).

FWIW, Gram stain showed GPCs in pairs and GNRs but no GPR. Abscess was acute onset in an otherwise healthy patient, and it was drained in the ED.

A few quick questions:

1) Do you consider Actinomyces sp. as components of mixed organisms rather than reporting it out as a specific organism recovered?

2) Do you routinely rule-out mixed cultures for the presence of Actinomyces?

3) Has MALDI-TOF changed how you report out mixed cultures given the ease of IDing orgs?

To: ClinMicroNet <clinmicronet@mail.asmusa.org>

Subject: RE: [clinmicronet] Recovery / Reporting of Actinomyces

Actinomycosis, to my knowledge, is a specific disease entity with certain clinical features, one of which is chronicity.

We only look for *Actinomyces* spp. when the gram stain shows a predominance of branching GPR.

All other polymicrobial abscesses may contain *Actinomyces* spp. but the significance of their presence is not clear and, as you know, ruling out the presence of an organism in a mixed abscess is a painstaking job at the bench.

If the gram shows no predominant organism we just call it polymicrobial and do not do any further work up.

I would also like to hear what other people do.

ClinMicroNet: What to do with those GPRs!?

To: ClinMicroNet <clinmicronet@mail.asmusa.org>

Subject: RE:[clinmicronet] Recovery / Reporting of Actinomyces

I didn't disclose what we actually did in my post, but I looked at the Gram stain and reviewed the chart. I decided based upon the clinical presentation (acute onset, no apparent sinus tract) and culture/stain (very mixed, no sulfur granules, 4+ of a bona fide pathogen (B.fragilis), that the actino should be considered part of the mixed.

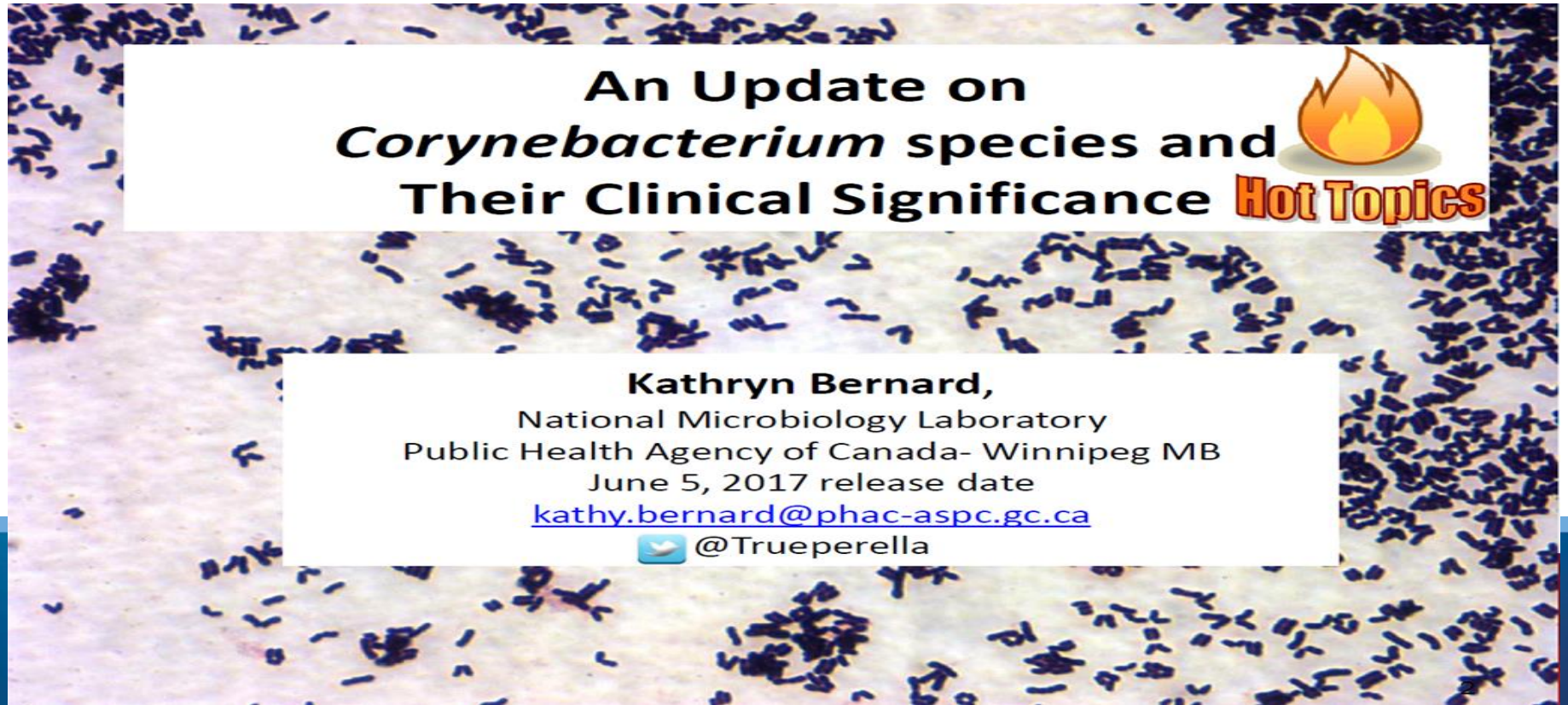
It should not have been worked up according to our protocols, but some love the MALDI so much, they over-ID things "just to find out."

What to do with those GPRs!?

The ASM presents the

Clinical Microbiology Portal's

June 2017 Hot Topic



And mixed aerobes and anaerobes…?

The ASM presents the

Clinical Microbiology Portal's

August 2017 Hot Topic

Best Practices in Diagnosis of Anaerobic Infections

Morgan A. Pence, PhD, D(ABMM)

Director, Clinical and Molecular Microbiology

Cook Children's Medical Center

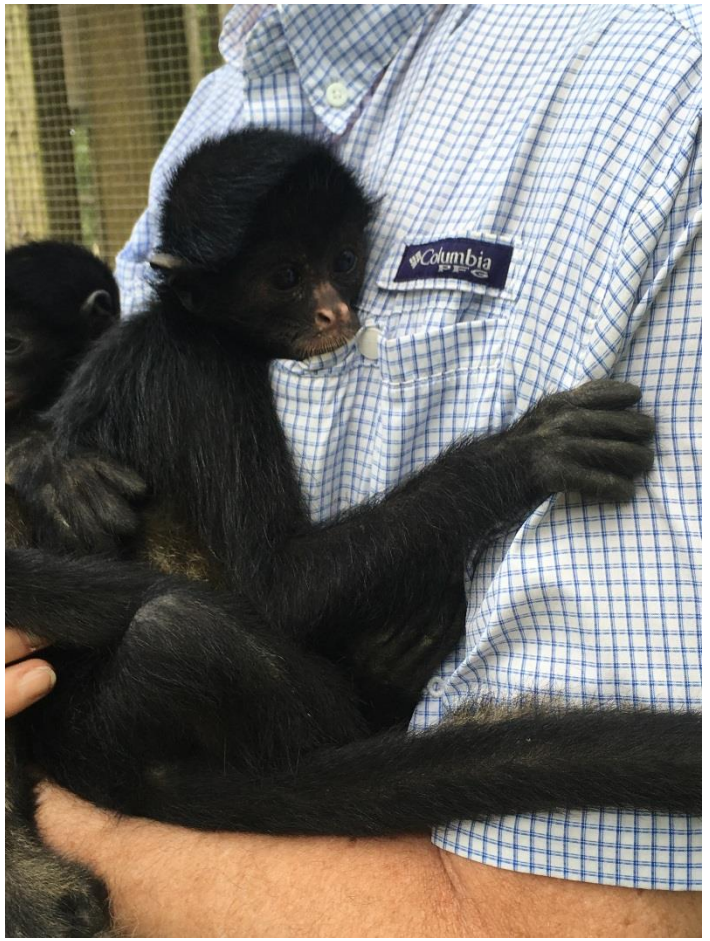
Fort Worth, TX

morgan.pence@cookchildrens.org

Cook Children's Medical Center

Questions?

Isla de la Monos
Peruvian Amazon
River Basin
June 2017



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