The Culture of Your Wound Culture

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Red Bluff, CA
No Conflicts of Interest
Today’s Topics

• Problem
• Hx of culture/techniques
• Colonization vs Infection
• Microbiome
• Sequence testing vs Wound Cultures
• Chronic wounds
• Culture technique
The Problem Scenarios

• My antibiotic dilemma

• “Your culture was negative. There is no infection.”

• “It just grew normal skin flora”

• “You should be really sick or dead based on your culture result”
Bacterial Cultures

• Standard since 1800’s
• Detect roughly 1% of bacteria in chronic wounds
• Select for bacteria that thrive in nutritional and physical parameters set by a lab
• These organisms may not be relevant
• Reported organism - outcompetes others
• Anaerobes cultivation is problematic
• Ignores all other life forms

“I just really hoped you’d have come up with something better by now.”

Van Leeuwenhoek
Posthumous thoughts
The Great Plate Count Anomaly

• Observation that most environmental microorganisms seen in the microscope cannot be grown under laboratory conditions
Your Aerobic Wound Culture

- Gram stain
- Blood Agar
- Chocolate Agar
- CNA (gram +)
- MAC (gram -)
- Thiol
Your Anaerobic Wound Culture

- Gram stain
- Brucella blood agar
- CNA
- Laked Blood agar

*Both cultures – 24, 48, 72 hour reads*
Wound Swabs

• Cotton, calcium alginate, Dacron-Rayon
• Collect < 0.1ml
• Tend to retain collected specimen
• Sterile loop is diluted (+1,+2,+3,+4)
• More testing = less material (aerobic, anaerobic, mycobacterial, and fungal)
• Transport dilemmas
Are Quantitative Bacterial Wound Cultures Useful?

George Kallstrom
Summa Health System, Department of Pathology, Akron, Ohio, USA

Determining if a nonhealing wound is infected can be difficult. The surface of a wound is not sterile and can be colonized with numerous commensal, environmental, and potentially pathogenic microorganisms. Different types of wounds have various clinical presentations, with some signs and symptoms more likely to be present than others depending on the type and location of the wound. Clinicians often order microbiology wound cultures to assist in determining if a nonhealing wound is infected. This minireview briefly summarizes the clinical microbiology of wound cultures, with an emphasis on the history and utility (or lack thereof) of the quantitative wound culture.

Quantitative bacteriology cultures are an important part of the modern clinical microbiology laboratory. Quantitative cultures assist clinicians in determining the threshold above which the bacterial burden of a culture will likely demonstrate clinical significance. Bacterial growth below established thresholds in quantitative cultures typically represents “background noise” of subclinical colonization or inconsequential growth of normal commensal microbiota. The most frequently used quantitative bacterial cultures are urine cultures, where a calibrated inoculation loop is used to inoculate media in order to yield accurate quantitative culture per milliliter of urine. Other less commonly utilized quantitative culturing techniques may be routinely performed depending on the size and scope of the clinical laboratory and can include the use of high-quality liquid specimens such as protected bronchial brushings. Quantitative wound culture techniques were described in large part by research microbiology laboratories in the 1960s and 1970s and were adopted into clinical use thereafter. Quantitative culturing of wounds, particularly biopsy specimens of wounds, involves extensive processing techniques that can be difficult for most clinical microbiology laboratories. Therefore, most nonurine bacterial cultures, including wound cultures, are plated using a semiquantitative technique where cultures are inoculated onto media using a sterile loop that sequentially selects, processing, and inoculation can often confuse the interpretation of quantitative wound culture results.

Some clinicians are reluctant to perform tissue biopsy procedures in order to minimize patient discomfort, while others fear complications such as introducing bacteria deeper into noninfected tissue, so swab specimens are submitted for culture. It has been my observation that it is not uncommon for clinicians to aspirate wounds producing a purulent drainage with a syringe (ideal specimens) and then inoculate the aspirate onto a swab (a less than ideal specimen) for culture submission. Traditional swabs are made from cotton, calcium alginate, and Dacron-Rayon. Swabs tend to collect a small fraction of a milliliter of specimen (<0.1 ml), which greatly reduces the amount of bacteria that can be recovered from the swab for bacterial culture. In addition to limited volume collection, traditional swabs tend to retain the collected specimen. A newer generation of swabs made from a flocking process which allows more-efficient specimen release has emerged over the past decade. However, flocked swabs share most of the collection limitations of traditional swabs as they do not collect adequate specimens for comprehensive clinical microbiology wound cultures. Swab culture yields are reduced as multiple types of cultures (aerobic, anaerobic, mycobacterial, and fungal) are requested from a single swab, thus requiring inoculation of many different types of media.
Colonization vs Infection

• Infection is your diagnosis. Not the lab’s

• Organisms cultured from wounds do not define infection

• Antibiotics can have lasting effects
C. Diff Risk with Antibiotic

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Odds of CDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flouroquinolones</td>
<td>2.8-5.2</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2.8-20.3</td>
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<tr>
<td>3\textsuperscript{rd} Gen Cephal</td>
<td>3.2-4.6</td>
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<td>Penicillins</td>
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<td>Macrolids</td>
<td>1.4</td>
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<td>TMP-SMX</td>
<td>1.78</td>
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<tr>
<td>Proton inhibitors</td>
<td>1.7-2.2</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Distinguishing Colonization from Infection

Colonization:
Microbial Co-habitation on or in host tissue \textit{without} significant disruption to host tissue function

Infection:
Microbial \textit{invasion} of viable host tissue with consequent \textit{injury as a result} of the microbe and microbe-specific host response
Healthy Skin
Chronic Wounds
Infection/Inflammation
Sequenced Based Testing
16S rRNA

- “gold standard” among microbiologists
- >500,000 in public database (NCBI)
- Reportedly > 2 million in private database
- GreenGenes, EZ-Taxon e, Ribosomal Database Project, SILVA
16S rRNA

- 16S rRNA present in prokaryotes
- Encodes part of a ribosome
- Allows for identification and amplification (PCR)
- Slow rate of evolution
1) Extract DNA from wound sample

2) Amplify bacterial DNA using 16S rRNA gene primers

3) Sequence the PCR products

4) Analyze sequencing data:
   a) Identify the microbial taxa by querying the sequenced amplicons against 16S rRNA gene databases
   b) Measure similarity between wound microbiomes by analyzing shared phylogeny
   c) Analyze microbial community membership, structure, and diversity
Nucleotide

The Nucleotide database is a collection of sequences from several sources, including GenBank, RefSeq, TPA and PDB. Genome, gene and transcript sequence data provide the foundation for biomedical research and discovery.

Using Nucleotide

Quick Start Guide
FAQ
Help
GenBank FTP
RefSeq FTP

Nucleotide Tools
Submit to GenBank
LinkOut
E-Utilities
BLAST
Batch Entrez

Other Resources
GenBank Home
RefSeq Home
Gene Home
SRA Home
INSDC
Pros and Cons of DNA/RNA sequencing

Pros
• Eliminates bias of culture techniques
• Not limited to bacteria
• Microbial load
• Microbial diversity
• Identifies “Pathogens”
• Cost is reasonable
• Primer tailored

Cons
• Possible human contamination
• Viable vs non-viable
• ID’d organism may not be clinically relevant
• “Chain of Evidence”
• Primer bias*
Baseline information

Cost of genome sequencing compared with Moore’s law for computers

Cost of computing (Moore’s law)

$ per million DNA bases

Source: Broad Institute
Mycobacteria (acid-fast)

- Good for slow growers
- Rapidly growing mycobacteria (RGM)- 65-KDa heat shock protein and RNA polymerase Beta subunit genes*

*Differentiate between *M. abscessus*, *M. chelonae*, *M. bolletii*, and *M. massiliense*. 
Yeasts & Molds

- Phenotypic testing can be difficult
- Phenotypic variation within species*
- Can take weeks
- 26S ribosomal RNA (rRNA) and Internal Transcribed Spacer 1 and 2 regions (ITS1 & ITS2)
Culture-based and Sequence-based

- Who is there?
- Not what’s going on
Skin & Soft Tissue Infections (SSTI) by Real-Time PCR

- Bacteroides fragilis,
- Enterococcus faecalis,
- Escherichia coli,
- Group A Streptococcus,
- Group B Streptococcus,
- Klebsiella
- Prevotella Groups 1 & 2,
- Proteus mirabilis,
- Pseudomonas aeruginosa,
- Staphylococcus aureus,
- MRSA
Skin & Soft Tissue Infections (SSTI) by Real-Time PCR

- Bacteroides fragilis
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- Pseudomonas aeruginosa
- Staphylococcus aureus,
- MRSA

PRIMER BIAS!
We are...

• 2 -5 lbs of bacteria

• 90% bacteria, 10% human by cell count

• 99% bacteria, 1% human by genes

• Largely ignorant of our microbiome

• 99.6% of human microbiome species cannot be cultured
Square CM of Your Skin

- Hundreds of distinct species
- Estimated 1 million bacteria
- Very site specific
- Quite resilient to change (*forehead licking)
- May affect immunity
- May affect physiology of keratinocytes
Human Microbiome

- 2007 NIH
- 242 **healthy** adults
- Gut
- Genitourinary
- Skin
- Spatial niches
### HMP Reference Genomes

#### Dataset: HMP reference genomes

<table>
<thead>
<tr>
<th>HMP...</th>
<th>GOLD ID</th>
<th>Organism Name</th>
<th>Domain</th>
<th>NCBI Superkingdom</th>
<th>HMP Isolation Body</th>
<th>Project Status</th>
<th>Current Finishing</th>
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<td>Acinetobacter calcoaceticus RUH22...</td>
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<td>G02731</td>
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<td>589</td>
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<td>Microbacterium lactum SK124</td>
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<td>Level 2: High-</td>
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<td>Complete</td>
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</tbody>
</table>

**Current News**
- June 2016: Poster and Booth at ASM 2016
- January 2015: Metagenome Analysis Workshop March 3-6
- September 2014: IHMC 2015 from Mar. 31 to Apr. 2

**Publications**
- GABA-producing *Bifidobacterium* dentium modulates visceral sensitivity...
- Mobile genes in the human microbiome are structured from global to ind...
- Correlation detection strategies in microbial data rate...

Top 4 Skin Phyla

• Actinobacteria
• Firmicutes
• Bacteroidetes
• Proteobacteria
Top 4 Skin Phyla

- **Actinobacteria** – *Propionibacterium*, *Mycobacterium*, *Corynebacterium*, *Nocardia*
- **Firmicutes** – *clostridium*, *staph*, *strep*
- **Bacteroidetes** – *b.fragilis*, *prevotella*
- **Proteobacteria** – *e.coli*, *pseudomonas*
Palm Microbiome

• 51 healthy subjects
• 4742 distinct species
• Average 158 species coexisting on single palm

The Belly Button Biodiversity Project

Generalities

- *Propionibacterium* – sebaceous areas
- *Staphylococcus* – moist areas/intertriginous
- *Corynebacterium* - same as staph
- Antecubidal fossa – highest diversity among subjects
- Partially occluded sites (axilla/inguinal)- more stable


Surprise!

• Gram-negatives found in dry areas (forearm and legs)

• Not always fecal contaminant


• Low-abundance species may be “linchpins” of the skin ecosystem (soil fungal studies)

Baldrain, et al. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J 2012;6:248-58
Phylogenetic Diversity

Tree of Life

Eukaryotes
- Rhizaria
- Plantae
- Alveolata
- Heterokonta

Excavates
- Opisthokonta
- Animalia
- Fungi
- Oomycota
- Haplosporidia
- Chytridiomycota
- Cryptophyta
- Chromalveolata
- Haptophyta
- Dinophyceae
- Dinobryon

Bacteria
- Crenarchaea
- Methanomicrobia
- Methanopyra
- Thermoplasma
- Archaeoglobus
- Bacteria
- CFB Group
- Chloroflexi (16S rRNA group)
- Chloroarchaeota
- Chloroflexi (16S rRNA group)
- α-proteobacteria
- β-proteobacteria
- γ-proteobacteria

Archaea
- Crenarchaeota
- Euryarchaeota

Root
• “Culture Everything!”
• Microbial Load
• Sensitivity Data
• Biofilm analysis
• Reasonable cost
In a First, Test of DNA Finds Root of Illness

Joshua Gribbin, 14, lay in a coma at American Family Children’s Hospital in Madison, Wis., with his father, Mark Gribbin. Joshua suffered from swelling in the brain, low level of a specific gap and a high fever. Nonspecific, 2013

By CARL JOHNSON  JUNE 8, 2014

The doctors told his parents, Clark and Julie, that they wanted to run one more test with an experimental new technology: scientists would search Joshua’s cerebrospinal fluid for pieces of DNA. Some of them might belong to the pathogens causing his encephalitis.

The Coburns agreed, although they were skeptical that the test would succeed where so many others had failed. But in the first procedure of its kind, researchers at the University of California, San Francisco, managed to pin down the cause of Joshua’s problem — within, 48 hours. He had been infected with an obscure species of bacteria. Once identified, it was eradicated within days.

The case, reported on Wednesday in The New England Journal of Medicine, signals an important advance in the science of diagnosis. For years, scientists have been sequencing DNA to identify pathogens. But until now, the process has been too cumbersome to yield useful information about an individual patient in a life-threatening emergency.

“This is an absolutely great story — it’s a tremendous test de force,” said Thomas Insel, director of the National Institutes of Health.

The test, called HybriSeq, is used to detect viral sequences in the cerebrospinal fluid of patients with encephalitis. The researchers looked at 77 patients, 13 of whom had encephalitis. In all but one of the cases, the test identified the correct pathogen. The one failure was a case of West Nile virus, which had spread through the brain and spinal fluid. But the researchers say they are confident they can improve the test to detect West Nile virus.

The researchers are now testing the test in other hospitals and hope to have it available for clinical use by next year. They are also testing it in patients with meningitis and other brain infections.

The test is not yet approved by the Food and Drug Administration, but it is being used in clinical trials. The researchers say they hope to have it approved for use in clinical settings within a year.
Wound culture: Pseudomonas

<table>
<thead>
<tr>
<th>Level 1Q Results</th>
<th>Amount</th>
<th>Level 2 Results</th>
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</thead>
<tbody>
<tr>
<td>Bacterial Load (High)</td>
<td>$&gt; 10^7$</td>
<td>Detected Bacteria:</td>
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<tr>
<td></td>
<td></td>
<td>Fusobacterium ulcerans: 63%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteroides ovatus: 8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Porphyromonas somerae: 5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudomonas aeruginosa: 5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clostridium bolteae: 2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteroides stercoris: 1%</td>
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<tr>
<td></td>
<td></td>
<td>Bacteroides xylanisolvens: 1%</td>
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<tr>
<td></td>
<td></td>
<td>Clostridium ramosum: 1%</td>
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<td></td>
<td></td>
<td>Peptostreptococcus anaerobius: 1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prevotella nanceiensis: 1%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>$3.6 \times 10^6$</td>
<td>Anaerococcus lactolyticus: 1%</td>
</tr>
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</table>
Virtually all bacteria/fungi are screened for and the most predominant populations are reported.

<table>
<thead>
<tr>
<th>Level 1 Swab Results</th>
<th>Amount (N/A)</th>
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<tbody>
<tr>
<td>Total Bacterial Load</td>
<td>Med</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>Low</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>Not Detected</td>
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<tr>
<td>Streptococcus agalactiae</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Vancomycin resistance</td>
<td>Not Detected</td>
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<tr>
<td>Candida albicans</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Methicillin resistance</td>
<td>Not Detected</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Level 2 Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected Bacteria:</td>
</tr>
<tr>
<td>- Staphylococcus epidermidis 42%</td>
</tr>
<tr>
<td>- Enterococcus faecalis 24%</td>
</tr>
<tr>
<td>- Staphylococcus hominis 24%</td>
</tr>
<tr>
<td>- Staphylococcus lugdunensis 4%</td>
</tr>
<tr>
<td>- Corynebacterium tuberculostearicum 4%</td>
</tr>
<tr>
<td>NO FUNGAL SPECIES DETECTED</td>
</tr>
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</table>

Only relative Level 1 Quantitation is obtainable from swab samples.

Antibiotics Report On Attached Sheet

Clinical erythrasma
### Level 1 Swab Results

<table>
<thead>
<tr>
<th></th>
<th>Amount (N/A)</th>
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<td>Total Bacterial Load</td>
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<tr>
<td>Enterococcus faecalis</td>
<td>Not Detected</td>
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<tr>
<td>Klebsiella pneumoniae</td>
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<tr>
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<td>Not Detected</td>
</tr>
<tr>
<td>Methicillin resistance</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

### Level 2 Results

- **Detected Bacteria:**
  - Proteus mirabilis: 78%
  - Staphylococcus aureus: 13%
  - Proteus vulgaris: 2%

**NO FUNGAL SPECIES DETECTED**

Wound culture: “normal skin flora”

Only relative Level 1 Quantitation is obtainable from swab samples.

Antibiotics Report On Attached Sheet
**Finegoldia magna**

- Normal skin flora

---

Methods are used to identify the pathogens' genetic signatures and the estimated percentage of organisms present in the specimen. Virtually all bacteria/fungi are screened for and the most predominant populations are reported.

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<th>Level 1 Swab Results</th>
<th>Amount (N/A)</th>
<th>Level 2 Results</th>
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<td></td>
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<td>Finegoldia magna</td>
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<td>Streptococcus parasanguinis</td>
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<td></td>
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<td>Veillonella atypica</td>
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<tr>
<td></td>
<td></td>
<td>Corynebacterium tuberculostearicum</td>
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<tr>
<td></td>
<td></td>
<td>Actinomyces neui</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus warneri</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peptoniphilus gorbachii</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO FUNGAL SPECIES DETECTED</td>
</tr>
</tbody>
</table>

---

Additional Information.
<table>
<thead>
<tr>
<th>Level 1Q Results</th>
<th>Amount per g</th>
<th>Level 2 Results</th>
<th>Additional Information</th>
</tr>
</thead>
</table>
| Bacterial Load (Medium) | $10^5$-$10^7$ | Detected Bacteria: Brevisbacterium luteolum 37%  
|                   |              | Staphilococcus warneri 11%  
|                   |              | Corynebacterium jeikeium 9%  
|                   |              | Staphilococcus epidermidis 7%  
|                   |              | Kocuria kristinae 7%  
|                   |              | Kocuria rosea 4%  
|                   |              | Staphilococcus lugdunensis 3%  
|                   |              | Brevisbacterium paucivorans 3%  
|                   |              | Corynebacterium tuberculostearicum 2%  
|                   |              | Detected Fungi: Debaryomyces hansenii  
|                   |              | Alternaria malorum  
|                   |              | Stagonosporopsis  
|                   |              | cucurbitacearum  
|                   |              | Candida smithsonii  
|                   |              | Malassezia restricta  
|                   |              | Pyrenochea acicola  |
Top 5 Nail Fungus by Next Gen Sequencing

- Trichophyton Rubrum 38%
- Leptosphaerulina chartarum 17%
- Cladosporium uredinicola 14%
- Epicoccum nigrum 13%
- Malassezia restricta 9%
National Human Genome Research Institute (fungal studies)

- Heel – largest fungal diversity, 80 species
- Nail clippings – 60 species
- Toe web – 40 species

- (Head and trunk hosted between 2-10)

“Normal Skin Flora?”

- Propionobacterium acnes – orthopedic and neurosurgery infections
- Elaborate biofilms in nonunion open fractures
- Very difficult to culture

100 Adults Toe Web Spaces

- *Candida albicans*
- *Rhodotorula rubra*
- *Torulopsis and Trichosporon cutaneum*
- *Microsporum gypseum,*
- *Trichophyton rubrum*
- *Rhizopus stolonifer*
- *Trichosporon cutaneum*
- *Fusarium*
- *Scopulariopsis brevicaulis*
- *Curvularia*
- *Alternaria alternata*
- *Paecilomyces*
- *Aspergillus flavus*
- *Penicillium*

### Microbial Diversity in Venous Ulcers

#### Number of taxa identified by each analytic method

<table>
<thead>
<tr>
<th>Taxon</th>
<th>No. identified by:</th>
<th>Pyrosequencing</th>
<th>Ibis T5000</th>
<th>Culture</th>
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<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Range</td>
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<td>2–5</td>
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<td>15</td>
<td>3–8</td>
<td>5.71 (0.51)</td>
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<td>3–12</td>
<td>7.86 (0.78)</td>
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<tr>
<td>Genus</td>
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<td>43</td>
<td>3–17</td>
<td>9.64 (1.04)</td>
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<tr>
<td>Species</td>
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<td>55</td>
<td>4–15</td>
<td>8.78 (0.87)</td>
</tr>
</tbody>
</table>

Two Faces of Same Microbe

Planktonic
- Acute infections
- Grows easily

Biofilm
- Chronic infections
- Difficult to grow & treat
- Express a radically different phenotype than planktonic
- Only diagnostic tool is molecular
Top 10 Chronic Wound Genera

- **Fusobacterium**
- **Finegoldia**
- **Oligella**
- **Achromobacter**
- **Brevibacterium**

- **Peptoniphilus**
- **Pseudomonas**

- **Raistonia**
- **Morganella**
- **Janthinobacterium**

- **Anaerococcus**
- **Staphylococcus**
- **Corynebacterium**

- **Bacteroides**
- **Peptostreptococcus**
- **Sphingomonas**
- **Unc. Actinomycetales**
- **Stenotrophomonas**

Han et al. 2011 [14]
Price et al. 2011 [16]
Take your Sample

• “Garbage in. Garbage out”
• Surface or deep?
• Are they on antibiotics?
• Immune status?
• How old is the wound?
• What are you looking for?
• Who is taking sample?
• Deep-tissue or punch biopsy
• Needle aspiration
• Swab culture (levine’s vs “Z” technique)
• Sequence based swab/tissue
Wound Swab
(FYI – no standardized technique)

• Avoid a superficial sample
• Collect the culture before topical or systemic antibiotics.
• Viable wound bed – deep sample more useful
• No necrotic debris
• Swab 1cm² (or Z-technique) for 5 seconds hard enough to get exudate
• Room temp – 2 hours


Tissue Biopsy for Culture

- Debride and clean superficial area
- Resect viable tissue with aseptic technique
- Aerobic & Anaerobic orders
Needle Aspiration for Culture

- Disinfect overlying tissue
- Use 18-22 gauge needle to aspirate fluid
- Aerobic & Anaerobic orders
Superficial Swabs

• Carefully swab surface of wound

• Throw swab into garbage can
Sequencing Sample Collection

• Swab the deck!
• Throw everything in!
• Try to give get as much as possible
• Remember: a chronic wound is an ecosystem
• Topical lidocaine will degrade DNA
Summary

• Infection is a clinical diagnosis and not a culture diagnosis
• Most wounds will culture something
• Chose your culture/sequence technique wisely
• Comprehensive sequencing is available
• Today’s dogma is tomorrow’s heresy
• Bergan et al. Chronic venous disease. NEJM 355:488-98
• Ehrlich G. D., Demeo PJ. The problem of culture negative infections. Biofilm Infections. Springer series on biofilm 7. DOI 10.1007/978-3-642-29554-6_1
• Bowler PG, Davies BJ. The microbiology of infected and non infected leg ulcers. Int J of Dermatol 38:573-6