Culture of Orthopaedic Infections

Microbiology Testing in the Diagnosis of Prosthetic Joint Infections

December 9, 2013

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Disclosure

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December 9, 2013

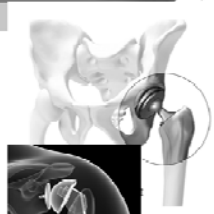
No relevant financial relationships to disclose.

Will mention some products by manufacturer name.

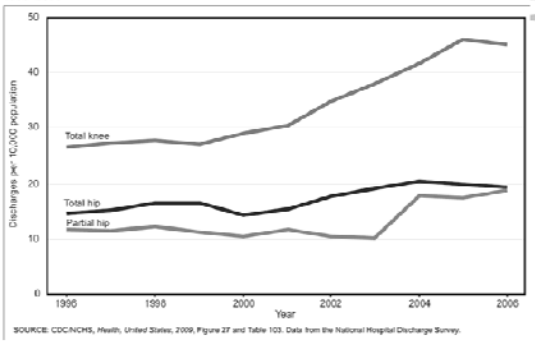
Objectives

- Examine the prevalence of prosthetic joint procedures, prosthetic joint infections (PJI), and bacteria involved.
- Understand the role of *P. acnes* in PJI.
- Review collection and transport devices for joint specimens.
- Describe the strengths and weakness of the Gram stain in PJI.
- Review guidelines around culturing of specimens from prosthetic joints and the strengths and weaknesses of culture.
- Review data on culture conditions needed for the isolation of *P. acnes* from infected prosthetic joints.
- Discuss some of the reasons for "culture negative" results from infected prosthetic joints.

Prosthetic Joint Infections

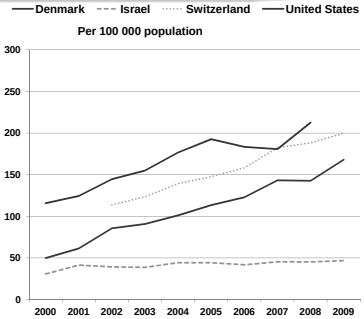


Discharges with at least one knee or hip replacement procedure in nonfederal short-stay hospitals among adults 45 years of age and over, by type of procedure: United States, 1996–2006



SOURCE: CDC/NCHS, *Health, United States, 2009*, Figure 27 and Table 103. Data from the National Hospital Discharge Survey.

Knee Replacement Surgery



OCED Library: Health at a Glance 2011

Number of prosthetic joint infections

US incidence PJI hip/knee, 2001 – 2009, 2.0% to 2.4% and increasing
Kurtz, et. al. 2012. J Arthroplasty. 27(8 Suppl):61-65.

PJI Hospitalizations average 17, 600 - 1997 to 2000
29,200 – 2001 to 2004
Hellmann et. al. 2010. J Arthroplasty. 25(5):766-71.

Discharges for hip (partial and total) total knee 2010: 1,300,000
Health, United States, 2012. DHHS, CDC, NCHS

If 2.4% become infected = 31,500 hip/knee PJI

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Bacteria Associated with PJI

TABLE 100-1 Bacteriology of Prosthetic Joint Infection

Pathogens	*Frequency (%)*
Coagulase-negative staphylococci	22
Staphylococcus aureus	22
α-Hemolytic streptococci	9
β-Hemolytic streptococci groups A, B, G	5
Enterococci	7
Gram-negative aerobic bacilli	25
Obligate anaerobes	10

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Bacteria Isolated Synovial Fluid Cultures*

S. aureus	**57.1%**
Coagulase-negative *Staphylococcus spp.*	**19.0%**
α-hemolytic Streptococcus	**6.4%**
Group B Streptococcus	**4.8%**
Corynebacterium striatum	**4.8%**
E. coli	**3.2%**
Streptococcus gallolyticus	**1.6%**
Pseudomonas aeruginosa	**1.6%**
Serratia marcesens	**1.6%**

Mixed infection (2 organisms) 1 culture

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* 1/1/2005 – 6/11/2006

Propionibacterium acnes and PJI

P. acnes is an anaerobic Gram positive rod that is normally found on the skin and many other body sites.

Relatively recent studies demonstrate that *P. acnes* is a significant cause of delayed PJI (2.8% - 12%).

P. acnes is a slow growing, biofilm producing, low virulence bacteria, with an indolent clinical presentation that frequently lacks the classical clinical presentation of a PJI.

PJI caused by *P. acnes* are frequently associated with the shoulder, but infections of hip and knee can also occur.

Because *P. acnes* is a well known contaminate of bacterial cultures, it can be difficult to determine it's significance when isolated (x cultures).

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Orthopaedic Specimen Transport Containers

2013

Synovial Fluid

- **Hematology**
- Cell Count & Differential
- Crystals

- **Microbiology/Culture**

For quality microbiology/culture, send fluid or tissue.

Tissue Specimens

- **Histology**

- **Microbiology/Culture**

Tissue transport devices (pea sized or smaller)

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10/13/20113OA, RPP

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 microliters of fluid.

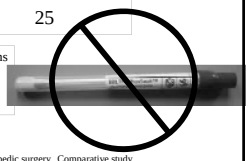
FOR QUALITY RESULTS, SEND TISSUE AND FLUIDS TO MICROBIOLOGY

Waukesha Memorial Hospital 12

Orthopaedic Surgery Specimen Study

Specimen Pairs	T&S	TO	SO	NG
noABX 57	41	8	0	8
ABX 67	27	15	0	25

T&S = same growth in tissue and swab specimens
TO = growth in tissue specimen only
SO = growth in swab specimen only
NG = no growth in either specimen



Ochs, BG., et. al. 2005. Improving microbiological diagnostics in septic orthopedic surgery. Comparative study Of patients receiving systemic antibiotic therapy. Orthopade 34:345-351

Recent PJI Guidelines

AAOS
American Academy of Orthopaedic Surgeons

THE DIAGNOSIS OF PERIPROSTHETIC JOINT INFECTIONS OF THE HIP AND KNEE
GUIDELINE AND EVIDENCE REPORT
Adopted by the American Academy of Orthopaedic Surgeons Board of Directors June 18, 2010

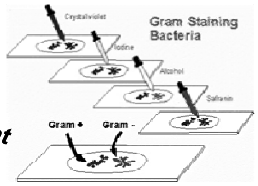
Clinical Infectious Diseases Advance Access published December 6, 2012

IDSA GUIDELINES

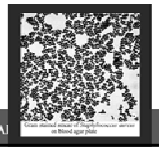
Diagnosis and Management of Prosthetic Joint Infection: Clinical Practice Guidelines by the Infectious Diseases Society of America[a]

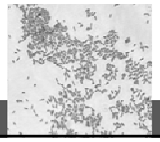
Are Gram Stains Really That Hard To Do?

Are they that hard to interpret?

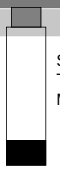


Why do they take so long?





Cytospin Gram Stain



Saline
TSB
MHB

Cytospin sensitivity

TABLE 2. Comparison of HSC and cytocentrifuge smears with culture results

Result	No. (%) isolates
Culture, HSC, and cyto[a] positive	24 (6.9)
Culture and cyto positive, HSC negative	18 (5.1)
Culture and HSC positive, cyto negative	1 (0.2)
Culture positive, HSC and cyto negative	7 (2.0)
Culture negative, HSC and cyto positive	3 (0.8)
Culture and HSC negative, cyto positive	1 (0.2)

[a] cyto, cytocentrifugation.

Chapin-Robertson et. al., 1992. JCM 30:377-380.

Cytospin sensitivity

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Cytospin sensitivity

TABLE 2. Sensitivities of unconcentrated, conventional centrifuge, and Cytospin-concentrated smears of CSF containing known concentrations of bacteria

Species in sample	Bacterial concn (CFU/ml)	Results								
		Unconcentrated			Conventional centrifuge			Cytospin centrifuge		
		Smear results[a]	Bacteria seen		Smear results[a]	Bacteria seen		Smear results[a]	Bacteria seen	
			Range[b]	Mean[c]		Range[b]	Mean[c]		Range[b]	Mean[c]
S. aureus	10^3	0/6			0/3			4/6	8–20	14
	10^4	4/6	4–22	13	3/3	8–20	12	6/6	8–>100	63
	10^5	6/6	23–>100	99	3/3	15–90	62	6/6	>100	>100
E. coli	10^3	0/6			0/3			3/6	8–19	12
	10^4	3/6	2–4	3	1/3	5	5	6/6	36–>100	82
	10^5	6/6	2–20	8	3/3	11–83	37	6/6	20–>100	>100
S. aureus and *E. coli*[d]	10^3	0/6			0/3			4/6	4–11	6
	10^4	0/6			0/3			5/6	3–11	7
	10^4	0/6			3/3	4–7	5	6/6	2–>100	34
	10^4	0/6			1/3	5	5	6/6	4–25	13
	10^5	5/6	10–>100	46	3/3	25–>100	59	3/3	>100	>100
	10^5	6/6	2–20	7	3/3	7–25	14	3/3	>100	>100

[a] Number positive/number of evaluations.
[b] Number of bacteria seen per positive slide during a 2-minute evaluation.
[c] Total number of bacteria seen divided by number of positive evaluations.
[d] Mixture of *S. aureus* and *E. coli*; results are shown as $\frac{S.\ aureus}{E.\ coli}$. Some positive smears showed only one of the two species present.

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Shanholtzer et. al., 1982. JCM 16:1052-1056.

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Cytospin sensitivity

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	10^4	0/6			0/3			5/6	3–11	7
	10^4	0/6			3/3	4–7	5	6/6	2–>100	34
	10^4	0/6			1/3	5	5	6/6	4–25	13
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Shanholtzer et. al., 1982. JCM 16:1052-1056.

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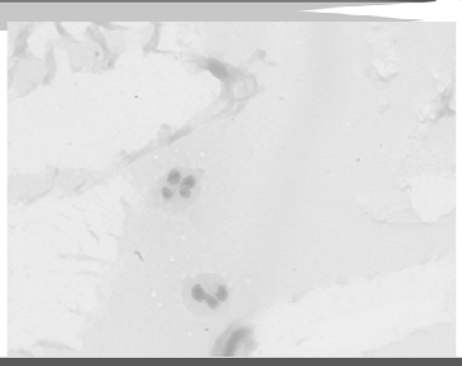
Drop of Synovial Placed on Slide



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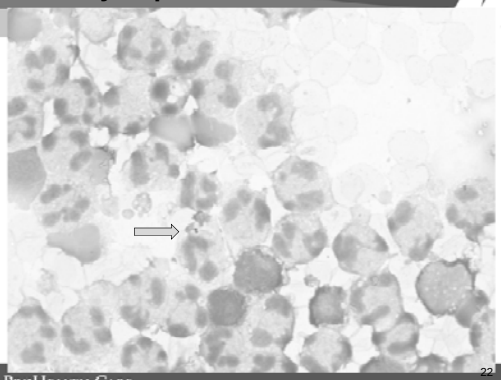
Cytospin Gram Stain



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Gram Stain and PJI

Study	Sensitivity	Specificity
Chimento et. al. 1996	0%	0%
Atkins et. al. 1998	12%	99%
Della Valle et. al. 1999	15%	99%
Spangehl et. al. 1999	19%	98%
Ghanem et. al. 2008	31%	99%
Morgan et. al. 2009	27%	99.9%
Johnson et. al. 2010	10%	100%

Poor Negative Predictive values Associated with the Gram Stain

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Intraoperative Gram Stains and PJI

AAOS 2010 Guidelines – *We recommend against the use of intraoperative Gram stain to rule out periprosthetic joint infection.*

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ClinMicroNet Chatter

False Positive Gram Stains

"We just had an unfortunate series of experiences in which Gram positive cocci were falsely reported to be present in specimens submitted to Microbiology from Orthopedic Surgery". (TSB)

"….we reported 7 positive (probably false positive) Gram stains on allograft tissue being used for knee repair". (TSB)

"Recently, after several cases of having reported Gram positive cocci in the direct Gram stain and no growth on the cultures, we tracked down the source to dead organisms in the 'sterile' saline." (Saline)

"We have detected another lot of highly contaminated, yet sterile media from XX. Out of 10 broths that we did Gram stains on, 9 had Gram positive cocci." (Saline)

"….dead organisms came from glue on the swabs they were using (resulted in false positive Gram stains), the company freely admitted that they can't keep them (dead bacteria) out of the product." (specimen collection swabs)

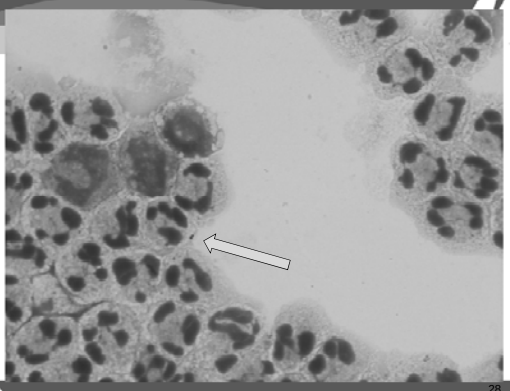
THIS IS A SERIOUS PROBLEM!

Sources of Gram Stain Contamination

- Elution/Dilution fluids – Saline, TSB, MHB
- Gram Stain Reagents
- Rinse Water
- Slides
- Swabs
- Transport Media
- Cytocentrifuge Funnels
- Tissue Grinder
- Specimen Digestion Reagents
- "Blue Blobs"

Sources of Gram Stain Contamination

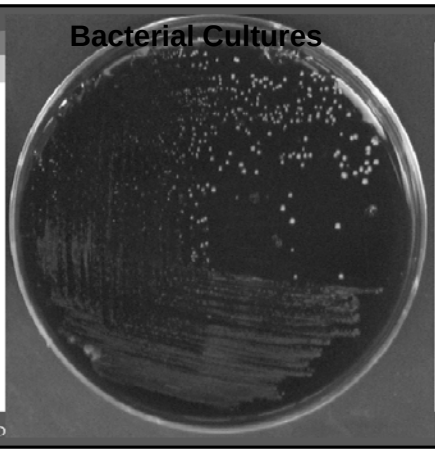
Major manufacturer 1 ml tube a sterile saline



Gram Stain Contamination

1 ml 0.85% saline, filter sterilized

Bacterial Cultures



Joint Fluid Bacterial Cultures

Joint (synovial) Cytospin Gram stain, 0.5 – 3.0 ml inoculate a Peds Plus blood culture bottle; if < 0.5 ml inoculate onto Blood agar, Chocolate agar, incubate Peds bottle for 7 days, plates in 35º C, 5% CO_2 for 7 days

ASM manual - BAP, CAP, plate inc. time not clear, 35º, 5% CO_2, use BC bottles for large vol., broth ≥ 5 days up to 14 days to cover *P. acnes*

CMPH - BAP, CAP, inc. plates 4 - 7 days, 35º, 5% CO_2, Use BC bottles for lg. vol. incubate for 5-7 days, up to 14 to cover *P. acnes*

Joint Tissue Bacterial Cultures

Joint tissue Gram stain, blood agar, chocolate agar, MacConkey Agar, anaerobic CDC-Blood agar, anaerobic CDC-PEA agar, anaerobic CDC-LKV agar, anaerobic Thioglycolate broth, CNA agar, Incubate for 7 days, aerobic plates in 5% CO_2, anaerobic plates in jars

ASM manual - BAP, CAP, Mac, CNA, Thio, BBA, LKV, BBE, plate inc. time not clear, broth ≥ 5 days up to 14 days to cover *P. acnes*

CMPH - BAP, CAP, 35 5% CO_2, BBA, LKV, BBE inc. plates 4 days, broth anaerobic BHI/TSB with 0.1% agar/Thio (7 days), incubate for days, up to 14 to cover *P. acnes*

Why Multiple intraoperative cultures?

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1998, p. 2930–2939 Vol. 36, No. 10
0095-1137/98/$04.00+0

Prospective Evaluation of Criteria for Microbiological Diagnosis of Prosthetic-Joint Infection at Revision Arthroplasty

BRIDGET L. ATKINS,[1,2] NICHOLAS ATHANASOU,[3,4] JONATHAN J. DEEKS,[5] DERRICK W. M. CROOK,[2] HAMISH SIMPSON,[3,6] TIMOTHY E. A. PETO,[2] PETER McLARDY-SMITH,[4] ANTHONY R. BERENDT,[4,6] AND THE OSIRIS COLLABORATIVE STUDY GROUP†

Public Health Laboratory,[1] Department of Pathology,[2] Nuffield Department of Orthopaedic Surgery,[3] and Bone Infection Unit,[4] Nuffield Orthopaedic Centre, Academic Unit of Microbiology and Infectious Diseases, John Radcliffe Hospital,[5] and Centre for Statistics in Medicine, Institute of Health Sciences,[6] Oxford, United Kingdom

"We recommend five or six specimens be sent, ….."

Multiple positive specimens with an indistinguishable organism for a definite diagnosis.

Why Multiple intraoperative cultures?

	1/2 vs 5/6
Changed Micro. Diagnosis	**34%**
Changed Antibiotic Therapy	**30%**
Negative Predictive value 5/6	**95%**

A. DeHann et. al., 2013. J. Arthroplasty, 28:59-65

Why Multiple intraoperative cultures?

IDSA Guidelines 2012 – *At least 3 and optimally 5 or 6 periprosthetic intraoperative tissue samples or the explanted prosthesis itself should be submitted for aerobic and anaerobic culture at the time of surgical debridement or prosthesis removal.*

AAOS Guidelines 2010 – *Multiple cultures should be obtained at the time of reoperation in patients being assessed for PJI.*

ASM Manual 2011 - *Collect up to 5 separate pieces of tissue from surgical site.*

Definition of a PJI

IDSA 2012 – Two or more intraoperative cultures/aspirations that yield the same organism may be considered definitive evidence of PJI.

CMPH 2010 - One or two colonies on a single plate, with multiple plates, and not growing on broth generally represent contamination when the bacteria are ones not typically associated with joint infections. Growth of one or two colonies on agar media in area outside the specimen inoculation area also likely represent contamination.

Bacterial contaminates are not typically detected in original Gram stain.

Bacterial Culture of Joint Hardware

Table 2. Comparative studies of sonication and yield of positive cultures in patients with PJIs

	No. of patients	+ Sonication (prosthesis)		− Sonication (tissue)	
		Sensitivity	Specificity	Sensitivity	Specificity
Piper *et al.* (2009) 95 % CI	134	67 % (22/33) (48; 82)	98 % (99/101) (93; 100)	55 % (18/33) (36; 72)	95 % (96/101) (89; 98)
Esteban *et al.* (2008) 95 % CI	31	94 % (16/17) (71; 100)	50 % (7/14) (23; 77)	88 % (15/17) (64; 99)	100 % (8/8) (63; 100)
Trampuz *et al.* (2007) 95 % CI	331	78 % (62/79) (68; 87)	99 % (249/252) (97; 100)	61 % (48/79) (49; 72)	99 % (250/252) (97; 100)
Trampuz *et al.* (2006) 95 % CI	78	75 % (18/24) (53; 90)	87 %* (47/54) (75; 95)	54 % (13/24) (33; 74)	98 %* (53/54) (90; 100)

*Excluding the patients who received antibiotics, the specificity was 100 % for all types of samples.

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L. Larsen et. al., 2012. J. Med. Microbiol. 61:309-316

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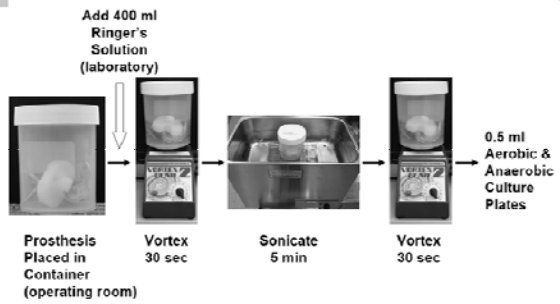
Bacterial Culture of Joint Hardware



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A. Trampuz et. al., 2007. N. Engl. J. Med. 357:654-663

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Bacterial Culture of Joint Hardware

Prosthetic Joint Infection Diagnosis **January 4, 2010**
Type: Hot Topic Video
Presenter/Author: Robin Patel, MD

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How Sensitive is Culture?

TABLE 4 Comparison of microbiologic tools for diagnosis of PJI

Test	No. of patients with positive specimens and: Aseptic failure (n = 290)	PJI (n = 161)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Synovial fluid culture*	5/161	59/89	66.3 (55.5–76.0)	96.9 (92.9–99.0)	92.2 (82.7–97.4)	83.9 (77.8–88.8)
Three culture						
≥2 positive tissues (same organism)	6	101	70.1 (62.0–77.5)	97.9 (95.6–99.2)	94.4 (88.2–97.9)	86.9 (82.7–90.3)
Sonicate fluid culture	5	105	72.9 (64.9–80.0)	98.3 (96.0–99.4)	95.5 (89.7–98.5)	88.0 (83.9–91.3)
Sonicate fluid PCR (10-assay panel)						
Any positive result	6	111	77.1 (69.3–83.7)	97.9 (95.6–99.2)	94.9 (89.2–98.1)	89.6 (85.7–92.7)

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C. Cazanave et. al., 2013. J. Clin. Microbiol. 51:2280-2287

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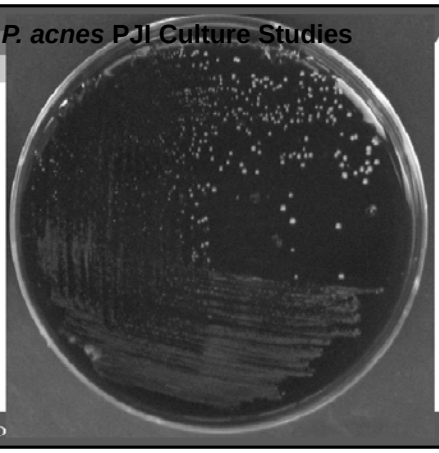
P. acnes PJI Culture Studies



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P. acnes PJI Culture Studies

Study	Schäfer, et. al	Wu, et. al.	Shannon, et. al.
P. acnes Cases	6	17	14
Media	BAP, CAP Mac, BHI broth Schaed. Agar Schaed. Broth	BAP, CAP BHI broth Bruc. Agar	BAP, ana. Thio, CDC ANA plate
Incubation	14 days	28 days	14 days
P. acnes grow by Day 7	most not	80%	100%

P. Schäfer et. al., 2008. Clin. Infect. Dis. 47:1403-1409

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S. Butler-Wu et. al., 2011. J. Clin. Microbiol. 49:2490-2495
S. Shannon et. al., 2013. J. Clin. Microbiol. 51:731-732

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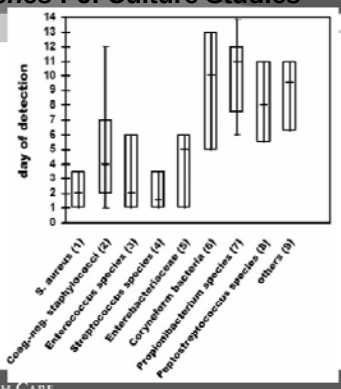
P. acnes PJI Culture Studies



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P. acnes PJI Culture Studies

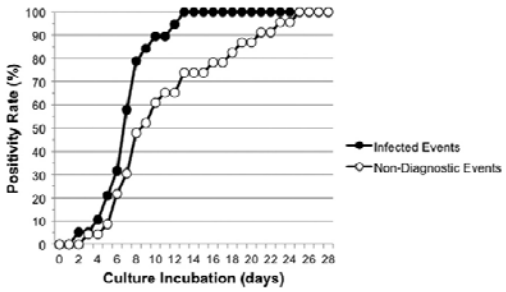
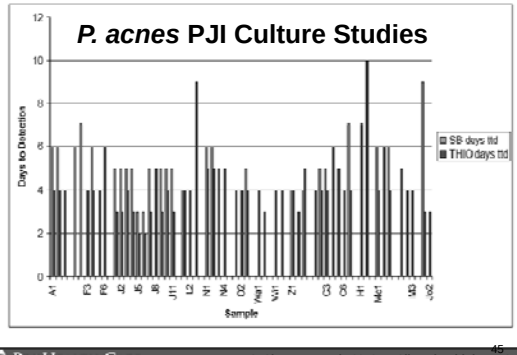


FIG. 1. Time to culture positivity for *P. acnes*-positive patient

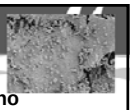
S. Butler-Wu et. al., 2011. J. Clin. Microbiol. 49:2490-2495

Plate vs. Broth Days to Detection



S. Shannon et. al., 2013. J. Clin. Microbiol. 51:731-732

Culture Negative PJI



Antibiotic therapy within 14 days of surgery – no antibiotics 23% false negative cultures, antibiotics 55% false negative cultures

Bacteria are in a biofilm and not free in sampled tissue or fluid

Inability to culture fastidious/unusual bacteria that do not grow on routine media under standard incubation conditions

Transport conditions do not maintain the viability of bacteria