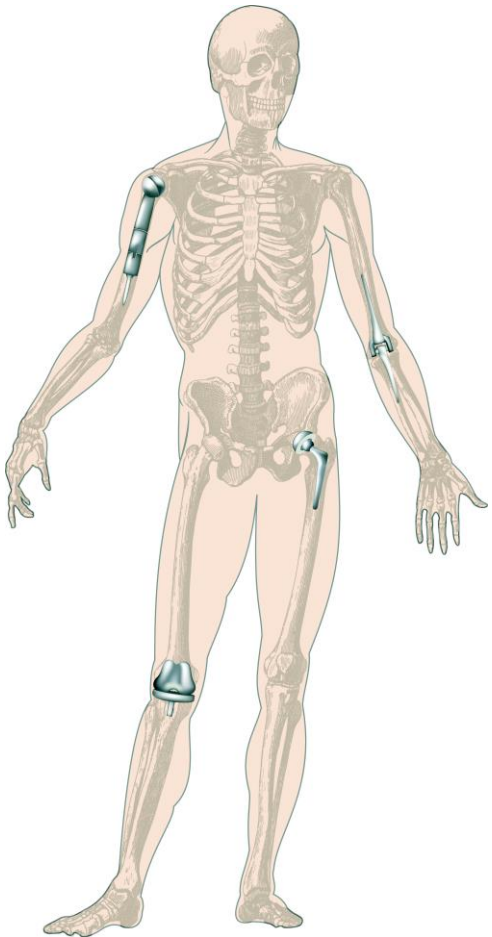
New Approaches to the Diagnosis of Prosthetic Joint Infection



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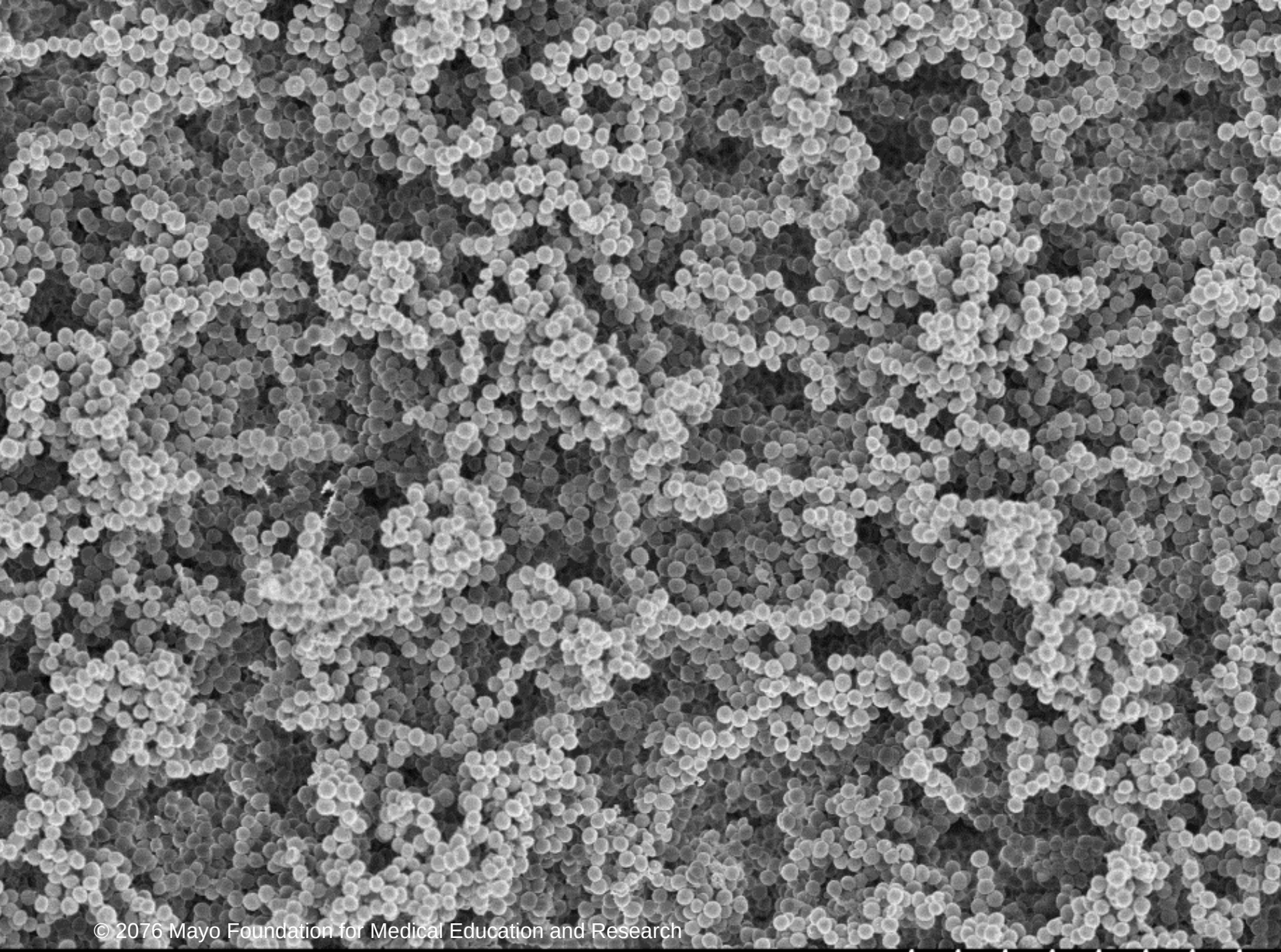
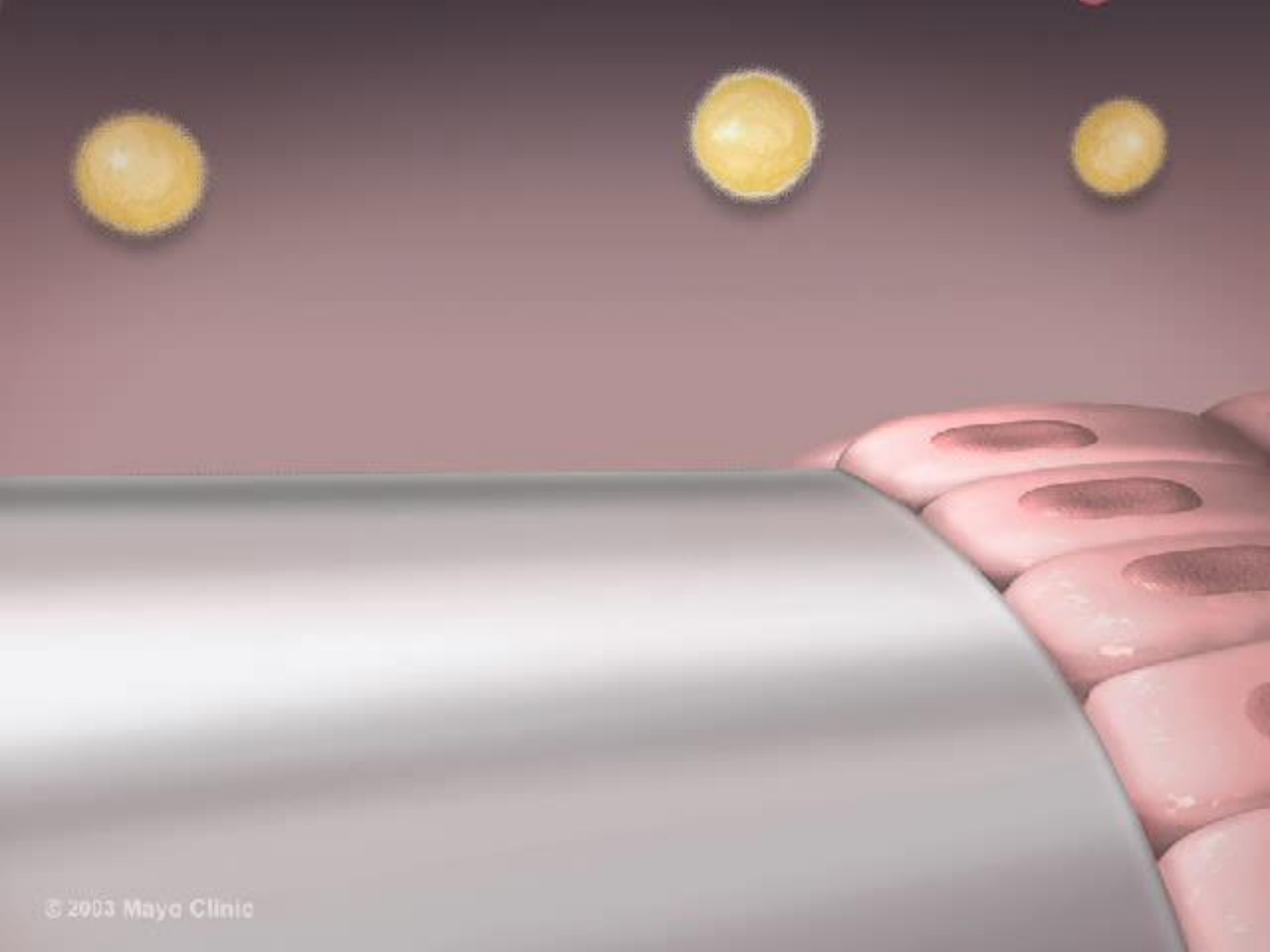
Disclosures

- Employee: Mayo Clinic
- Grants: CD Diagnostics, BioFire, Check-Points, Curetis, 3M, Merck, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, Allergan, The Medicines Company, Zimmer, Specific Technologies, EnBiotix
- Consultant: Curetis and Qvella (paid to Mayo Clinic)
- Patents: *Bordetella pertussis/parapertussis* PCR issued, device/method for sonication with royalties (paid by Samsung to Mayo Clinic), anti-biofilm substance issued
- Data safety monitoring board: Actelion
- Travel reimbursement/editor's stipends: ASM & IDSA
- Honoraria: NBME, Up-to-Date, Infectious Diseases Board Review Course

Objectives

1. Learn the epidemiology, pathogenesis, and microbiology of prosthetic joint infection
2. Interpret synovial fluid biomarkers in patients with a prosthetic joint
3. Determine appropriate specimen types for microbial diagnostics for prosthetic joint infection

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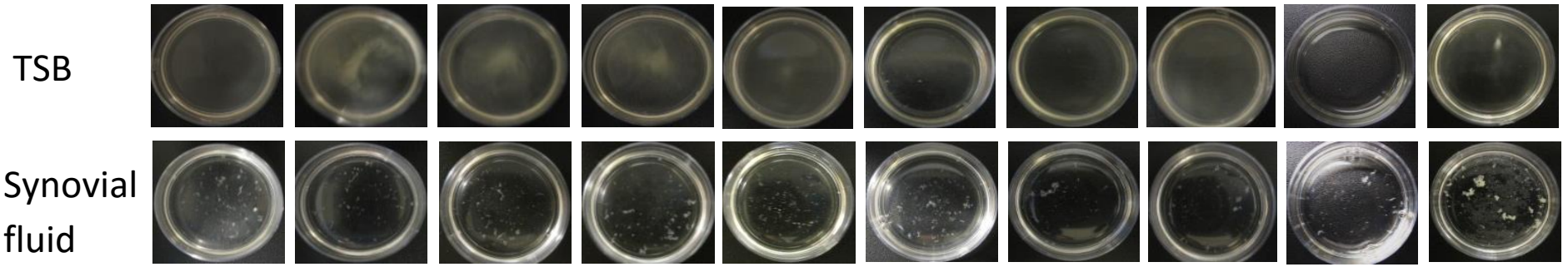
Biofilm-Like Aggregation

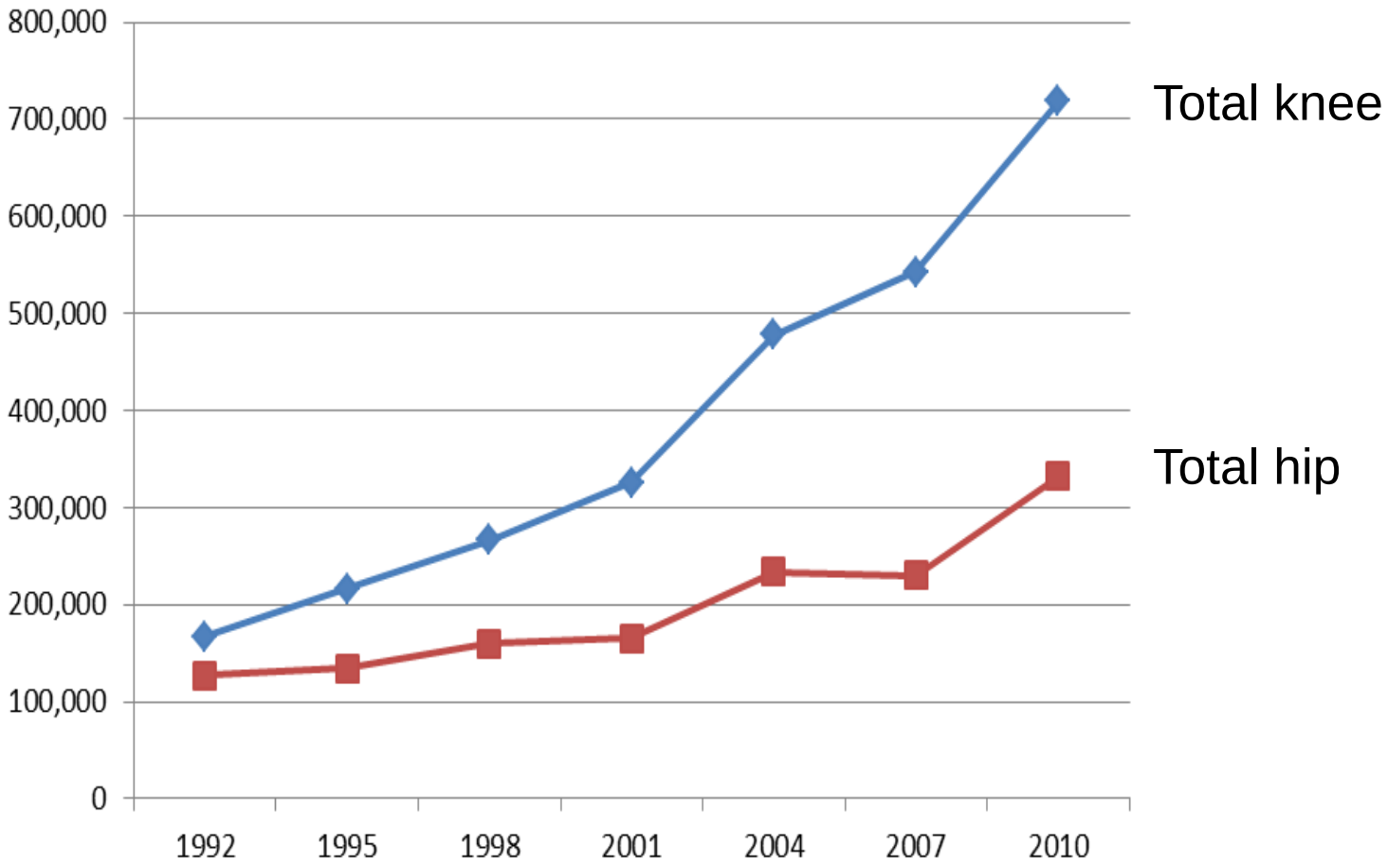
Staphylococcus epidermidis, *Staphylococcus aureus* in Synovial Fluid



Perez and Patel. J Inf Dis 2015;212:335

Total Hip & Knee Replacement Procedures United States

National Hospital Discharge Survey http://www.cdc.gov/nchs/about/major/hdasd/listpubs.htm



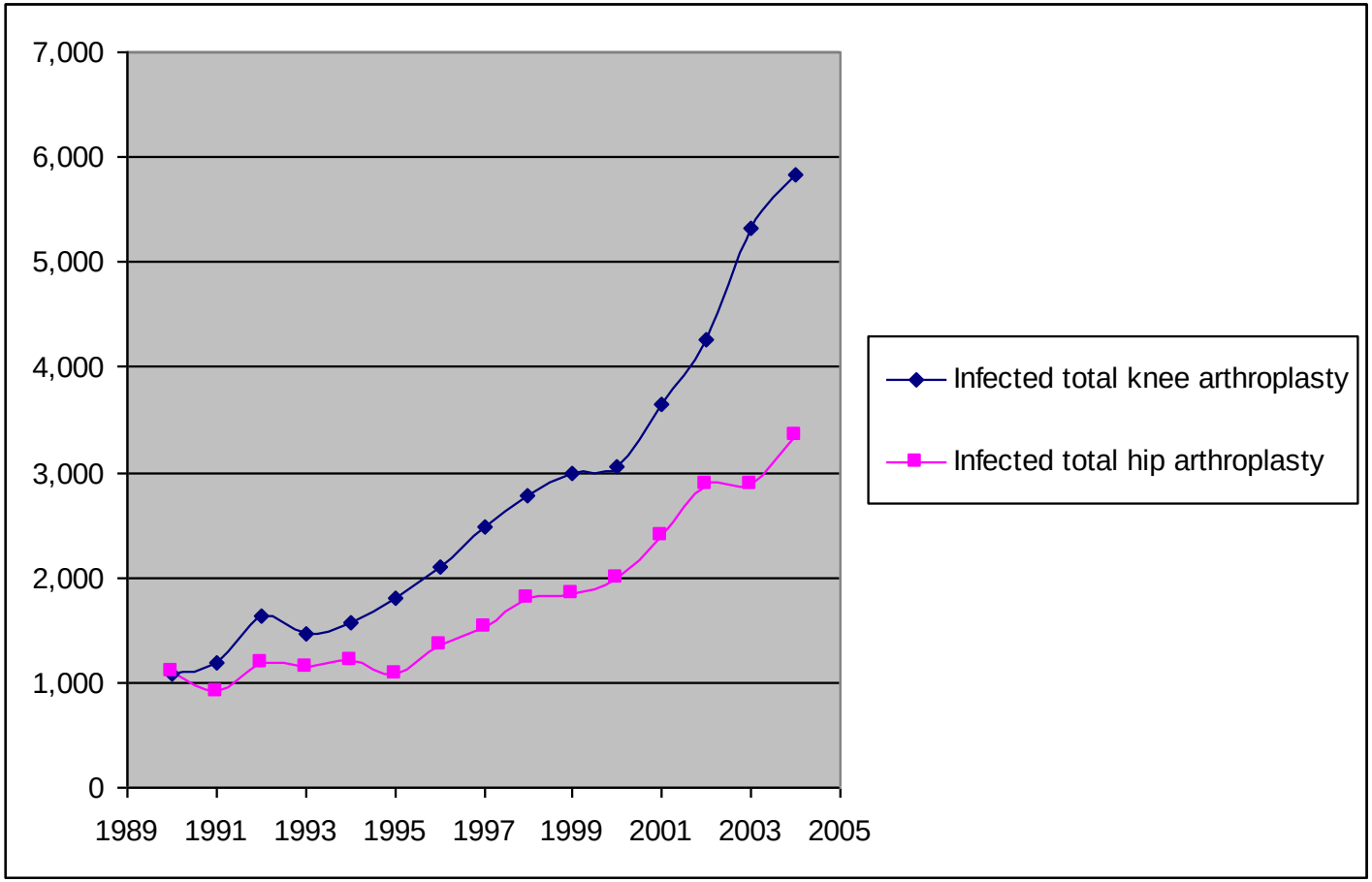
Prosthetic Joint Infection Rates

	Years*	
	0-2	2-10
Prosthetic knee infection	1.55%	0.46%
Prosthetic hip infection	0.78%	0.33%

*Medicare Population
Primary Elective Arthroplasty
1997-2006

Kurtz et al. Clin Orthop Relat Res 2010:468:52
Ong et al. J Arthroplasty 2009;24:105

Prosthetic Hip and Knee Infections United States



Kurtz et al. J Arthroplasty 2008;23:984

Prosthetic Joint Infection Microbiology

	Hip and Knee		Hip	Knee	Shoulder	Elbow
	All time periods	Early				
Number of joints	2435	637	1979	1427	199	110
Staphylococcus aureus	27	38	13	23	18	42
Coagulase negative staphylococci	27	22	30	23	41	41
***Streptococcus* species**	8	4	6	6	4	4
***Enterococcus* species**	3	10	2	2	3	0
Aerobic gram negative bacilli	9	24	7	5	10	7
Anaerobic bacteria	4	3	9	5		
Propionibacterium acnes					24	1
Other anaerobes					3	0
Culture negative	14	10	7	11	15	5
Polymicrobial	15	31	14	12	16	3
Other	3					

Tande and Patel. Clin Microbiol Rev 2014;27:302

PJI Definitions

	Definitions of Prosthetic Joint Infection					
	2011 Musculoskeletal Infection Society[1]		2013 Infectious Diseases Society of America[2]		2013 International Consensus[3]	
	Definitive evidence	Supportive evidence	Definitive evidence	Supportive evidence	Definitive evidence	Supportive evidence
Sinus tract communicating with the prosthesis	X		X		X	
Identical microorganism isolated from ≥2 cultures	X		X		X	
Purulence surrounding the prosthesis		X	X			
Acute inflammation of periprosthetic tissue		X		X		X
A single culture with any microorganism		X				X
A single culture with a virulent microorganism				X		
Elevated synovial fluid leukocyte count		X				X
Elevated synovial fluid neutrophil percentage		X				X
Elevated serum ESR and CRP		X				X

[1]Parvizi et al. Clin Orthop Relat Res 2011;469:2992
[2]Parvizi & Gehrke. Proceedings of the International Consensus Meeting on Periprosthetic Joint Infection 2013
[3]Osmon et al. Clin Infect Dis 2013:56:e1

Musculoskeletal Infection Society (MSIS) and Infectious Diseases Society of America (IDSA) Diagnostic Criteria for Prosthetic Joint Infection

		MSIS	
		Aseptic Failure	Prosthetic Joint Infection
IDSA	Aseptic Failure	297	1
	Prosthetic Joint Infection	4	69
			p=0.56

Melendez et al. IDWeek poster 2014

Musculoskeletal Infection Society (MSIS) and International Consensus Meeting (ICM) Diagnostic Criteria for Prosthetic Joint Infection

		MSIS	
		Aseptic Failure	Prosthetic Joint Infection
ICM	Aseptic Failure	298	0
	Prosthetic Joint Infection	3	70 p=0.65

Melendez et al. IDWeek poster 2014

Infectious Diseases Society of America (IDSA) and International Consensus Meeting (ICM) Diagnostic Criteria for Prosthetic Joint Infection

		IDSA	
		Aseptic Failure	Prosthetic Joint Infection
ICM	Aseptic Failure	294	4
	Prosthetic Joint Infection	4	69 p=0.9

Melendez et al. IDWeek poster 2014

Test Characteristics and Relative Costs of Preoperative Tests for PJI Diagnosis

Test	Joint	Threshold value	Sensitivity	Specificity	+ LR	- LR	Cost	Comments
PERIPHERAL BLOOD								
WBC	Hip and knee	11,000 x 10^9/L	45	87	3.5	0.6	$	1796 patients in 15 studies
CRP		10 mg/L	88	74	3.4	0.2	$	3225 patients in 23 studies
ESR		30 mm/hr	75	70	2.5	0.4	$	3370 patients in 25 studies
IL-6		10 pg/mL	97	91	10.8	0.0	$	432 patients in 3 studies
Procalcitonin		0.3 ng/mL	33	98	16.5	0.7	$	78 patients in single study
IMAGING								
Plain radiograph	Hip	Lucency or periosteal new bone formation	75	28	1.0	0.9	$	65 patients in single study
Triple phase bone scan	Late hip	Increased uptake on all 3 phases	88	90	8.8	0.1	$$$	46 patients in single study
Bone scan/labeled leukocyte scan	Late hip and knee	Incongruent images	64	70	2.1	0.5	$$$	166 patients in single study
FDG-PET scan	Hip and knee		82	87	6.1	0.2	$$$$$	635 patients in 11 studies
SYNOVIAL FLUID								
Cell count	Knee	1100 cells/μL	91	88	7.6	0.1	$$	429 patients in single study
Neutrophil percentage		64%	95	95	17.9	0.1	$$	
Cell count	Hip	4200 cells/μL	84	93	12.0	0.2	$$	201 patients in single study
Neutrophil percentage		80%	84	82	4.7	0.2	$$	
Cell count	Knee (<6 weeks)	27,800 cells/μL	84	99	84.0	0.2	$$	146 patients in single study
Neutrophil percentage		89%	84	69	2.7	0.2	$$	
Culture	Hip and knee		72	95	14.4	0.3	$$	3332 patients in 34 studies

Tande and Patel. Clin Microbiol Rev 2014;27:302

Multiplex Antibody Detection

- Developed multiplex antibody detection-based immunoassay by “comparative immunoproteomics” – compared antibody response profile of arthroplasty patients with and without PJI

	Sensitivity	Specificity
Staphylococci targeted	68/94 (72%)	213/264 (81%)
S. aureus	36/54 (67%)	
S. epidermidis	26/35 (74%)	
S. lugdunensis	9/9 (100%)	
S. agalactiae	6/8 (75%)	250/270 (93%)
P. acnes	5/13 (39%)	235/277 (85%)

Marmor et al. J Clin Microbiol. 2016;54:1065

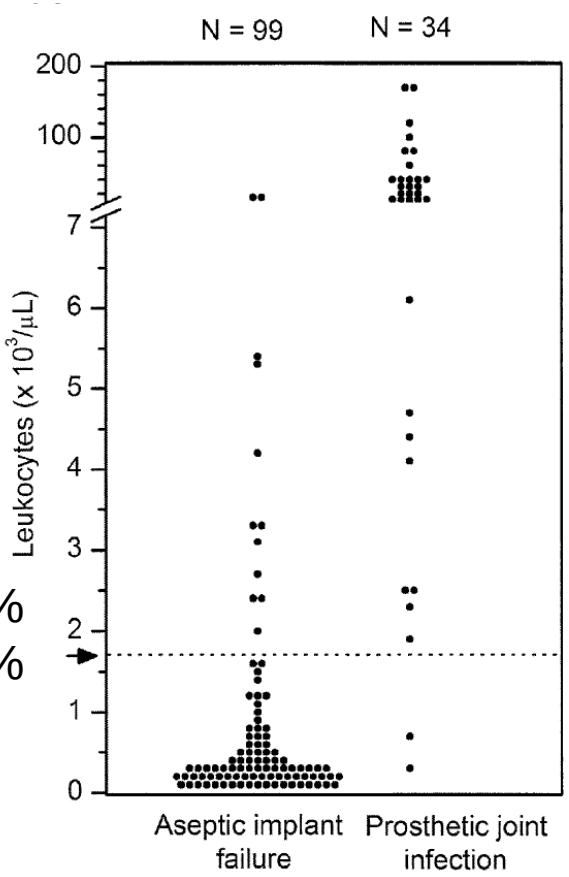
Synovial Fluid Should be Cultured in Blood Culture Bottles

- 219 subjects, revision hip or knee arthroplasty
- Intraoperative fluid inoculated into aerobic and anaerobic blood culture bottles (15 ml each)

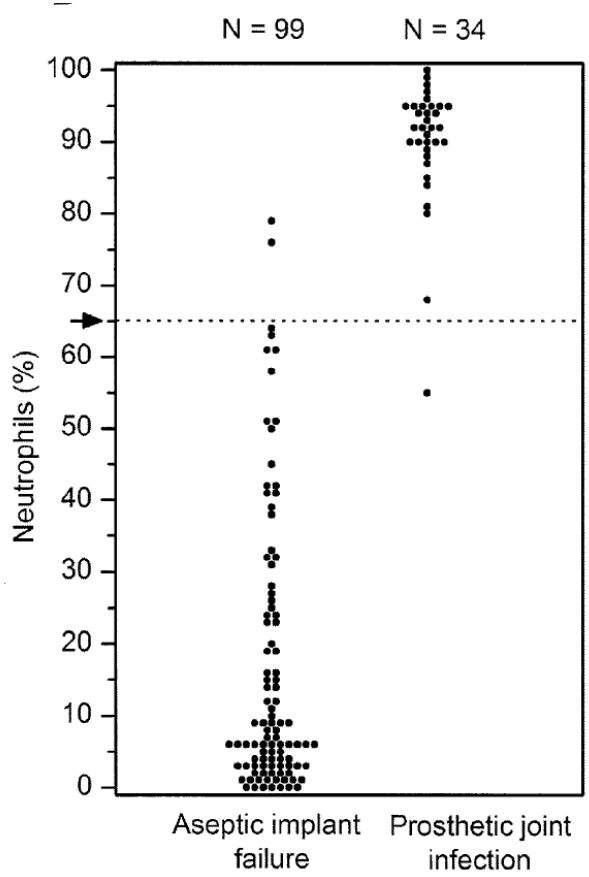
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Synovial fluid culture in blood culture vial	58	100	100	71
Synovial fluid culture standard	47	100	100	61

Jordan et al. BioMed Res Internat 2014;article ID 180416

Synovial Fluid Leukocyte/Differential Prosthetic Knee (>6 months from index surgery)



Sensitivity, 94%
Specificity, 88%



Sensitivity, 97%
Specificity, 98%

Trampuz et al. Am J Med 2004;117:556

Synovial Fluid Leukocyte/Differential

	WBC (cells/μl)	Sensitivity	Specificity	Neutrophil percentage	Sensitivity	Specificity	Joint type	Time from surgery
Trampuz Am J Med 2004;117:556	**1,700**	94	88	**65**	97	98	Knee (133)	>6 months
Ghanem JBJS 2008;90:1637	**1,100**	91	88	**64**	95	95	Knee (429)	Varied
Schinsky JBJS 2008;90:1869	**4,200**	84	93	**80**	84	82	Hip (201)	Varied
Bedair CORR 2011;469:34	**10,700**	95	91	**89**	84	69	Knee (146)	<6 weeks
Cipriano JBJS 2012;94:594 **Non-inflammatory arthritis**	**3,450**	91	93	**78**	96	87	Hip/Knee (810)	Unknown
Cipriano JBJS 2012;94:594 **Inflammatory arthritis**	**3,444**	88	80	**75**	100	82	Hip/Knee (61)	Unknown
Zmistowski J Arthroplasty 2012;27:1589	**3,000**	93	94	**75**	93	83	Knee (150)	Unknown
Dinneen Bone & Joint J 2013;95:554	**1,590**	90	91	**65**	90	87	Hip/Knee (75)	Unknown

Natural Progression of Synovial Fluid Leukocytes in TKA

WBC, Neutrophil Percentage and Total Neutrophil Count by Time Period*				
	First 45 Days	Days 46 to 90	3 Months to 1 Year	1 to 2 Years
WBC count/µL	3037±3786	1119 ±1325†	474 ±894‡	428±706
Neutrophil %	69±27	49±23†	41±27	28±32‡
Neutrophils µL	2533±3483	649±871†	270±598‡	241±552

*Values are means and standard deviations
†Significantly different from the first 45 days ($p<0.05$)
‡Significantly different from days 46 to 90 ($p<0.05$)

Christensen et al. JBJS 2013;95:2081

Synovial Fluid CRP

CRP (mg/L)	Aseptic Failure	PJI	
Knee	N = 11	N = 10	Ronde-Oustau et al. Orthopaed & Traumatol: Surg and Res 2014;100:217
Serum	4.69 (0.94-66.50)	52.00 (19.50-225.00)	
Synovial Fluid	0.69 (0.17-30.80)	13.95 (4.39-92.10)	
Knee and Hip	N = 43	N = 20	Parvizi et al. J Arthoplasty 2012;27:12
Serum	1.1 (0-125)	98 (3-263)	
Synovial Fluid	2 (0-13)	40 (0-103)	
Knee			Parvizi et al. Clin Orthop Relat Res 2012;470:54
Serum	9.6 (N = 30)	91.7 (N = 25)	
Synovial Fluid (Individual ELISA)	0.002 (N = 5)	0.322 (N = 10)	
Synovial Fluid (Multiplex ELISA)	1.19 (N = 34)	22.49 (N = 25)	
Knee			Vanderstappen et al. Acta Orthop Belg 2013;79:326
Serum	3.38 (0.50-10.10) (N = 17)	32.70 (6.70-145.10) (N = 7)	
Synovial Fluid	1.08 (0.30-5.00) (N = 33)	11.98 (2.00-30.20) (N = 11)	
Hip	(N = 27)	(N = 38)	Buttaro et al. Clin Orthop Rel Res 2015;473:3876
Synovial Fluid	Cutoff 9.5 mg/L, sensitivity 90%, specificity 94%		

Novel Synovial Fluid Biomarkers

Biomarker	Cutoff	Sens-itivity (%)	Spec-ificity (%)	
α-Defensin	4.8 µg/mL	100	100	Deirmengian et al. Clin Orthop Rel Res 2014;472:3254 (95 subjects; 29 PJI; hip/knee)
Neutrophil elastase 2	2.0 µg/mL	100	100	
Bactericidal/permeabiity-increasing protein	2.2 µg/mL	100	100	
Neutrophil gelatinase-associated lipocalin	2.2 µg/mL	100	100	
Lactoferrin	7.5 µg/mL	100	100	
IL-8	6.5 ng/mL	100	95	
CRP	12.2 mg/L	90	97	
Resistin	340 ng/mL	97	100	
α-Defensin	5.2 mg/L	97	96	Deirmengian et al. JBJS 2014;96:1439 (149 subjects; 37 PJI; hip/knee; did not report WBC)
CRP	3 mg/L	97	79	
α-Defensin → if positive, CRP		97	100	
α-Defensin	5.2 mg/L	100	100	Deirmengian et al. CORR 2015;473:198 (46 subjects; 23 PJI; hip/knee; did not report WBC performance)
α-Defensin	7720 ng/mL	95	100	Bingham et al. CORR 2014;472:4006 (57 subjects; 19 PJI; hip/knee; did not report %neutrophils)
α-Defensin	0.48 S/CO	63	95	Frangiamore et al. J Shoulder Elbow Surg 2015;24:1021 (33 cases; 11 PJI; shoulder)
α-Defensin	1 S/CO	99		Shahi et al. CORR 2016;474:1610 (106 PJI subjects; hip/knee)
α-Defensin	1 S/CO	97	97	Bonanzinga et al. CORR 2017;475:408 (156 subjects; 33 PJI; hip/knee)
α-Defensin		77	82	Suda et al. Int Ortho 2017 In Press (28 subjects – 30 joints; hip/knee; 13 PJI)
IL-6	359.3 pg/mL	87	90	Frangiamore et al. J Bone Joint Surg Am 2015:97:63 (35 subjects; 15 PJI; shoulder)
IL-1β IL-6	8.26 pg.ml 8.7 ng/ml	90 81	87 96	Frangiamore et al. Clin Orthop Relat Res 2016;474:1630 (90 subjects; 31 PJI; hip or knee)

Intraoperative Frozen Section Histopathology

Reference	Specimen	Joint	#PMN*	n	Sensitivity	Specificity	PPV**
Feldman et al. JBJS(Am) 1995;77:1807	JC, IM	Hip/knee	5	33	100	100	100
Athanasou et al. JBJS(Br) 1995;77:28	JC, IM	Hip/knee	1	106	90	96	88
Lonner et al. JBJS(Am) 1996;78:1553	JC, IM, ASPI	Hip/knee	5	175	84	96	70
			10		84	99	89
Pace et al. J Arthroplasty 1997;12:64	JC, IM	Hip/knee	5	18	82	93	82
Abdul-Karim et al. Mod Pathol 1998;11:427	IM, ST, UDT	Hip/knee	5	64	43	97	60
Banit et al. CORR 2002;401:230	JC, ASPI	Knee	10	55	100	96	82
		Hip	10	63	45	92	55
Musso et al. Postgrad Med J 2003;79:590	JC, IM, ASPI	Hip/knee	5	45	50	95	60
Wong et al. J Arthroplasty 2005;20:1015	JC, IM, SS	Hip/knee	5	33	93	77	68
			10		86	85	75
Ko et al. J Arthroplasty 2005;20:189	JC, IM, ASPI	Hip/knee	5	40	67	97	86
Frances Borrego et al. Int Orthop 2007;31:33	PST	Hip	10	63	67	90	80
		Knee		83	50	100	100
Nunez et al. Acta Orthop 2007;78:226	JC, IM, ASPI	Hip	5	136	86	87	79
Tohtz et al. CORR 2010;468:762	IM	Hip	10	52	87	100	100

*Some studies used >, others ≥ the number shown, **Positive predictive value
JC, joint pseudocapsule; IM, interface membrane; ASPI, any area that appears suspicious for possible infection; ST, synovial tissue; SS, synovial surface; UDT, unusually discolored tissue; PST, periprosthetic soft tissue

Periprosthetic Tissue and Device Culture

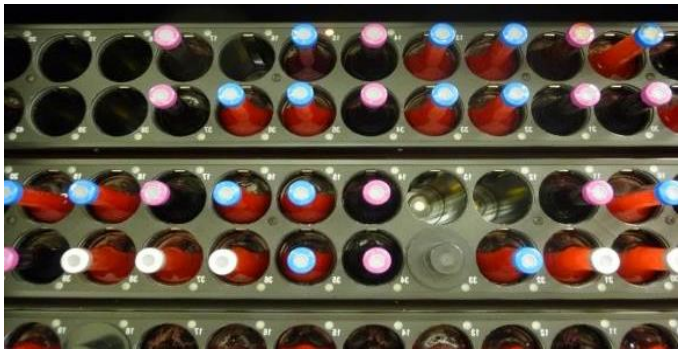
- **IDSA Guidelines** - At least 3 and optimally 5 or 6* periprosthetic intraoperative tissue samples or the explanted prosthesis itself should be submitted for aerobic and anaerobic culture at the time of surgical débridement or prosthesis removal to maximize the chance of obtaining a microbiologic diagnosis (B-II)

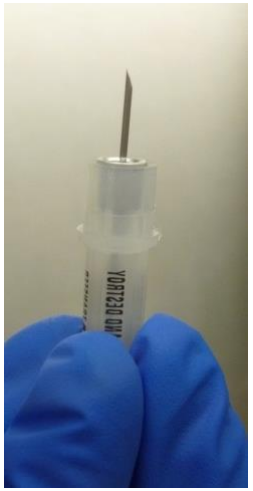
  Osmon et al. Clin Infect Dis 2013:56:1
  *Atkins et al. J Clin Microbiol 1998;36:2932

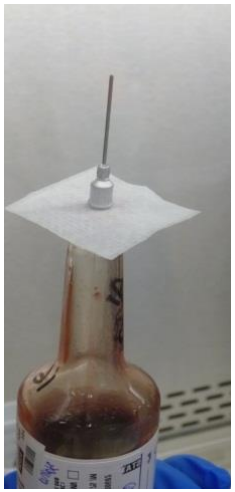
- **ESCMID Guidelines** - Peri-implant tissue (AIII) and device/prosthesis sonication (BII)

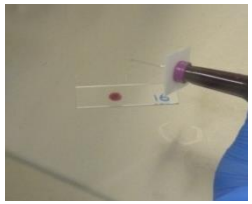
  Høiby et al. Clin Microbiol Infect 2015;21:S21

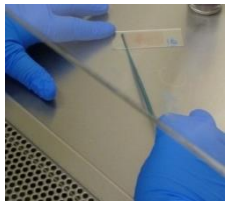
Periprosthetic Tissue Culture Blood Culture Bottles











Periprosthetic Tissue Culture Blood Culture Bottle Study (Oxford #1)

- Tissue + sterile glass beads (Ballotini) 5 ml saline (shaking)
  - 1 ml each into Robertson's cooked meat broth, fastidious anaerobic broth, BACTEC Anaerobic/F & Aerobic/F bottles
  - 0.25 ml each onto chocolate, aerobic & anaerobic (2) blood agars
  - 5 day incubation
  - 141 elective joint revisions (mean, 4.9 specimens/case)
  - 23 PJI cases

Sensitivity/ Specificity (≥2 positive)	Direct plates	Fastidious anaerobic broth	Cooked meat broth	BACTEC blood culture bottles
Sensitivity, %	39	57	83	87
Specificity, %	100	100	97	98

Hughes et al. Clin Microbiol & Infect 2011;10:1528

Periprosthetic Tissue Culture Blood Culture Bottle Study (Oxford #2)

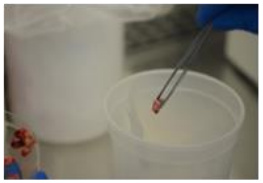
- Tissue + 3 ml saline + sterile glass beads → vortexed 15 sec
  - 0.5 ml BACTEC Lytic/10 Anaerobic/F & Plus Aerobic/F bottles
  - 14 day incubation
- 322 patients (mean, 4 specimens/case)
- 66/79 PJI culture-positive (sensitivity, 84%)
  - Positive within 3 days of culture in all cases except 1 (detected at 8 days)
- 7/243 non-PJI culture-positive (specificity, 97%)
- *Propionibacterium* species
  - Sub-culturing negative bottles after 14-days' incubation detected a single additional *Propionibacterium* isolate from 1000 bottles

Minassian et al. BMC Infect Dis 2014;14:233

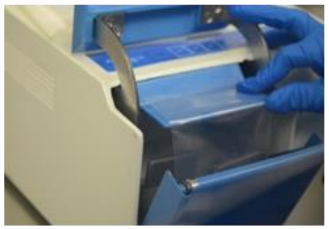

Periprosthetic Tissue Blood Culture Bottle Study (Mayo Clinic)


- Patients undergoing revision arthroplasty, Mayo Clinic, 8/2013–4/2014
  - 369 subjects - 138 hip, 160 knee, 49 shoulder, 22 elbow arthroplasties
  - 117 (32%) met IDSA diagnostic criteria for PJI

Peel et al. mBio 2016;7:e01776

Peri-prosthetic tissue sample

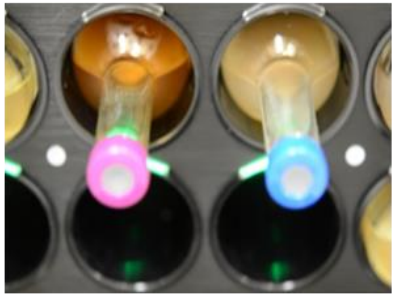


Tissue sample homogenized

Conventional Culture Technique:
Homogenate inoculated onto agars and broth

Media manually checked daily for growth and sub-cultured if growth detected

New Technique:
Homogenate inoculated into blood culture bottles and placed onto the semi-automated machine

Bottles continuously monitored for growth and sub-cultured if flagged positive




Periprosthetic Tissue Blood Culture Bottle Study (Mayo Clinic) Bayesian Latent Class Modeling

Media Combination	Sensitivity	Specificity
Aerobic and anaerobic agars	48.9	99.7
Aerobic and anaerobic agars and thioglycollate broth	62.6	98.1
Aerobic and anaerobic blood culture bottles	92.1	99.7
Aerobic and anaerobic blood culture bottles and thioglycollate broth	92.1	98.8
Aerobic and anaerobic blood culture bottles and aerobic agar	94.6	99.7
Aerobic and anaerobic blood culture bottles and anaerobic agar	96.8	99.8
Aerobic and anaerobic blood culture bottles and aerobic and anaerobic agars	99.1	99.7
All media combined	99.1	97.3

Peel et al. mBio 2016;7:e01776

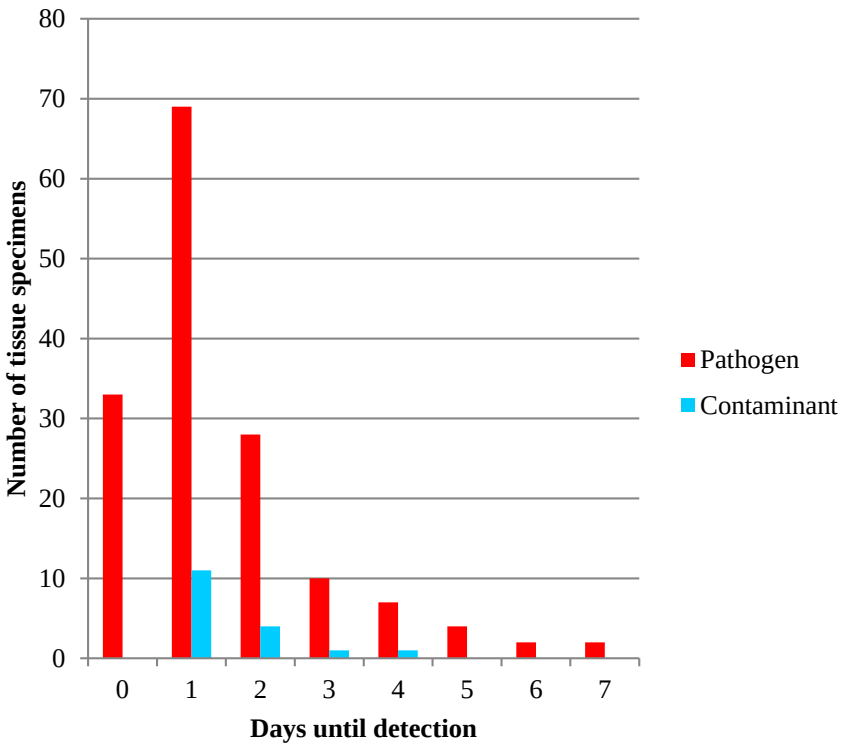



Sensitivity and Specificity using PJI (IDSA Criteria) as the Gold Standard

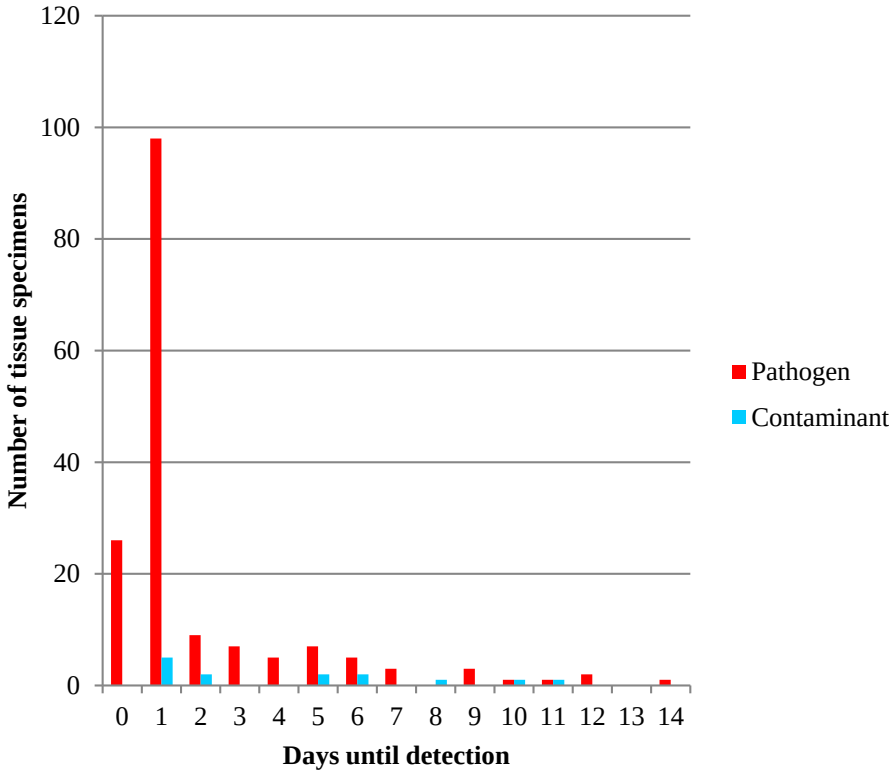
Media Combination	Sensitivity	Specificity
Aerobic and anaerobic agars	33.3	100.0
Aerobic and anaerobic agars and thioglycolate broth	44.4	98.8
Aerobic and anaerobic blood culture bottles	60.7	98.8
Aerobic and anaerobic blood culture bottles and thioglycolate broth	63.3	98.8
Aerobic and anaerobic blood culture bottles and aerobic agar	62.4	98.8
Aerobic and anaerobic blood culture bottles and anaerobic agar	62.3	98.0
Aerobic and anaerobic blood culture bottles and aerobic and anaerobic agars	64.1	98.0
All media combined	67.5	96.8

Peel et al. mBio 2016;7:e01776


Periprosthetic Tissue Blood Culture Bottle Study (Mayo Clinic) Time-to-Detection

Aerobic bottles

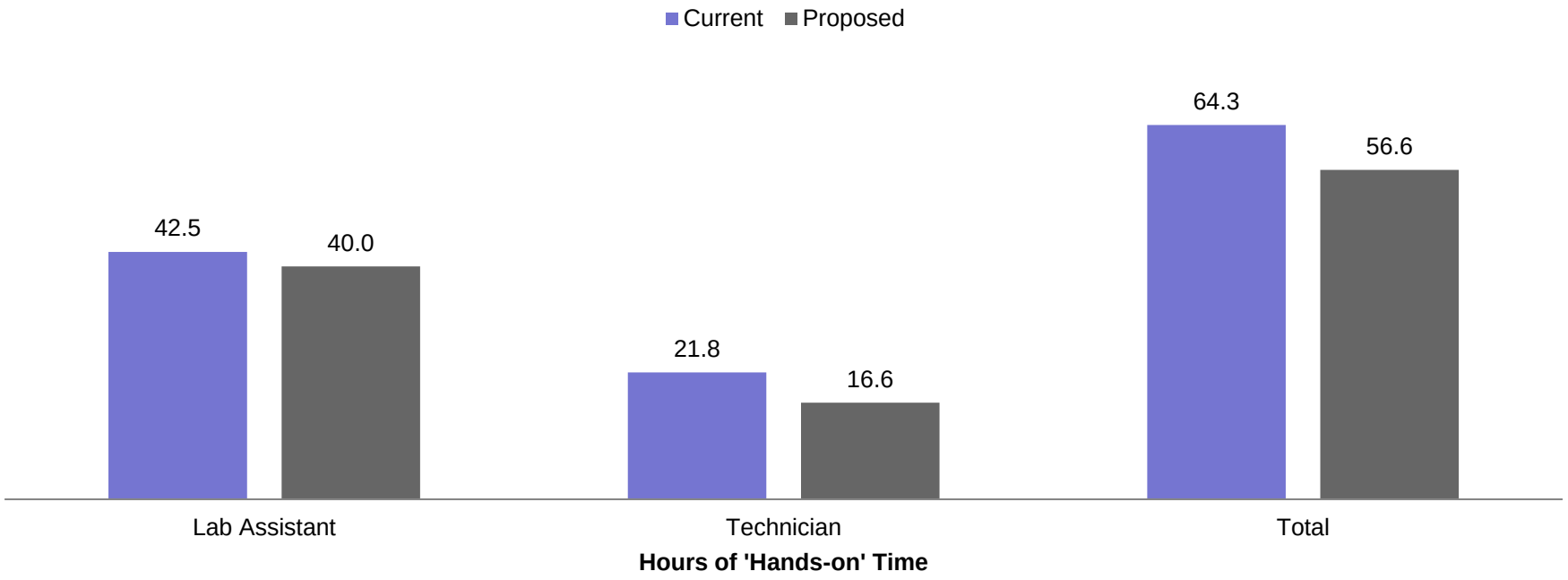


Anaerobic bottles

Peel et al. mBio 2016;7:e01776

Laboratory Workflow Analysis of Culture of Periprosthetic Tissues in Blood Culture Bottles

- 10-11% reduction in total staff time
- 20-24% reduction in technician time



Prospective Multicenter Trial Assessing Sample Number

- 215 PJI subjects
- 5 samples/patient - assessed using random selection 2, 3, 4 or 5 samples
- Results with individual culture media compared
- Aerobic bacteria: Blood agar, 70%; chocolate agar, 69%; Schaedler broth, 69%; pediatric blood culture bottle, 83%
- Anaerobic bacteria: Blood agar, 31%; chocolate agar, 53%; Schaedler broth, 75%; pediatric blood culture bottle, 54%
- Agreement of 98 and 100%, respectively, for bacteriological criterion and confirmed PJI diagnosis obtained with 4 perioperative samples
- Highest percentages of agreement obtained with association of 3 culture media, blood culture bottle, chocolate agar and Schaedler broth, incubated for 5, 7 and 14 days, respectively

→ **4 samples seeded in/on 3 culture media sufficient for diagnosing PJI**

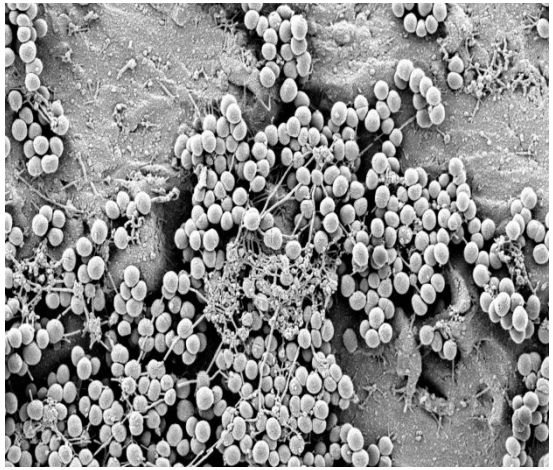
Bémer et al. J Clin Microbiol 2016;54:385

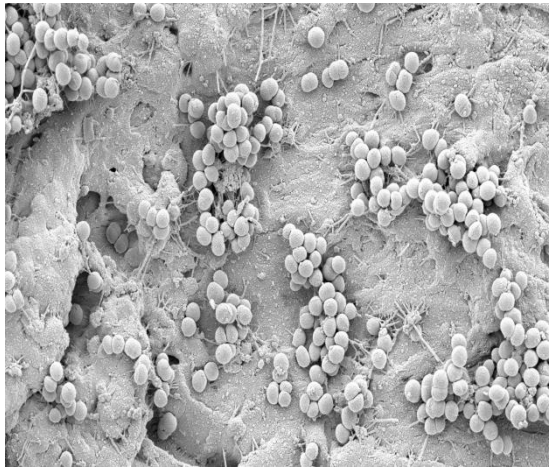
Periprosthetic Tissue Culture

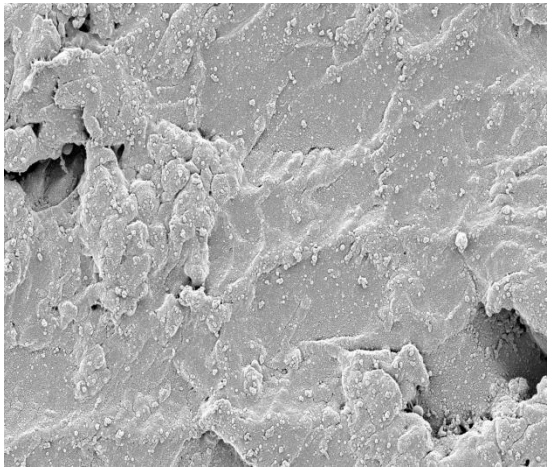
- 499 consecutive patients undergoing revision arthroplasty (August 2013 through April 2014)
- 1,437 periprosthetic tissue samples
- Conventional culture techniques (aerobic and anaerobic sheep blood agars, thioglycolate broth)
  - Greatest accuracy with four specimens (91%; 95% Credible Interval: 77-100%)
- Inoculation of tissues into aerobic and anaerobic blood culture bottles
  - Greatest accuracy with three specimens (92%; 95% Credible Intervals: 79-100%)

Peel et al. J Clin Microbiol 2017;55:234-43

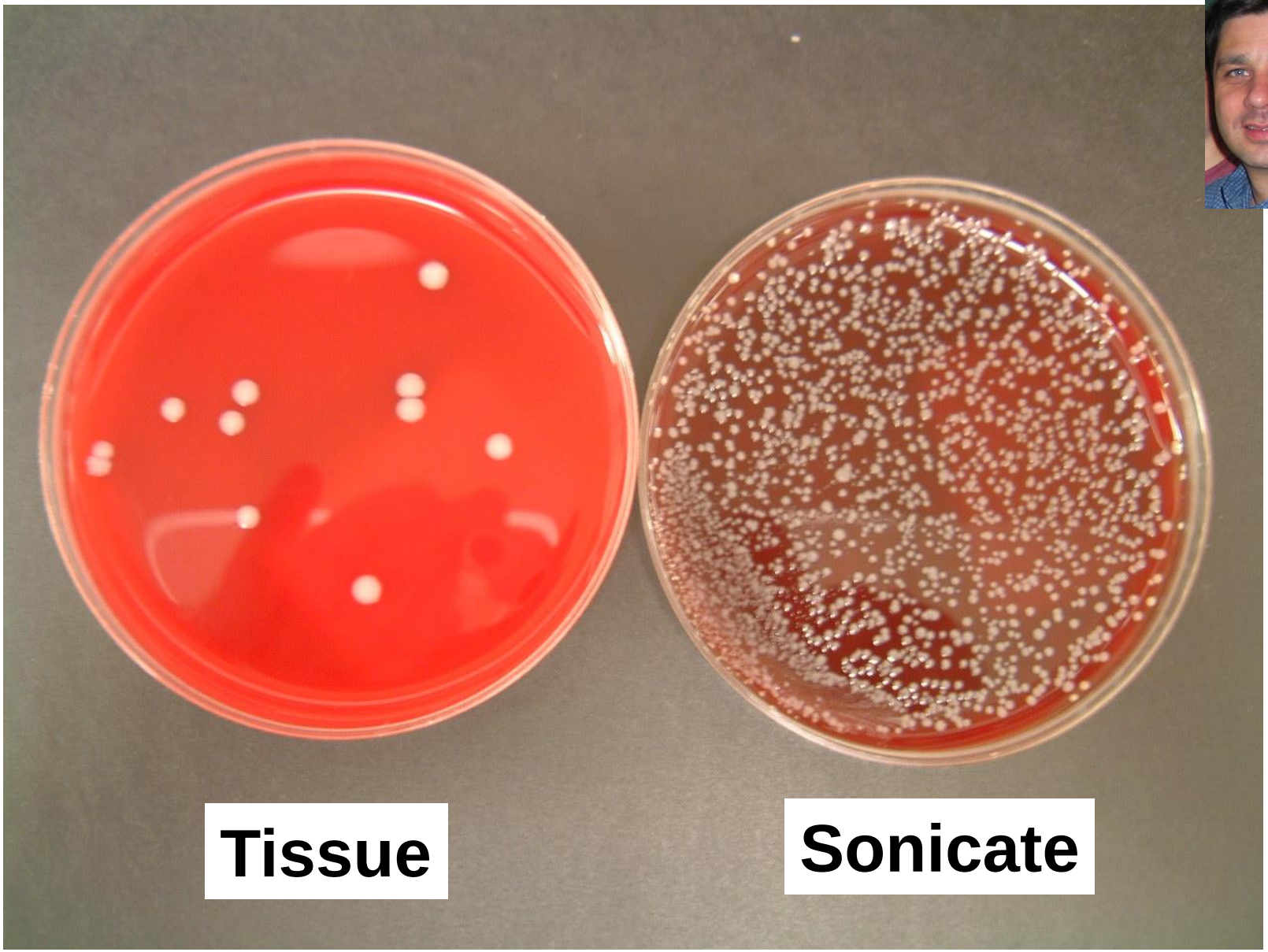
Staphylococcus epidermidis Biofilm on Polycarbonate Coupons Scanning Electron Microscopy







Soaking	Scraping	Sonication



Current Orthopedic Implant Processing - Mayo Clinic

400 ml Ringer's Solution Added

Prosthesis Placed in Container (Operating Room)

Vortex 30 sec

Sonicate 5 min

Vortex 30 sec

Centrifuge 5 min

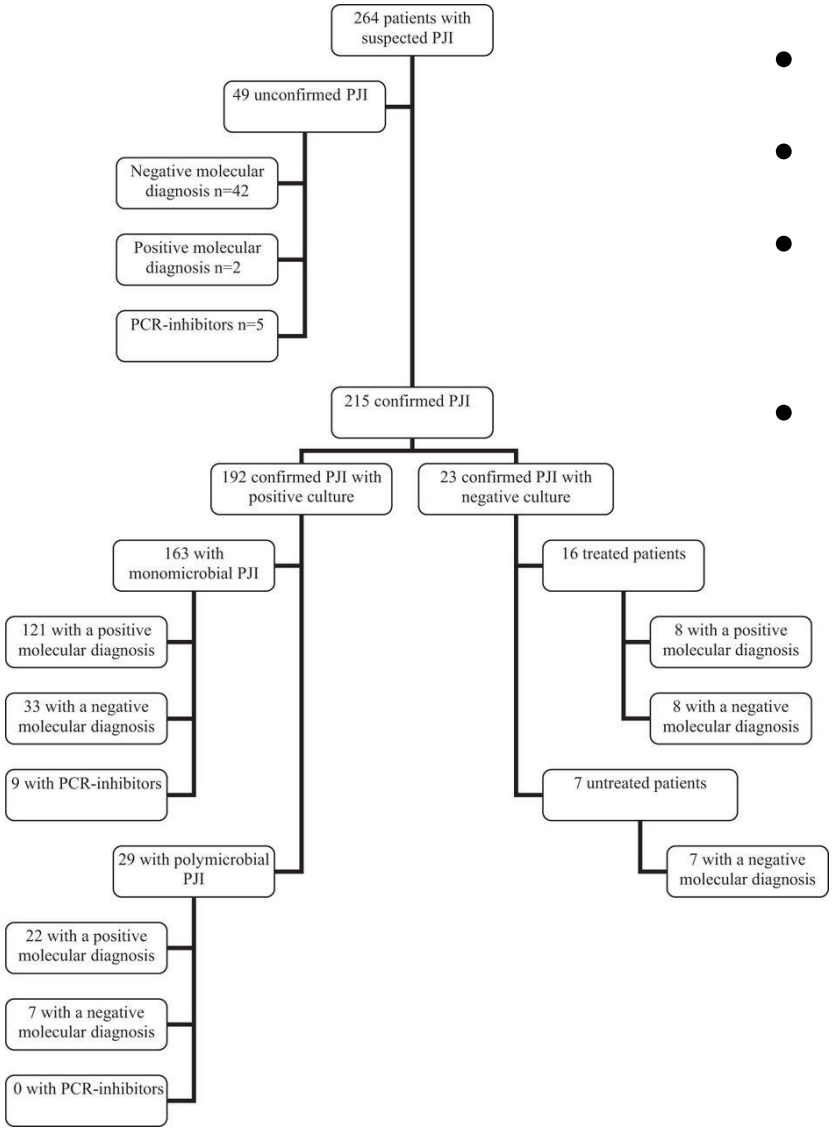
Aspiration

Plating

Comparison of Sonicate Fluid and Tissue Culture Diagnosis of Orthopedic Foreign Body Infection

Implant type		Sonicate fluid	Periimplant tissue	p value	Reference
Prosthetic hip/knee joint	Sensitivity	79%	61%	<0.001	Trampuz et al, NEJM 2007 Vol 357;654
	Specificity	99%	99%		
Prosthetic shoulder joint	Sensitivity	67%	55%	0.046	Piper et al, JCM 2009 Vol 47:1878
	Specificity	98%	95%		
Spine implant	Sensitivity	91%	73%	0.046	Sampedro et al, Spine 2010 Vol 25:1218
	Specificity	97%	93%		
Prosthetic elbow joint	Sensitivity	89%	55%	0.18	Vergidis et al, JSES 2011 Vol 20;1275
	Specificity	100%	93%		
Prosthetic hip/knee/shoulder joint, mega-prostheses, osteosynthesis, spine implant	Sensitivity	83%	61%	0.034	Holinka et al. J Orthop Res 2011 Vol 29:617
	Specificity	95%	95%		
Prosthetic hip joint	Sensitivity	75	69		Bogut et al. Polish J Microbiol 2014:63:299
	Specificity	97	100		
Prosthetic hip/knee joint, internal device	Sensitivity	77	34	<0.002	Scorzolini et al. New Microbiol 2014;37:321
Fracture fixation implant (plate and screws, spine implant, intramedullary nail)	Sensitivity	90	57	<0.001	Yano et al. J Clin Microbiol 2014;52:4176
	Specificity	91	96		

Lack of Sensitivity of Periprosthetic Tissue Broad-Range Bacterial PCR



- 2-year period
- 5 tissues/patient
- Culture, 16S rRNA gene PCR/sequencing
- 264 suspected cases of PJI
  - PJI confirmed (215)
    - Culture positive, 192 (89%)
    - PCR positive, 151 (73%)
  - Non-PJI (49)
    - PCR positive, 2 (specificity, 96%)

Bémer et al. J Clin Microbiol 2014:52:3583

Sonication and Broad-Range PCR Hip/Knee Prostheses

- Sonication fluid more sensitive than periprosthetic tissue

Rak et al. Acta Orthopaedica 2016;87:339

Test	Sensitivity 135 PJI	Specificity 231 Aseptic Failure	Accuracy
	% (95% Confidence Interval)		
Tissue culture	70.4 (64.5-76.3)	98.7 (97.2-100)	88.3 (84.2-92.4)
Sonicate fluid culture	72.6 (66.8-78.4)	98.3 (96.6-100)	88.8 (84.7-92.9)
Sonicate fluid broad-range PCR	70.4 (64.5-76.3)	97.8 (95.9-99.7)	87.7 (83.5-91.9)
Combination of two tests above	83.0 (78.2-87.8)	95.7 (93.1-98.3)	91.0 (87.3-94.7)
Sonicate fluid culture plus PCR	78.5 (73-2-83.8)	97.0 (94.8-99.2)	90.2 (86.4-94.0)
Synovial fluid culture	64.7 (56.5-72.9)	96.9 (93.9-99.9)	84.1 (77.8-90.4)
Sonicate fluid PCR - lower cutoff (CP <27.59 cycles)	80.0 (74.8-85.2)	90.9 (87.2-94.6)	86.8 (82.5-91.3)

Gomez et al. J Clin Microbiol 2012:50:3501

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Sonication Studies – PJI PCR Panel Hip/Knee Prostheses

Test	Aseptic failure (290)	PJI (144)	Sensitivity	Specificity	PPV	NPV
	No. of patients with positive specimens		% (95% confidence interval)			
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)
Tissue culture						
Any growth	45	119	82.6 (75.4-88.4)	84.5 (79.8-88.5)	72.6 (65.1-79.2)	90.7 (86.6-93.9)
≥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)			77.1 (69.3-83.7)	97.9 (95.6-99.2)	94.9 (89.2-98.1)	89.6 (85.7-92.7)
Any positive result	6	111				
Staphylococcus sp	2	75				
S. aureus	0	28				
Coagulase-negative staphylococci	2	47				
Streptococcus sp	3	11				
Enterococcus/*Granulicatella*/*Abiotrophia* sp	0	11				
Enterobacteriaceae	1	8				
Gram-positive anaerobic cocci	0	8				
Propionibacterium sp	0	8				
P. aeruginosa	0	5				
Corynebacterium sp	0	4				
C. jeikeium/*urealyticum*	0	0				
Non-*jeikeium* sp	0	4				
Proteus sp	0	1				
B. fragilis group	0	0				

Cazanave et al. J Clin Microbiol 2013;51:2280

Commercial Multiplex PCR Assays

- Unyero i60 implant and tissue infection (ITI) multiplex PCR (Curetis)
  - 28 subjects; 30 hip/knee athroplasties, 13 PJI
    - **Tissue** “synovia” (2 mm punch press)
      - Sensitivity, 31%; specificity, 100%

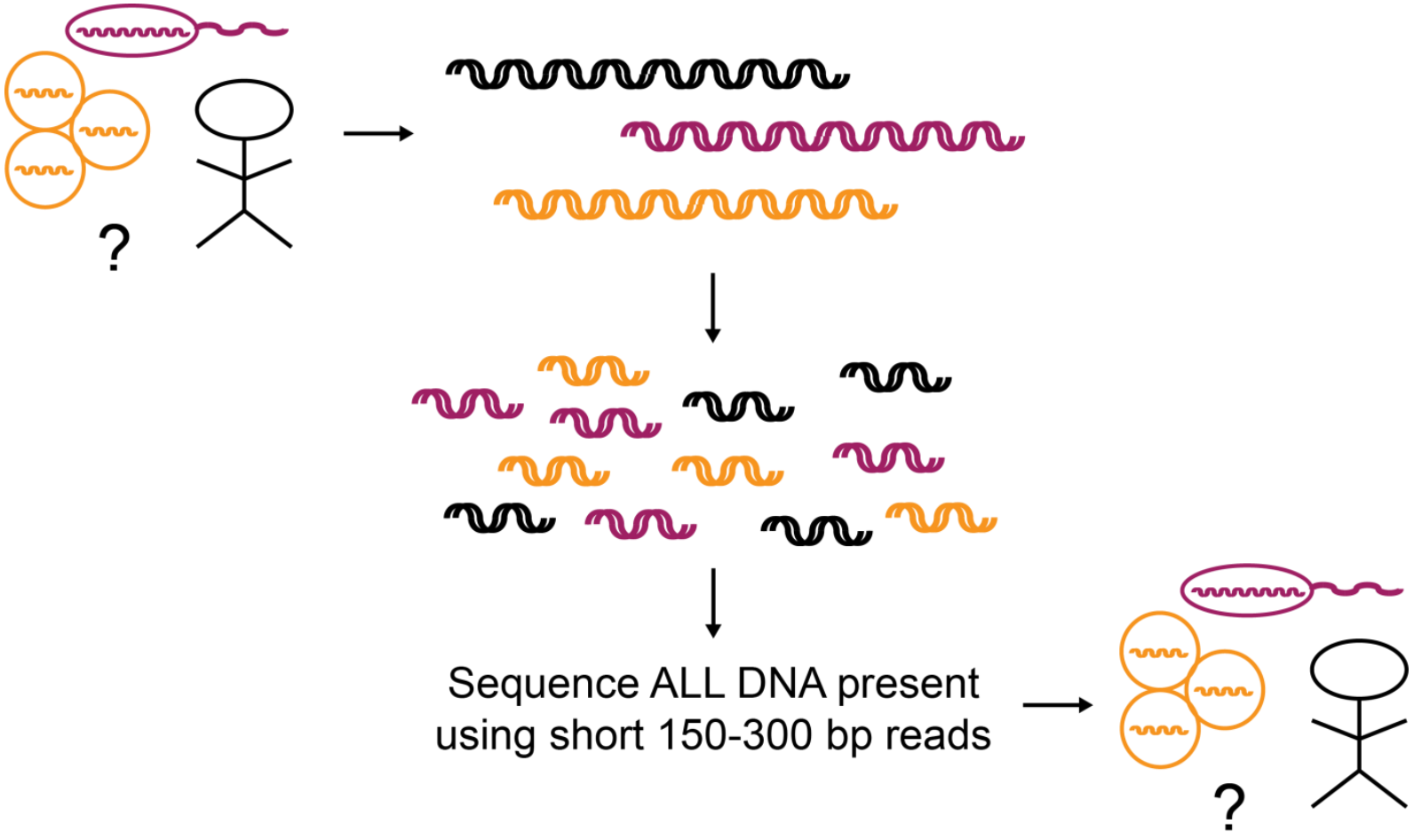
        Suda et al. Int Ortho 2017 In Press
  - 28 subjects, 79 samples
    - **Tissue** $(3\ mm)^3$
      - 7 culture positive – 3 concordant positive
      - 21 culture negative – 20 concordant negative

        Borde et al. Infection 2015;43:551
  - 31 subjects
    - **Sonication fluid and synovial fluid**
      - Sensitivity, 67%; specificity 100%

        Hischebeth et al. J Microbiol Meth 2016;121:27

MAYO CLINIC

Metagenomic Shotgun Whole Genome Sequencing



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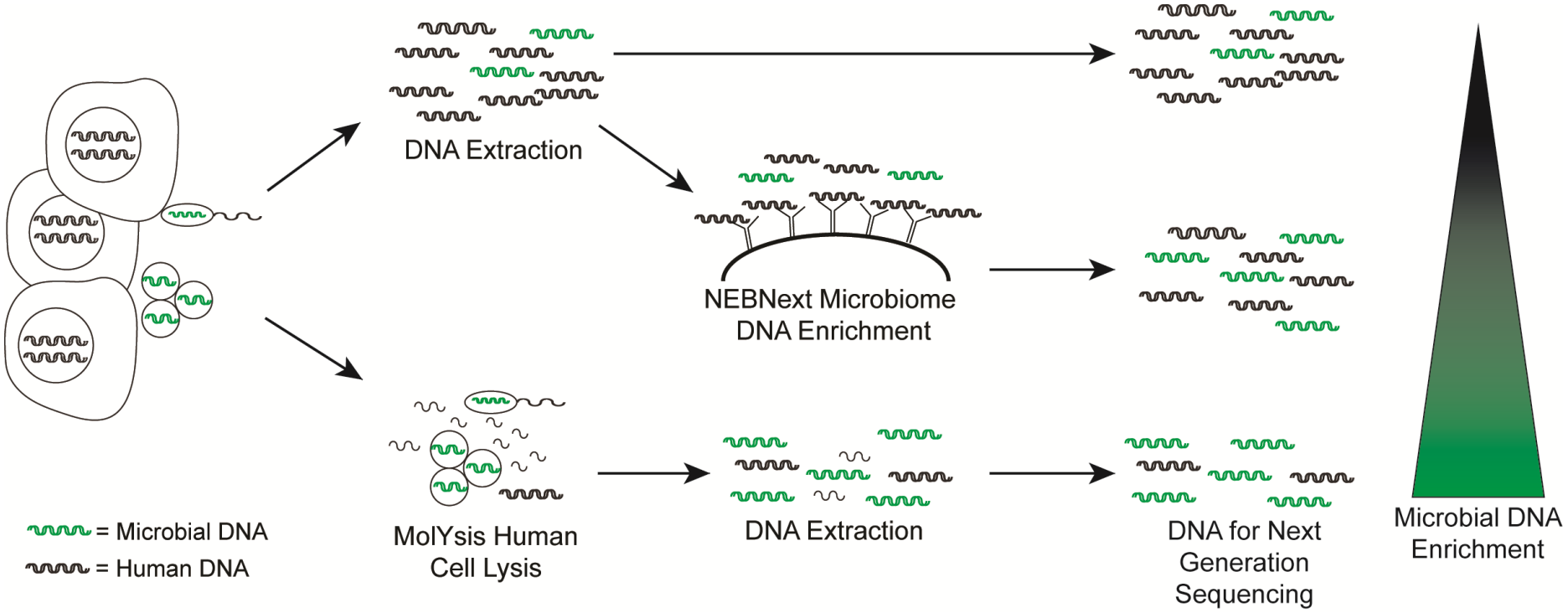
Methods

- Paired-end libraries prep: **NEBNext Ultra DNA Library Prep Kit**
- Sequencing**: Illumina HiSeq 2500** in rapid run mode with 2×250 bp reads, as single samples or multiplexed with 2 or 6 samples per lane, based on number of samples available
- Paired-end reads pre-processing: **seqtk**, **Trimmomatic** & **Livermore Metagenomics Analysis Toolkit** with pre-processing scripts
- Pre-filter human and PhiX reads: **BioBloom tools**
- Taxonomy assignment: **LMAT with kML + Human.v4-14.20.g10.db database**

MAYO CLINIC

Comparison of Microbial DNA Enrichment Tools for Metagenomic Whole Genome Sequencing



Thoendel et al. J Microbiol Methods 2016;127:141–5

MAYO CLINIC

Effect of Enrichment Methods by Metagenomic Whole Genome Sequencing

	No Enrichment	NEBNext Microbiome DNA Enrichment	MolYsis Enrichment
***S. aureus* PJI**			
% of reads	0.02% (4,158 of 25,609,460)	0.2% (350,625 of 169,981,133)	7.7% (2,286,890 of 29,530,730)
Enrichment factor		13X	481X
***S. epidermidis* PJI**			
% of reads	0.007% (1,682 of 23,606,476)	0.2% (133,680 of 74,544,475)	7.0% (2,268,087 of 32,184,381)
Enrichment factor		25X	986X
***E. faecalis* PJI**			
% of reads	0.006% (1,671 of 26,949,030)	0.5% (497,206 of 94,522,959)	59.4% (16,407,878 of 27,643,294)
Enrichment factor		85X	9,580X

Thoendel et al. J Microbiol Methods 2016;127:141–5

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S. aureus PJI

No Enrichment

Read Score	# of Reads	TaxID	Taxonomy group
877206	445817	9606	species,Homo sapiens
54347.8	29068	1301	genus,Streptococcus
25834.1	32491	2759	superkingdom,Eukaryota
9473.81	15978	131567	no rank,cellular organisms
7892.17	4622	671232	species group,Streptococcus anginosus group
4756.18	2901	1279	genus,Staphylococcus
1567.67	1007	1280	species,Staphylococcus aureus
1197.96	755	1338	species,Streptococcus intermedius
843.518	509	286	genus,Pseudomonas
534.066	346	76860	species,Streptococcus constellatus
367.308	232	1282664	no rank,Streptococcus tigurinus AZ_3a
361.242	530	1	depth=0,taxid=1,ktaxid=1,entries=-1
347.345	228	1282	species,Staphylococcus epidermidis
255.733	159	184250	subspecies,Streptococcus constellatus subsp. pharyngis
252.168	204	1328	species,Streptococcus anginosus
223.389	155	857292	no rank,Streptococcus intermedius F0395
221.627	170	883167	no rank,Streptococcus intermedius F0413
206.954	130	1248438	no rank,Pseudomonas alcaliphila 34
175.499	102	1743	genus,Propionibacterium
145.828	96	90964	family,Staphylococcaceae
131.955	141	1051072	no rank,Streptococcus equi subsp. zooepidemicus ATCC 35246
106.976	75	136841	species group,Pseudomonas aeruginosa group
79.7075	55	1182590	no rank,Pseudomonas pseudoalcaligenes CECT 5344
71.3676	49	1074494	no rank,Streptococcus salivarius M18
71.2256	52	1300	family,Streptococcaceae
70.0294	52	32630	species,synthetic construct
69.8811	44	1077464	species,Streptococcus tigurinus
51.1347	31	101564	species,Pseudomonas alcaliphila
48.2248	35	1390370	no rank,Pseudomonas mendocina EGD-AQ5
47.8976	86	1239	phylum,Firmicute

NEBNext Enrichment

Read Score	# of Reads	TaxID	Taxonomy group
8.14E+07	41684794	9606	species,Homo sapiens
807585	1210368	2759	superkingdom,Eukaryota
611285	342132	32630	species,synthetic construct
580565	336338	1280	species,Staphylococcus aureus
268536	481220	131567	no rank,cellular organisms
15809	10833	1279	genus,Staphylococcus
13898.3	18574	1	depth=0,taxid=1,ktaxid=1,entries=-1
13530.1	9200	286	genus,Pseudomonas
2688.34	2762	136843	species group,Pseudomonas fluorescens group
1816.09	1388	1156998	no rank,Staphylococcus aureus HIF003_B2N-C
1561.25	959	90964	family,Staphylococcaceae
1318.53	776	1743	genus,Propionibacterium
1236.36	1069	10003441	species,Staphylococcus aureus plasmid pTZ2162
1226.75	1799	294	species,Pseudomonas fluorescens
1029.9	1597	1292037	no rank,Amycolatopsis vancoresmycina DSM 44592
923.909	719	10003448	species,Staphylococcus aureus plasmid pSA1379
645.914	403	463794	no rank,Pseudomonas fluorescens BBc6R8
564.99	561	613	genus,Serratia
462.127	1310	2	superkingdom,Bacteria
408.322	283	1197727	species,Pseudomonas sp. Ag1
256.031	204	1284392	species,Pseudomonas sp. FH1
230.898	254	543	family,Enterobacteriaceae
217.701	156	10003454	species,Staphylococcus aureus plasmid EDINA
203.746	204	317	species,Pseudomonas syringae
196.998	215	1239	phylum,Firmicutes
181.019	146	68887	species,Torque teno virus
178.088	112	1206777	species,Pseudomonas sp. Lz4W
154.956	184	1163065	species,Pseudomonas sp. CBZ-4
144.54	125	135621	family,Pseudomonadaceae
112.687	87	99158	species,Hammondia hammondi
109.857	84	1240676	species,Pseudomonas sp. PAMC 26793
94.634	63	1301	genus,Streptococcus
93.5661	55	1248438	no rank,Pseudomonas alcaliphila 34
91.8243	74	80865	genus,Delftia
86.2212	81	308865	genus,Elizabethkingia
77.866	45	985010	species,Pseudomonas sp. TJI-51
74.2086	46	53335	genus,Pantoea
69.9358	45	91459	species,Methylobacterium sp. B1

MolYsis Enrichment

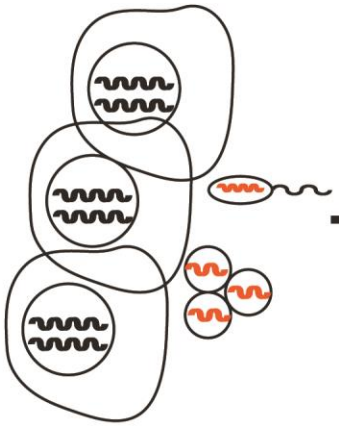
Read Score	# of Reads	TaxID	Taxonomy group
	219358		
3.21E+06	7	1280	species,Staphylococcus aureus
1.26E+06	713346	9606	species,Homo sapiens
193781	215573	2759	superkingdom,Eukaryota
96085.8	71730	1279	genus,Staphylococcus
86648.5	93461	131567	no rank,cellular organisms
17892.4	15986	1156998	no rank,Staphylococcus aureus HIF003_B2N-C
4970.16	3686	90964	family,Staphylococcaceae
3083.97	2641	10003441	species,Staphylococcus aureus plasmid pTZ2162
2110.41	1776	10003448	species,Staphylococcus aureus plasmid pSA1379
1768.6	2414	1	depth=0,taxid=1,ktaxid=1,entries=-1
1649.2	1185	32630	species,synthetic construct
958.691	1031	1239	phylum,Firmicutes
867.117	669	10003454	species,Staphylococcus aureus plasmid EDINA
716.226	2113	2	superkingdom,Bacteria
372.719	290	10003425	species,Staphylococcus aureus plasmid pBORa53
307.861	198	286	genus,Pseudomonas
200.233	175	68887	species,Torque teno virus
192.236	504	1385	order,Bacillales
99.5046	91	10003460	species,Staphylococcus aureus plasmid
77.8404	173	91061	class,Bacilli
50.2773	45	308865	genus,Elizabethkingia
47.0102	71	1282	species,Staphylococcus epidermidis
44.0471	40	1301	genus,Streptococcus
39.0756	22	1743	genus,Propionibacterium
37.8316	24	1248438	no rank,Pseudomonas alcaliphila 34
28.2731	18	136841	species group,Pseudomonas aeruginosa group
24.6249	18	1390370	no rank,Pseudomonas mendocina EGD-AQ5

Thoendel et al. J Microbiol Methods 2016;127:141–5

MAYO CLINIC

Host DNA Depletion Depletes DNA Quantities for Sequencing

Vortex + Sonication

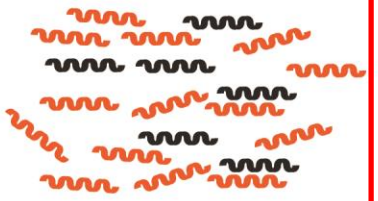


MolYsis Human Cell Lysis

= Microbial DNA

= Human DNA

DNA Extraction



Whole Genome Amplification

Next Generation Sequencing (HiSeq 2500, 2 x 250bp)

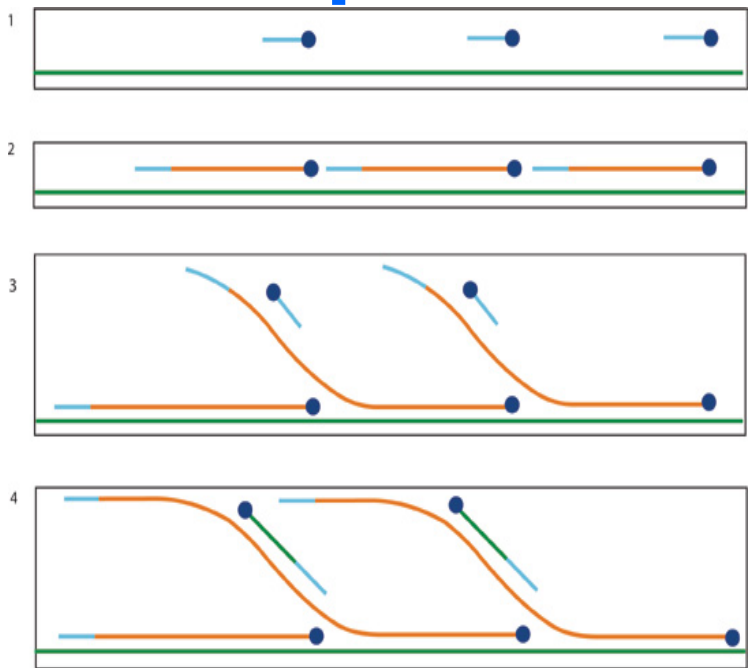
Data Analysis

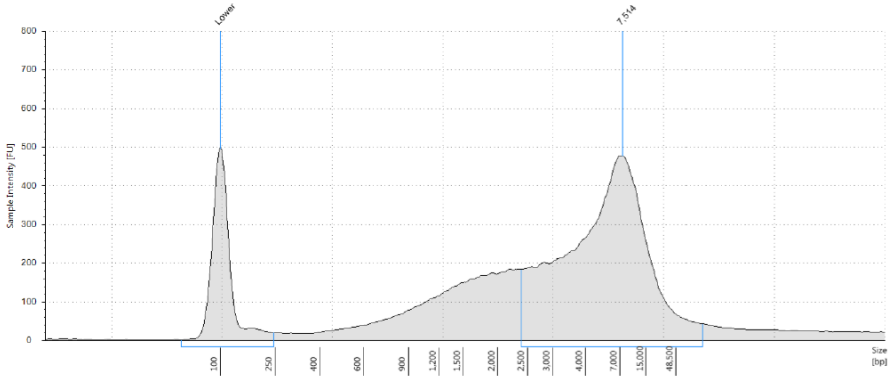
- Identify pathogens
- Antibiotic resistance genes
- Subpopulations

MAYO CLINIC

Whole Genome Amplification Using Multiple Displacement Amplification

- Random hexamers bind to template DNA
- Phi29 DNA polymerase amplifies DNA, displacing previous dsDNA
- High-fidelity, isothermal reaction
- Not completely unbiased
  - High GC content DNA, low abundance templates, small DNA fragments





Spits, et al. Nat Prot. 2006

MAYO CLINIC

Impact of Contaminating DNA in Whole Genome Amplification Kits on PJI Diagnosis

- **8 sonicate fluids, including culture-positive and –negative PJI, and aseptic failure**
- **DNA extraction: MoBio Bacteremia DNA isolation kit**
- **Whole genome amplification:**
  1. **Qiagen REPLI-g Single Cell kit (Qiagen, Hilden Germany)**
  2. **Illustra V2 Genomiphi kit (GE Healthcare Bio-Sciences, Pittsburgh PA)**
  3. **Illustra Single Cell Genomiphi kit**
- **Amplified DNA purification: Agencourt Ampure XP beads**
- **Paired-end library preparation: NEBNext Ultra DNA Library Prep Kit**
- **Sequencing: Illumina HiSeq 2500 rapid run mode, 2x250 bp reads (multiplexed 6 samples/lane)**
- **Controls (no WGA) - library preparation with NEBnext Ultra II DNA library Prep Kit (4 samples/lane)**
- **Illumina adapters removal: Trimmomatic**
- **Human and PhiX sequences prefiltration: BioBloom tools**
- **Taxonomic assignment of reads: Livermore Metagenomics Analysis Toolkit (LMAT)**
- **Trimming of low-quality reads: Trimmomatic**
- **Analysis of trimmed reads: MetaPhlan2**

MAYO CLINIC

Sonicate Fluid Samples Tested and Relative Read Counts (LMAT)

Sample	Site	Microbiology		Qiagen REPLI-g		Illustra Single Cell		Illustra V2	
		Sonicate Fluid	Positive tissue cultures	Total reads		Total reads		Total reads	
				Pathogen reads	Non-pathogen reads	Pathogen reads	Non-pathogen reads	Pathogen reads	Non-pathogen reads
Culture-positive PJI									
980	H	Group C Streptococcus <20 CFUs	1 of 3	27,244,012		36,576,986		25,754,214	
				2,694,906	103	286,577	556	3,075,181	566
982	K	*S. epidermidis* >100 CFUs	3 of 4	28,320,234		30,284,116		26,508,366	
				3,272,895	591	102,934	203	9,539,147	17,625
986	K	*S. aureus* <20 CFUs	1 of 5	26,835,306		28,991,289		27,391,148	
				26,397	77,581	424	308	168,046	240,703
996	H	*B. fragilis*, >100 CFUs	3 of 3	28,388,841		28,745,844		28,904,638	
				155,761	4,853	11,134	1,346	96,154	8,140
1002	K	*C. striatum*, 51-100 CFUs	3 of 3	32,165,206		27,925,551		32,338,186	
				536,155	2,594	5,503	422	1,000,810	53,019
Culture-negative PJI									
984	K	Culture negative	0 of 4	31,616,449		26,240,307		36,808,230	
				NA	2,821	NA	305	NA	16,974

MAYO CLINIC

Sonicate Fluid Samples Tested and Relative Read Counts (LMAT)

Sample	Site	Microbiology		Qiagen REPLI-g		Illustra Single Cell		Illustra V2	
		Sonicate Fluid	Positive tissue cultures	Total reads		Total reads		Total reads	
				Pathogen reads	Non-pathogen reads	Pathogen reads	Non-pathogen reads	Pathogen reads	Non-pathogen reads
Aseptic Failure									
983	K	Culture negative	0 of 3	32,924,419		29,076,492		32,128,178	
				NA	278	NA	522		45,539
987	K	Anaerobic organism, <20 CFUs	1 of 3, *Bacillus* species	30,316,155		30,587,175		27,955,363	
				NA	7,628	NA	265	NA	655,417
Controls									
C. glutamicum	NA	NA	NA	28,121,822		28,973,351		26, 986,400	
				26,912,706	2,145	22,276,446	856	26,149,302	1,311
Ringers	NA	NA	NA	27,488,254		29,531,289		27,810,322	
				NA	24,140	NA	3,560	NA	835,014
WGA no template	NA	NA	NA	25,669,747		53,626,034		24,512,662	
				NA	6,695	NA	46,759	NA	7,818,383

MAYO CLINIC

Taxonomic Identification of Reads
(Performed using LMAT, Grouped by Genus)

Sample	LMAT Identifications (Number of reads)		
	Qiagen REPLI-g	Illustra Single Cell	Illustra V2
Culture-positive PJI			
980	**Streptococcus (2,694,906)**	**Streptococcus (286,577),** Toxoplasma (489)	**Streptococcus (3,075,181),** Pseudomonas (232), Anaerococcus (135)
982	**Staphylococcus (3,272,895),** Propionibacterium (195), Mupapillomavirus (137), Finegoldia (115)	**Staphylococcus (102,934),** Toxoplasma (131)	**Staphylococcus (9,539,147),** Pseudomonas (15,292), Acinetobacter (739), Pseudoperonospora (346), Streptococcus (303), Elizabethkingia (242), Malassezia (176), Propionibacterium (142)
986	Streptococcus (37,268), **Staphylococcus (26,397),** Prevotella (13,472), Haemophilus (11,513), Campylobacter (3,344), Propionibacterium (2,298), Gemella (2,045), Alloprevotella (1,756), Chryseobacterium (1,531), Rothia (1,458), Neisseria (913), Capnocytophaga (911), Acinetobacter (297), Corynebacterium (248), Alloiococcus (156)	**Staphylococcus (424),** Toxoplasma (118)	**Staphylococcus (168,046),** Haemophilus (69,045), Streptococcus (66,370), Pseudomonas (58,244), Corynebacterium (18,344), Elizabethkingia (12,471), Propionibacterium (7,678), Gemella (2,298), Neisseria (2,092), Rothia (1,186), Empedobacter (835), Acinetobacter (479), Fusobacterium (434), Lactobacillus (237), Prevotella (185), Granulicatella (144), Delftia (130), Achromobacter (126)
996	**Bacteroides (155,761),** Clostridium (4,115), Parabacteroides (191), Corynebacterium (168), Blautia (143)	**Bacteroides (11,134),** Toxoplasma (669), Clostridium (454)	**Bacteroides (96,154),** Clostridium (4,052), Pseudomonas (1,483), Yarrowia (563), Corynebacterium (364), Propionibacterium (331), Staphylococcus (345), Parabacteroides (239), Streptococcus (116), Anaerotruncus (104)
1002	**Corynebacterium (536,155),** Lactococcus (2,195), Acinetobacter (187), Propionibacterium (108)	**Corynebacterium (5,503),** Toxoplasma (346)	**Corynebacterium (1,000,810),** Pseudomonas (32,865), Staphylococcus (5,704), Propionibacterium (5,396), Lactococcus (3,008), Streptococcus (2,121), Acinetobacter (1,293), Dermabacter (634), Malassezia (424), Elizabethkingia (382), Achromobacter (224), Actinobaculum (123), Delftia (108), Debaryomyces (102), Arcanobacterium (101)
Culture-negative PJI			
984	Streptococcus (2,425), Acinetobacter (167)	Toxoplasma (230)	Pseudomonas (12,472), Acinetobacter (1,395), Elizabethkingia (936), Alloiococcus (706), Streptococcus (395), Staphylococcus (264), Achromobacter (205), Propionibacterium (169), Delftia (134), Malassezia (111)

MAYO CLINIC

Taxonomic Identification of Reads
(Performed using LMAT, Grouped by Genus)

Sample	LMAT Identifications (Number of reads)		
	Qiagen REPLI-g	Illustra Single Cell	Illustra V2
Aseptic Failure			
983	Streptococcus (352), Gloeocapsa (137)	Toxoplasma (442)	Pseudomonas (42,315), Acinetobacter (788), Elizabethkingia (742), Staphylococcus (630), Streptococcus (475), Propionibacterium (289)
987	Acinetobacter (5,396), Streptococcus (1,179), Prevotella (433), Propionibacterium (346), Kurthia (122)	Toxoplasma (173)	Pseudomonas (642,812), Elizabethkingia (5,283), Propionibacterium (3,271), Peptoniphilus (1,742), Acinetobacter (560), Epilithonimonas (372), Pseudoperonospora (353), Anaerococcus (255), Streptococcus (188), Capnocytophaga (172), Malassezia (158)
Controls			
C. glutamicum	**Corynebacterium (26,912,706),** Rothia (1,070), Streptococcus (545), Neisseria (221), Haemophilus (240)	**Corynebacterium (22,276,446),** Streptococcus (333), Toxoplasma (126)	**Corynebacterium (26,149,302),** Pseudomonas (627), Streptococcus (289)
Ringers	Neisseria (10,575), Streptococcus (8,441), Dolosigranulum (3,912), Haemophilus (716), Propionibacterium (385)	Streptococcus (1,192), Toxoplasma (969), Staphylococcus (929), Propionibacterium (127), Corynebacterium (123)	Pseudomonas (301,181), Staphylococcus (286,646), Streptococcus (135,065), Yarrowia (40,273), Propionibacterium (27,201), Achromobacter (15,251), Elizabethkingia (8,617), Acinetobacter (4,813), Haemophilus (4,423), Alloiococcus (2,610), Granulicatella (2,381), Malassezia (2,557), Delftia (681), Fusarium (671), Corynebacterium (593), Veillonella (498), Alloprevotella (384), Chryseobacterium (216), Dolosigranulum (234), Neisseria (177), Aspergillus (165)
WGA no template	Achromobacter (5,072), Rothia (1,031), Micrococcus (451), Delftia (110)	Sphingomonas (35,596), Staphylococcus (6,414), Propionibacterium (3,596), Peptoniphilus (697), Finegoldia (196), Elizabethkingia (149)	Pseudomonas (5,297,561), Propionibacterium (1,557,410), Staphylococcus (407,691), Lactococcus (297,420), Elizabethkingia (134,333), Malassezia (89,791), Delftia (13,668), Acinetobacter (7,858), Phyllobacterium (2,808), Comamonas (3,175), Chryseobacterium (1,732), Achromobacter (1,734), Corynebacterium (1,061), Rhodococcus (1,231), Micrococcus (452), Polynucleobacter (426), Streptococcus (274), Wolbachia (223), Alternaria (220)

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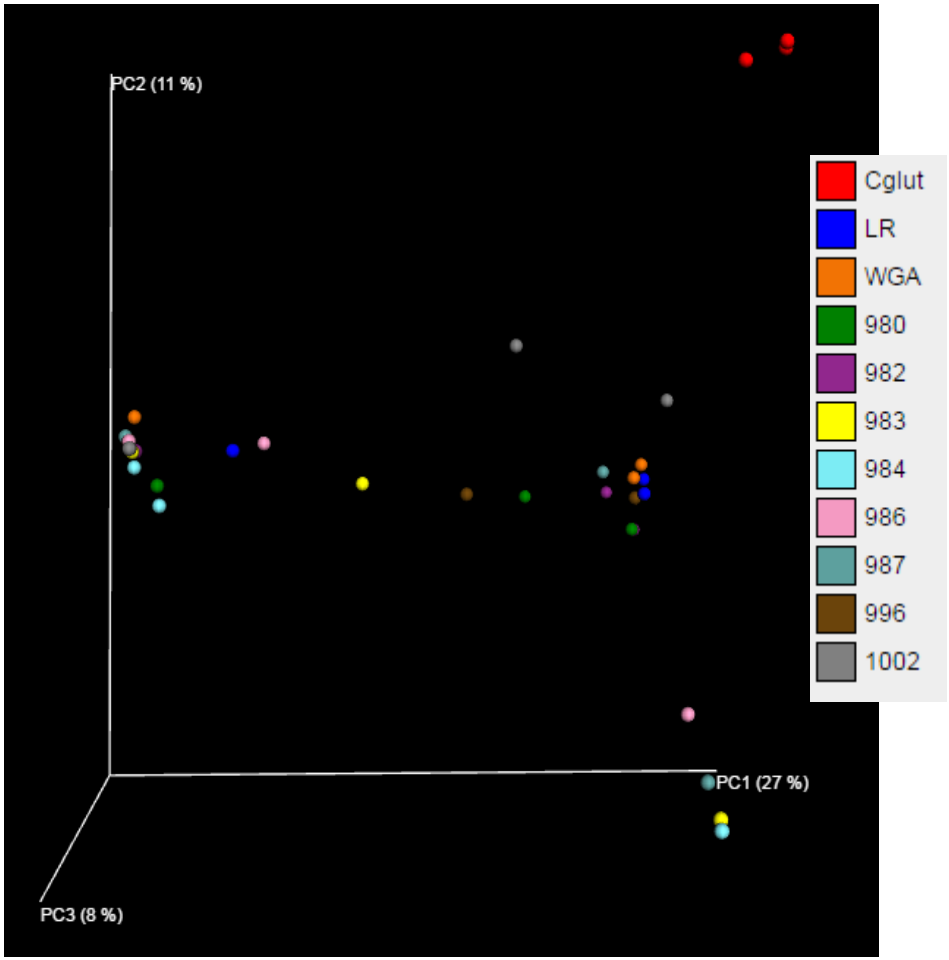
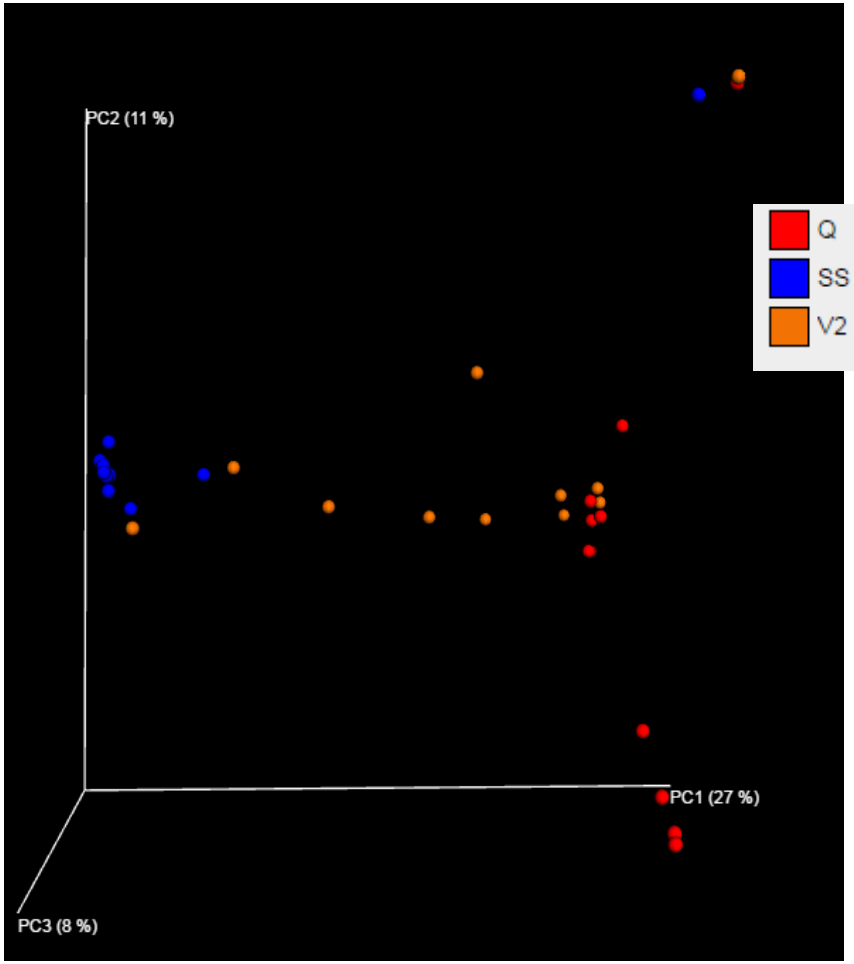
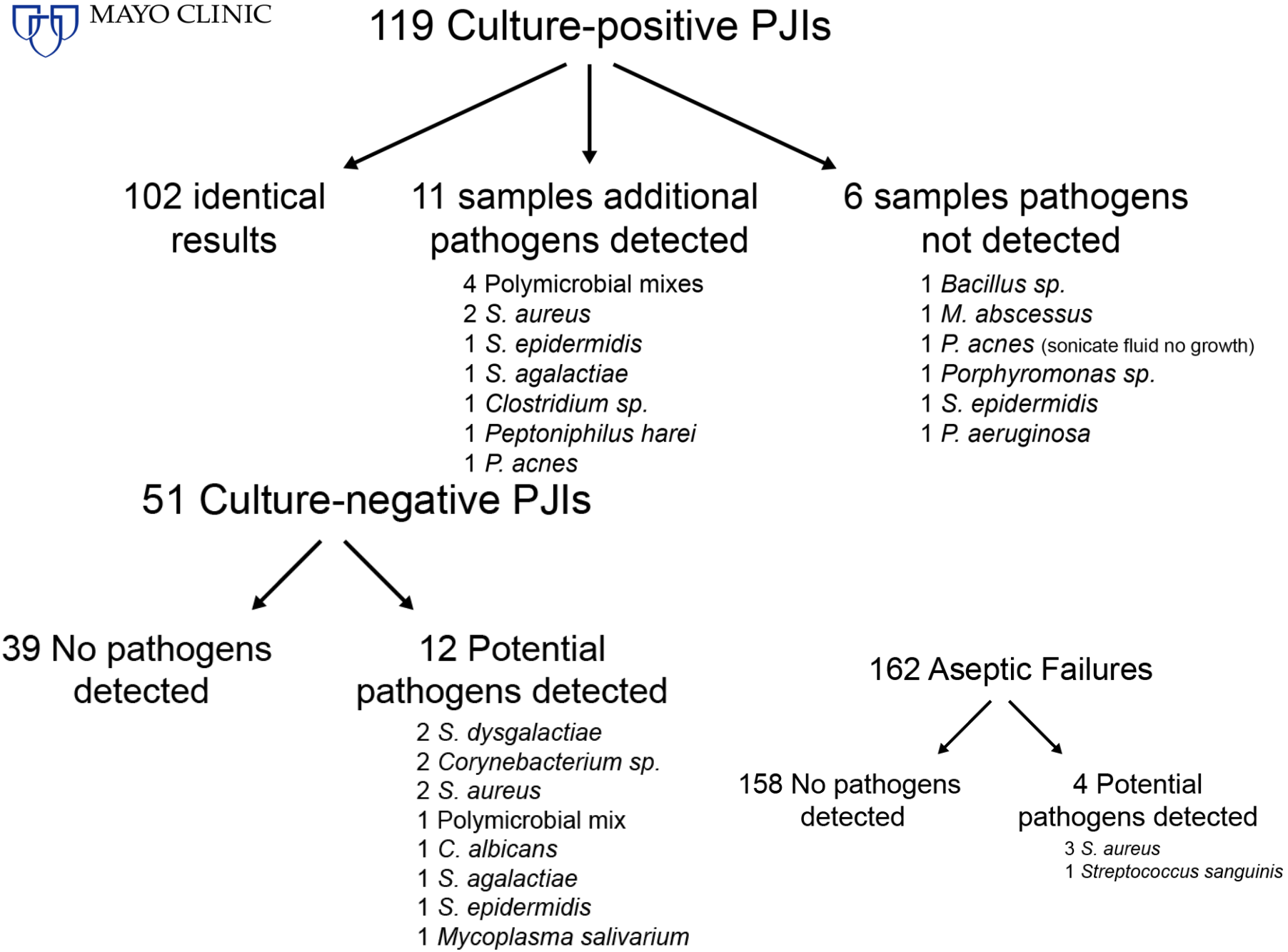
PCoA Plots - β-diversity Between Samples

Fastq files depleted of human and PhiX sequences concatenated and HUMAnN2 (version 0.5.0) used to determine gene abundances of organisms present; QIIME used to calculate β-diversity and generate PCoA plots.





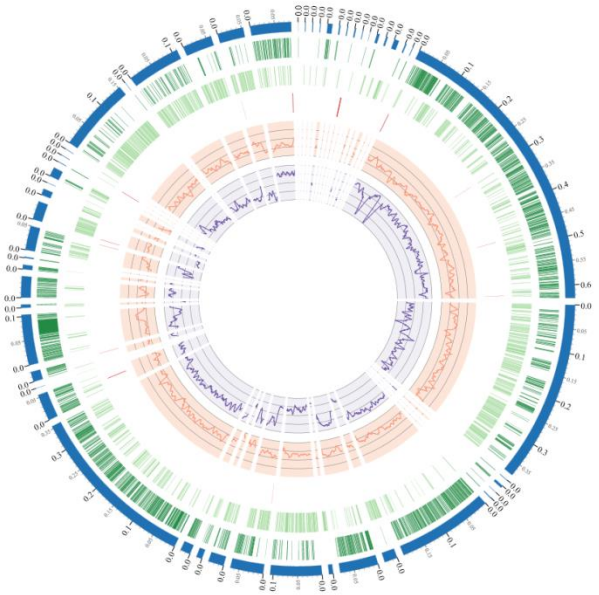
MAYO CLINIC



MAYO CLINIC

S. aureus genome assembly

- **Used PATRIC online tools to assemble and annotate genome**
- **2,822,458 bp genome**
  - **Typically 2.7 to 3.0 Mb**
- **Able to identify antibiotic resistance genes (*mecA*, *ermB*, *norA*)**



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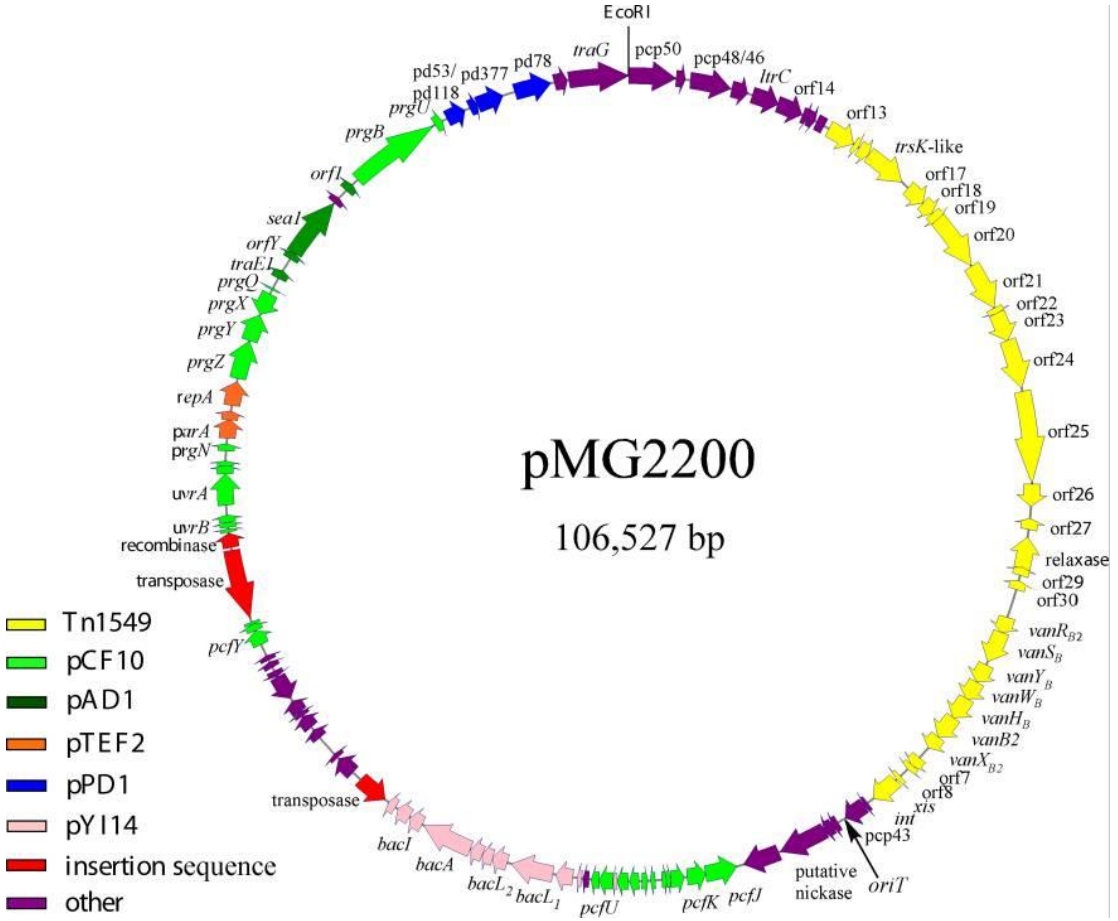
E. faecalis plasmid analysis

- **Reads identified mapping to multiple plasmids, pMG2200 most common**
- **pMG2200: plasmid with vancomycin resistance genes**



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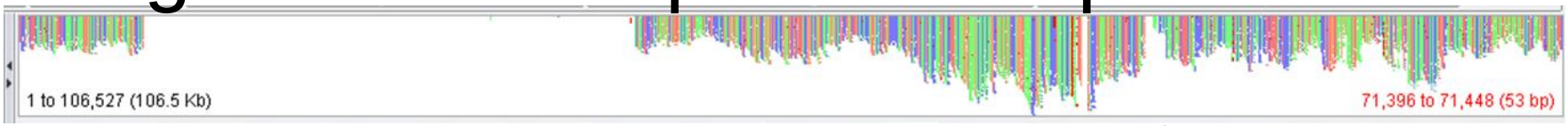
Analysis of pMG2200-Like Plasmid

Alignment of sequences to pMG2200



- **If pull sequences labeled as plasmids, can assemble into single 70,892 bp fragment**
- **High homology to pMG2200 with transposon-like element absent**

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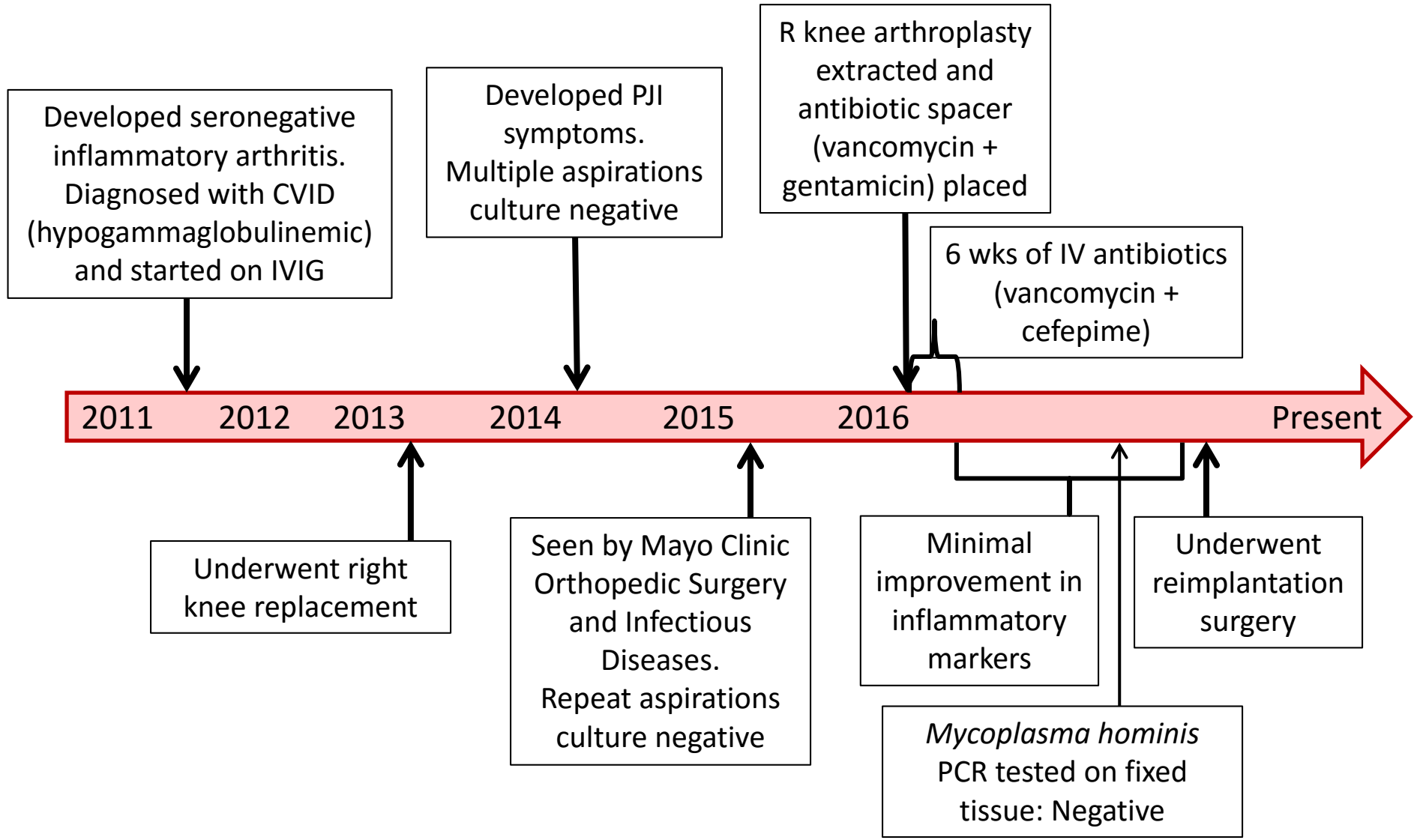
53 yo Man – Right Knee PJI



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Metagenomic Analysis From Resected Arthroplasty

Number of Reads (out of 27,984,652)	Taxonomic Identification
1,796	*Mycoplasma salivarium*
58	*Acinetobacter junii**
19	*Propionibacterium acnes**
5	*Plasmodium gaboni**
5	*Salpingoeca rosetta**
3	*Aspergillus* sp.*

* Known background

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Follow-up Studies

- Second sonicate fluid aliquot from resection surgery:
  - Metagenomic analysis without MolYsis enrichment
  - 16S rRNA gene PCR of sonicate fluid: *M. salivarium*

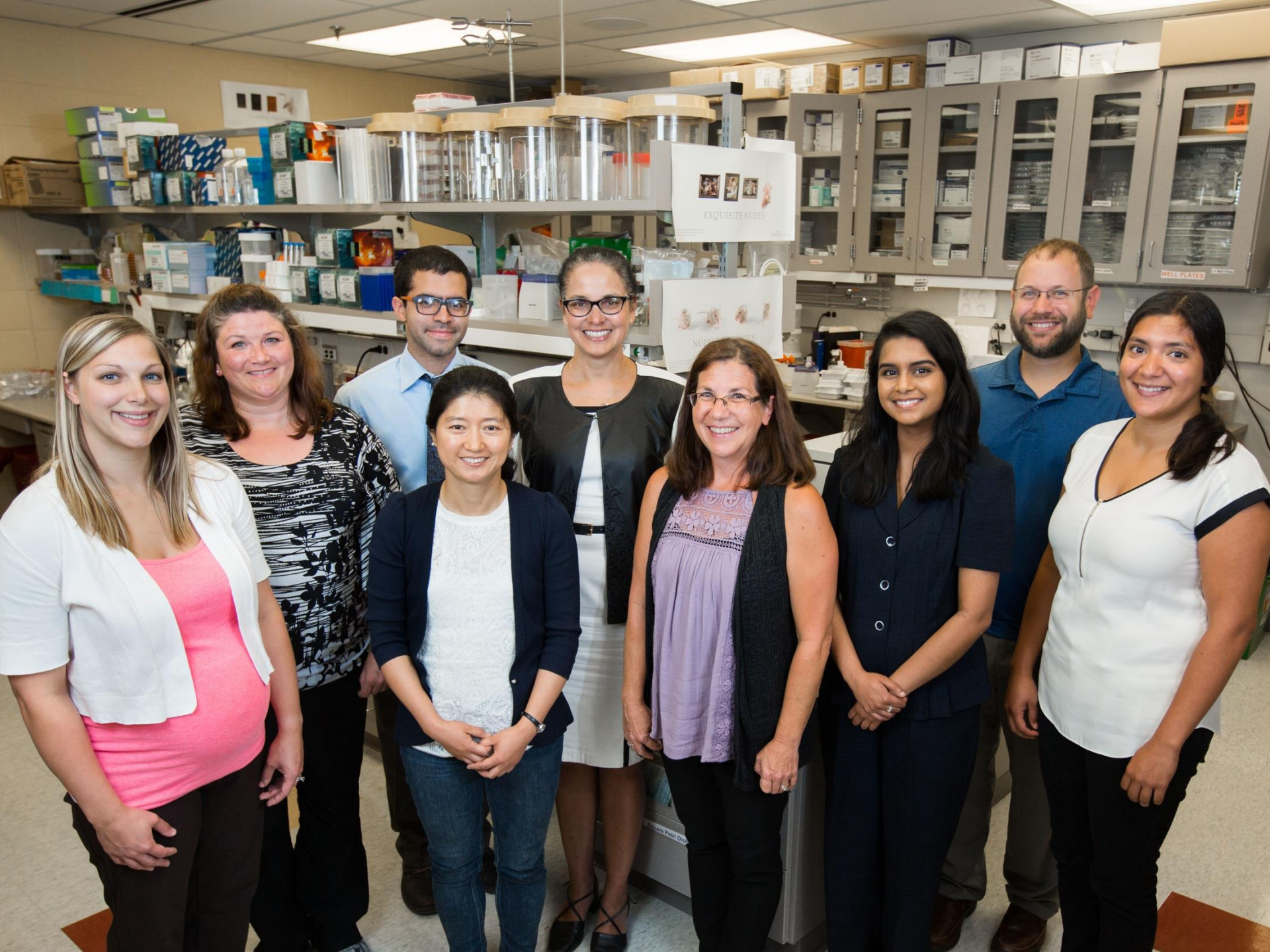
Metagenomic Analysis without MolYsis

Number of Reads (out of 234,088,137)	Taxonomic Identification
19,642	*Mycoplasma salivarium*
371	*Propionibacterium acnes**
247	*Pseudomonas stutzeri**
143	*Toxoplasma gondii**
86	*Corynebacterium* sp.*
73	*Acinetobacter junii**

* Known background

Take-Home Points
"Prosthetic Joint Infection - Think Biofilms"

- Device cultures
  - That's where the biofilms are
- Culture in blood culture bottles
  - Synovial fluid
  - Periprosthetic tissues (just 3 needed!)
- Molecular diagnostics
  - Specimen type matters (tissue poor specimen)
  - Not first-line (? role as "rapid" diagnostics)
  - ?Challenging cases


EXQUISITE NUDES
WELL PLATES

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