

Real-life Experience and Diagnostic Utility of the BioFire Joint Infection PCR Panel in Bone and Joint Infections: Analysis of a Prospective Validation Study

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Table S1. Patient characteristics

	Total (n=57)	Infected (n=39)	Uninfected (n=18)	P value
Gender, male (%)	35 (61.4)	21 (53.8)	14 (77.8)	0.142
Age, years (mean)	64.5	65.9	61.6	0.473
Diabetes (%)	17 (29.8)	13 (33.3)	4 (22.2)	0.537
Cardio- or cerebro-vascular disease (%)	20 (35)	14 (35.9)	6 (33.3)	1
Chronic kidney disease (%)	8 (14)	6 (15.4)	2 (11.1)	1
Inflammatory arthritis (%)	1 (1.8)	1 (2.6)	0 (0)	-
Cancer (%)	5 (8.8)	4 (10.3)	1 (5.6)	1
Immunocompromised (%)	3 (5.3)	2 (5.1)	1 (5.6)	1
Sample type (%)				0.02
Tissue sample	23 (40.4)	20 (51.3)	3 (16.7)	
Fluid sample	34 (59.6)	19 (48.7)	15 (83.3)	
Sample obtainment method (%)				0.012
Arthrocentesis	30 (52.6)	16 (41)	14 (77.8)	
Intra-operative	27 (47.4)	23 (59)	4 (22.2)	
Suspected infection site¹ (%)				
Bone	13 (22.8)	11 (28.2)	2 (11.1)	0.124
Joint	44 (77.2)	28 (71.8)	16 (88.8)	0.191
Native	22 (38.6)	14 (35.9)	8 (44.4)	1
Prosthetic	22 (38.6)	14 (35.9)	8 (44.4)	
Soft tissue	2 (3.5)	2 (5.1)	0 (0)	1

Suspected post-operative infection (%)	33 (57.9)	24 (61.5)	9 (50)	0.565
Multiple prior revision surgeries (%)	8 (24.2)	6 (25)	2 (22.2)	1
Antibiotics in preceding 14 days (%)	20 (35.1)	14 (35.9)	6 (33.3)	1
Blood leukocyte count, 10⁹/L (mean)	10.2	10.9	8.5	0.141
Blood C-reactive protein, mg/L (mean)	138	162	74	0.007
Arthrocentesis performed (%)	34 (59.6)	20 (51.3)	14 (77.8)	0.083
Synovial fluid leukocyte count, 10⁹/L (mean)	54.9	78	24.1	0.003
Synovial fluid neutrophil, percentage (mean)	78.8	84.6	71.5	0.172
Surgery performed (%)	40 (70.1)	35 (89.7)	5 (27.7)	<0.001
No infection found	1 (1.7)	0 (0)	1 (5.5)	
Debridement	32 (56.2)	29 (74.3)	3 (16.7)	
One-stage joint exchange	1 (1.7)	1 (2.6)	0 (0)	
Two-stage joint exchange	5 (8.8)	5 (12.8)	0 (0)	
Intramedullary nail exchange	1 (1.7)	0 (0)	1 (5.5)	
Empirical antibiotics administered (%)	45 (78.9)	37 (94.9)	8 (44.4)	<0.001
Final diagnosis¹				n/a
Native joint septic	14	14	0	

arthritis				
Prosthetic joint infection	14	14	0	
Spinal infection	8	8	0	
Osteomyelitis	1	1	0	
Device-associated osteomyelitis	2	2	0	
Soft tissue infection	9	9	0	
Uninfected	18	0	18	
Hospital length of stay, days (mean)	15	20	5	<0.001
Re-operation performed (%)	7 (12.3)	7 (17.9)	0 (0)	0.565
Length of follow-up, days (mean)	44	52	27	0.015
Outcome at last follow-up (%)				0.180
No evidence of infection	46 (80.7)	29 (74.3)	17 (94.4)	
Persistent or recurrent infection	7 (12.3)	6 (15.4)	1 (5.6) ²	
Death	4 (7)	4 (10.3)	0 (0)	
Ongoing antibiotics at last follow-up (%)	17 (29.8)	16 (41)	1 (5.6)	0.011

¹ More than one suspected infected site or diagnosis may apply to each patient

² A single patient who was uninfected at index event later developed persistent septic arthritis

Table S2. Pathogens identified in infected cases

<i>Staphylococcus aureus</i>	6
<i>Cutibacterium acnes</i>	3
<i>Staphylococcus epidermidis</i>	2
<i>Streptococcus pyogenes</i>	2
<i>Streptococcus agalactiae</i>	2
<i>Streptococcus dysgalactiae</i>	2
<i>Escherichia coli</i>	2
<i>Enterobacter cloacae complex</i>	2
<i>Pseudomonas aeruginosa</i>	2
<i>Klebsiella pneumoniae</i>	1
<i>Klebsiella aerogenes</i>	1
<i>Aggregatibacter aphrophilus</i>	1
<i>Staphylococcus hominis</i>	1
<i>Streptococcus intermedius</i>	1
<i>Streptococcus gallolyticus</i>	1
<i>Finegoldia magna</i>	1
<i>Bacteroides fragilis</i>	1
<i>Anaerococcus spp.</i>	1
<i>Ureaplasma urealyticum</i>	1
Mixed flora	1

Table S3. Description of BioFire-negative infected cases (n=17)

Case	Description
1	<i>Staphylococcus epidermidis</i> in culture and 16s rRNA PCR
2	<i>Cutibacterium acnes</i> in culture
3	<i>Staphylococcus epidermidis</i> in culture and 16s rRNA PCR
4	<i>Cutibacterium acnes</i> in culture
5	<i>Staphylococcus aureus</i> ¹ in culture
6	<i>Enterobacter cloacae</i> ¹ in culture and 16s rRNA PCR
7	<i>Cutibacterium acnes</i> in culture
8	<i>Aggregatibacter aphrophilus</i> in blood culture and tissue 16s rRNA PCR
9	<i>Ureaplasma urealyticum</i> in 16s rRNA PCR
10	<i>Staphylococcus hominis</i> in 16s rRNA PCR
11	BioFire, culture and 16s rRNA PCR were negative
12	BioFire, culture and 16s rRNA PCR were negative
13	BioFire, culture and 16s rRNA PCR were negative
14	BioFire, culture and 16s rRNA PCR were negative
15	BioFire, culture and 16s rRNA PCR were negative
16	BioFire, culture and 16s rRNA PCR were negative
17	BioFire, culture and 16s rRNA PCR were negative

¹ Organism is included in the BioFire panel

Table S4. Description of BioFire – culture discrepant cases (n=12)

Case	Infection status	BioFire result	Culture result	Description
1	Infected	Negative	Positive	<i>Staphylococcus epidermidis</i> in culture and 16s rRNA PCR
2	Infected	Negative	Positive	<i>Cutibacterium acnes</i> in culture
3	Infected	Negative	Positive	<i>Staphylococcus epidermidis</i> in culture and 16s rRNA PCR
4	Infected	Negative	Positive	<i>Cutibacterium acnes</i> in culture
5	Infected	Negative	Positive	<i>Staphylococcus aureus</i> in culture
6	Infected	Negative	Positive	<i>Enterobacter cloacae</i> in culture and 16s rRNA PCR
7	Infected	Negative	Positive	<i>Cutibacterium acnes</i> in culture
8	Infected	Positive	Negative	<i>Streptococcus</i> spp. in BioFire, <i>Streptococcus dysgalactiae</i> in 16s rRNA PCR, recent antibiotic treatment
9	Infected	Positive	Negative	<i>Streptococcus</i> spp. in BioFire, <i>Streptococcus dysgalactiae</i> in 16s rRNA PCR, recent antibiotic treatment
10	Uninfected	Negative	Positive	<i>Cutibacterium acnes</i> in single specimen culture
11	Uninfected	Negative	Positive	<i>Bacillus</i> spp. in single specimen culture
12	Uninfected	Negative	Positive	<i>Cutibacterium acnes</i> in single specimen culture

Table S5. Diagnostic modalities sensitivity rates according to specimen type, obtainment method, infection site and recent antibiotic exposure

Modality	Fluid sample	Tissue sample	Arthrocentesis	Intra-operative	Native joint	Prosthetic joint	Bone	Soft tissue	Recent antibiotics	No recent antibiotics
BioFire	58%	55%	63%	52%	43%	71%	44%	100%	62%	54%
Gram stain	26%	40%	13%	39%	29%	36%	22%	100%	31%	35%
Culture	68%	70%	63%	74%	43%	86%	67%	100%	62%	73%
16s rRNA PCR	64%	81%	64%	79%	45%	80%	100%	100%	82%	68%

Supplementary S6. 16s rRNA procedure

DNA Extraction

DNA Extraction was performed using Seegene's STARMAG (Seegene Inc., S. Korea) on a robotic Hamilton liquid handler. Homogenized ground solid tissue or synovial fluid were used according to availability in each case.

Initial PCR

5ul of DNA was used as template for initial PCR.

PCR reaction was as follows:

Reagent	ul
Roche Sybr ready-mix	15
16s F primer 10um	1
16s R primer 10um	1
DNA template	5
h20	8
total	30

PCR Thermocycler Program was as follows:

Temperature	Time	
95	5	min
95	15	sec
58	30	sec
72	30	sec
repeat	x29	
72	3	min

Primers used for 16s (~750bp):

16SAF CCAGACTCCTACGGGAGGCAG

16SAR ACATTTCAACAACACGAGCTGACGA