Comparing microbes detected by culture, next-generation sequencing, multiplex PCR, and antigen immunoassay in matched synovial fluid testing

Craig D Tipton[1], Andrew Bennett[1], Jacob Ancira[1,2], Caleb D Phillips[2], and Edward J McPherson[3]

[1]MicroGen DX, Lubbock, TX, United States, [2]Department of Biological Sciences, Texas Tech University, Lubbock, TX, United States, [3]Department of Orthopedic Surgery, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, United States

Introduction:

- Identifying an organism preoperatively provides clarity in treatment.
- Multiple testing strategies are now commonly used for characterizing microbes, but limited data exist to understand their comparability and reliability [1-3].

Primary Objectives:

a) Compare detection rates of microbes by 4 common microbial testing strategies
b) Evaluate the concordance of each test for mutually detectable microbes.
c) Quantify species-level accuracy of NGS against culture-positive cohort.

Methods:

- Retrospective diagnostic comparison of hip and knee synovial fluid aspirations performed by single surgeon split for parallel testing by two commercial labs.
- PJI classified based modified ICM-2018 Shohat definition [4], using synovial WBC (3 pts), PMN% (2 pts), CRP (1 pt), α-defensin (3 pts), and positive culture or NGS (2 pts).
- Description of microbial testing strategies used:
  - **NGS (16S+ITS):** V1-V2 16S rRNA gene for bacteria and ITS3-4 sequencing for fungi [1,5]
  - **Multiplex PCR (mPCR)**: Limited qPCR series provided as part of NGS panel, targeting 8 organisms: *Enterococcus faecalis*, *Streptococcus pyogenes*, *Cutibacterium acnes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Candida albicans*.
  - (**Fluid) culture**: Aerobic and anaerobic culture incubated for 7-14 days.
  - **Antigen immunoassay**: 4 targets; *Staphylococcus* sp, *C. acnes*, *Enterococcus* sp., and *Candida* sp.. [2-3]
- Microbial composite reference: The reference was defined as detection of a common microbe by two or more independent testing methods.
- Concordance was assessed through detection rates, percent agreement, cohen's Kappa, and McNemar testing.
- To evaluate performance of species-level reporting, NGS was compared to culture results by sensitivity, specificity, and accuracy.

Key Result #1: NGS and Antigen Immunoassay Improve Pre-Op Sensitivity for PJI Compared to Conventional Tests, but with Varying Trade-offs in Decreasing Specificity (Table 1)

- Antigen sensitivity highest (73%), followed by NGS (51%), culture (29%) and mPCR (10%)
- Antigen specificity lowest (91%), followed by NGS (94%), culture (97%), and mPCR (99%)

Table 1. Positivity rates and performance versus ICM-2018 (Shohat) reference for PJI

Diagnostic	Positive n (%)	Sensitivity (95%CI)	Specificity (95%CI)
Fluid Culture	64 (11%)	**0.29** [0.194-0.408]	**0.972** [0.928-0.989]
NGS (16s+ITS)	116 (20%)	**0.508** [0.408-0.608]	**0.94** [0.899-0.966]
Multiplex PCR	23 (4.0%)	**0.098** [0.056-0.168]	**0.987** [0.97-0.994]
Antigen	169 (30%)	**0.731** [0.641-0.805]	**0.906** [0.822-0.953]
ICM-18 (S)	183 (32%)	-	-

Key Result #2: Comparing detection rates of mutually observable microbes generally shows congruence between NGS, culture, and mPCR, but not antigen testing.

- Culture unable to detect *C. acnes*, whereas molecular methods generally report higher rates of *C. acnes* and *Candida* (Table 2, Figure 1).
- Only 42% of antigen detections can be reproduced by at least one other method (Tables 2, 3), with largest discrepancies driven by *Staphylococcus*, *Candida*, and *Enterococcus*.
- Antigen kappa values ranged -0.007:0.599, suggesting poor to moderate agreement (Table 3)
- NGS and culture report high kappa scores ranging 0.47:1 suggesting moderate to perfect agreement with other tests, except for *C. acnes* by culture (k = 0).

Table 2. Detection rates for mutually observable pathogen groups by each diagnostic

Taxa	Culture	NGS	mPCR	Antigen	2+ Tests Agree
Staphylococcus sp.	45 (7.9%)	68 (12.0%)	12 (2.1%)	126 (22.1%)	**66 (11.6%)**
Cutibacterium acnes	0 (0%)	13 (2.3%)	5 (0.9%)	4 (0.7%)	**4 (0.7%)**
Candida sp.	8 (1.4%)	6 (1.1%)	0 (0%)	45 (7.9%)	**10 (1.8%)**
Enterococcus sp.	2 (0.4%)	3 (0.5%)	1 (0.2%)	19 (3.3%)	**2 (0.4%)**

Table 3. Concordance of each test against (>1) reference for pathogen focus groups

Pathogen Group	Diagnostic	Agreement (%)	Cohen's K	K Interpretation	McNemar P-val
Staphylococcus sp.	**Culture**	95.958	0.771	Substantial	<0.0001
	NGS	98.946	0.949	Almost Perfect	0.68309
	mPCR	90.51	0.282	Fair	<0.0001
	Antigen	88.536	0.599	Moderate	<0.0001
Candida sp.	**Culture**	99.649	0.887	Almost Perfect	0.4795
	NGS	99.297	0.747	Substantial	0.13361
	mPCR	98.243	0	Poor	NA
	Antigen	93.827	0.345	Fair	<0.0001
Cutibacterium acnes	**Culture**	99.297	0	Poor	NA
	NGS	98.418	0.465	Moderate	0.00766
	mPCR	99.824	0.888	Almost Perfect	1
	Antigen	98.589	-0.007	Poor	1
Enterococcus sp.	**Culture**	100	1	Perfect	NaN
	NGS	99.824	0.799	Substantial	1
	mPCR	99.824	0.666	Substantial	1
	Antigen	97.002	0.185	Slight	0.0001

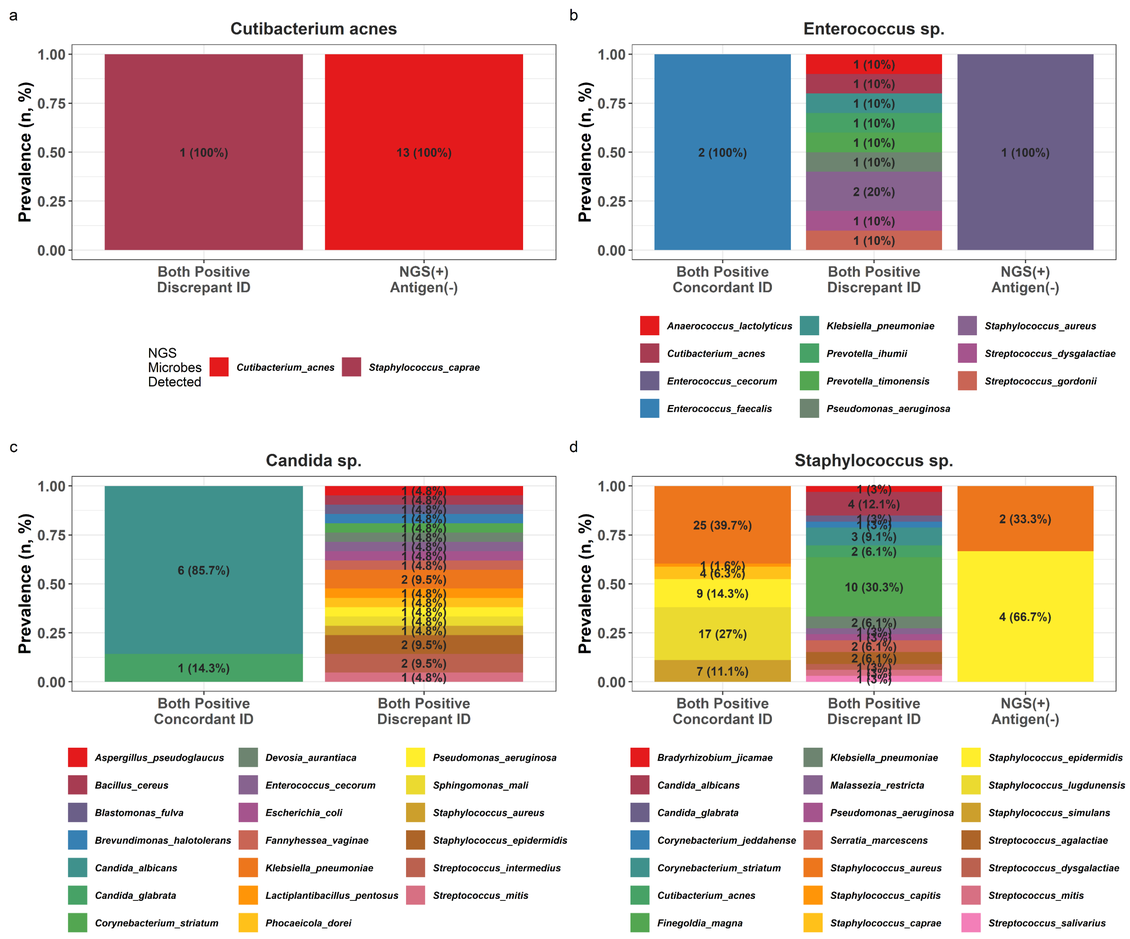

Figure 1. Comparing NGS with antigen immunoassay panel results for select comparable taxa: (a) *Cutibacterium acnes*, (b) *Enterococcus* sp., (c) *Candida* sp., and (d) *Staphylococcus* sp. Colored slices within each bar graph indicate species reported by NGS in each scenario.

Key Result #3: NGS shows 98.4% accuracy to species level in culture-positive PJI.

- Aggregated performance statistics show high sensitivity and specificity for NGS reproducing findings by culture (Table 4).
- In 6 instances, culture reported simply coagulase negative Staphylococcus and matching specific species (S. lugdunensis, S. simulans) by NGS were treated as (TP). Otherwise non-matching species names within same genus were treated as mismatches (FP or FN).

Organism	TN	TP	FP	FN	Sensitivity	Specificity	Accuracy
Bacillus cereus	52	1	0	0	1 (0.207, 1)	1 (0.931, 1)	1 (0.932, 1)
Candida albicans	50	3	0	0	1 (0.439, 1)	1 (0.929, 1)	1 (0.932, 1)
Corynebacterium striatum	51	2	0	0	1 (0.342, 1)	1 (0.93, 1)	1 (0.932, 1)
Enterococcus faecalis	51	2	0	0	1 (0.342, 1)	1 (0.93, 1)	1 (0.932, 1)
Klebsiella pneumoniae	51	1	1	0	1 (0.207, 1)	0.981 (0.899, 0.997)	0.981 (0.901, 0.997)
Pseudomonas aeruginosa	47	5	0	1	0.833 (0.436, 0.97)	1 (0.924, 1)	0.981 (0.901, 0.997)
Staphylococcus aureus	41	11	0	1	0.917 (0.646, 0.985)	1 (0.914, 1)	0.981 (0.901, 0.997)
Staphylococcus capitis	51	0	1	1	0 (0, 0.793)	0.981 (0.899, 0.997)	0.962 (0.872, 0.99)
Staphylococcus caprae	48	3	1	1	0.75 (0.301, 0.954)	0.98 (0.893, 0.996)	0.962 (0.872, 0.99)
Staphylococcus epidermidis	44	6	2	1	0.857 (0.487, 0.974)	0.957 (0.855, 0.988)	0.943 (0.846, 0.981)
Staphylococcus hominis	52	0	0	1	0 (0, 0.793)	1 (0.931, 1)	0.981 (0.901, 0.997)
Staphylococcus lugdunensis *	43	10	0	0	1 (0.722, 1)	1 (0.918, 1)	1 (0.932, 1)
Staphylococcus simulans *	47	6	0	0	1 (0.61, 1)	1 (0.924, 1)	1 (0.932, 1)
Pooled Diagnostic	**628**	**50**	**5**	**6**	**0.893 (0.785, 0.95)**	**0.992 (0.982, 0.997)**	**0.984 (0.972, 0.991)**

Discussion:

- Current study reflects the first major comparison in the performance of NGS and the antigen immunoassay panel to date.
- Among molecular tests compared, the antigen immunoassay panel and NGS showed improvements in overall sensitivity for microbial detection in PJI (Table 1).
- NGS shows good improvements in sensitivity while maintaining concordance with other tests and providing species-level clarity (Tables 2-4, Figure 1).
- The evaluated NGS application is well positioned to be used as a diagnostic criterion for PJI weighted similar to culture, given the improvements in sensitivity and reliability in findings.
- Antigen immunoassay panel offers strong sensitivity, but was the least reproducible among all methods evaluated and may not be specific to the intended targets (Tables 1-3, Figure 1).
- A positive antigen immunoassay finding should be treated as a signal to verify through further testing, such as specialized culture or NGS.

Key Takeaways:

- **Antigen immunoassay has poor agreement with other microbial tests**
- **NGS offers best improvement in sensitivity and identification while maintaining fidelity with other microbial tests**

Recommendation:

- **A positive NGS should be weighted similarly as culture for PJI diagnosis**

References:

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