

Same-Specimen NGS vs Culture and Biomarkers for Diagnosing PJI from Aspirated Synovial Fluid

Edward J McPherson¹, Jacob Ancira^{2,3}, Andy Bennett², Khalid Omeir^{2,3}, Craig D Tipton²

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

Introduction:

- **Preoperative** PJI diagnosis remains elusive because diagnostic schemes are weighted more on intraoperative parameters. When applied preoperatively, patient’s scores for PJI are frequently inconclusive across all systems [1].
- Furthermore, culture-negative aspirations occur in 28 to 46% of infected joints [2,3].
- Molecular methods to detect microbes have been developed to help improve diagnostic performance. Of these methods, Next Generation Sequencing (NGS) provides the broad identification of microbes in an efficient timeframe [3,4,5].
- Structural Equation Modelling (SEM) is a method of multivariate analysis to improve the diagnostic power of biomarker values in PJ, eliminating threshold values [6].
- **Primary Objectives:**
 1. Compare NGS and culture performance in PJI using **same specimen** synovial fluid
 2. Assessing the power of SEM for diagnosing PJI, leveraging the full range of a test values rather than rigid biomarker thresholds.

Methods:

- 569 hip and knee arthroplasty synovial fluid specimens were obtained from **painful** arthroplasties **concurrently submitted** to two independent commercial labs.
- Synovial biomarker profiling:
 - White blood cell (**WBC**) count, and polymorphonuclear (**PMN**) leukocyte percentage, C-reactive protein (**CRP**), and alpha defensin (**AD**)
- Microbial Identification
 - NGS profiling consisting of V1-V2 16S rRNA sequencing for bacteria, ITS 3-4 sequencing for fungi.
 - Synovial fluid was placed in aerobic and anaerobic culture **bottles** containing enriched liquid growth media for 7 days and extended to 14 days if fastidious organisms were suspected.
- PJI Diagnosis
 - Scored **only** on **synovial criteria** employing a modified ICM-18 score with two modifications: 1) Shohat modification with Synovial CRP = 1 point[7]. 2) a **positive NGS** was **weighted equal** to **culture** (2 points).
 - Samples with a score ≥6 were defined as infected.
- Statistical Analysis
 - Diagnostic performance metrics were calculated with 95% confidence limits accounting for same patient repeated sampling.
- Structural Equation Modelling (SEM)
 - SEM for PJI uses NGS, Culture, and biomarkers in combination.
 - A **latent variable** is a **directly unmeasurable** variable (PJI) estimated from biomarkers, each weighted by a β coefficient.
 - Models were specified using the biomarkers (CRP, WBC, and PMN) loading onto a latent variable to define Infection (base model), in the spirit of the **Unified PJI Definition**
 - The biomarker model was modified to include culture or NGS positivity for the respective model with NGS defined infection including a regression of **microbial load** values and **time in transit** to account for sample quality.
 - SEM was optimized in an initial training cohort and performance calculated in a second validation cohort.

Results:

- A **majority** of specimens originated from the **knee** (447, 79%) vs hip (122, 21%).
- Among **all samples**, NGS was positive in 20% and culture positive in 11%.
- 271 (48%) specimens showed all tests as normal: normal biomarkers, negative Cx & NGS.

Table 1. Patient Demographics and primary results by joint.

Characteristic	Overall, N = 569	Hip, N = 122	Knee, N = 447	p-value
Mean Age (SD)	71 (64, 77)	71 (65, 73)	71 (64, 78)	0.047 *
Sex (Male)	319 (56%)	56 (46%)	263 (59%)	0.011 *
NGS Positivity	116 (20%)	32 (26%)	84 (19%)	0.071
Culture Positivity	64 (11%)	23 (19%)	41 (9.2%)	0.003 **
PJI (ICM-18/Shohat)	183 (32%)	45 (37%)	138 (31%)	0.2

KEY Result 1: (See Table 2 in top-right)

- NGS showed **higher sensitivity than culture**, with comparable specificity and accuracy.
- NGS specificity is most comparable to that of PMN and **outperforms CRP and Alpha defensin**.
- Culture sensitivity was clearly outperformed by all biomarkers.
- **NGS was 1.8× more sensitive** than culture and provided greater depth and breadth of microbial detection.

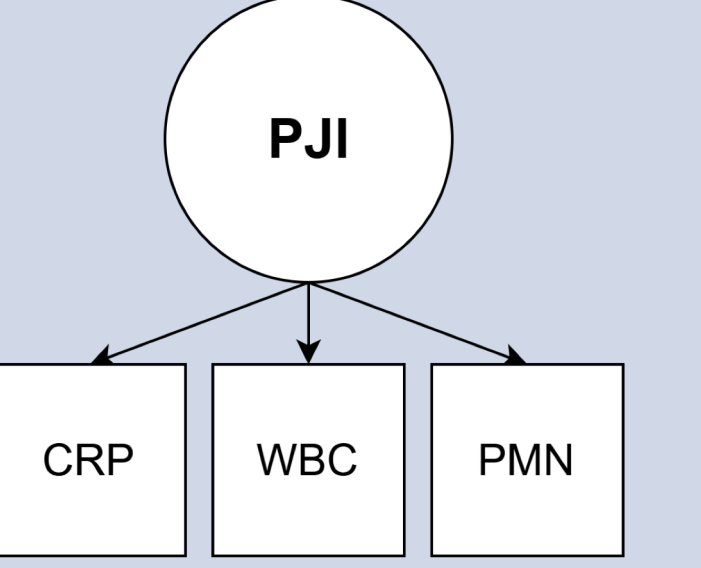
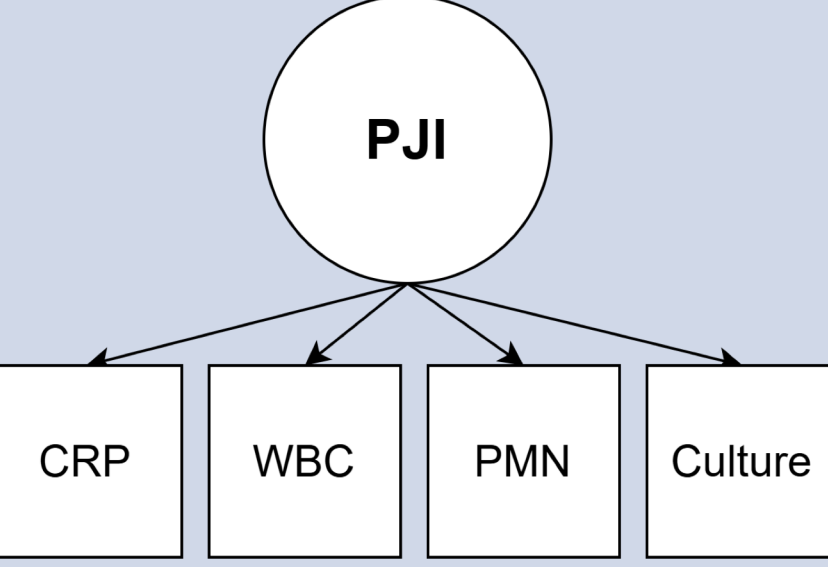
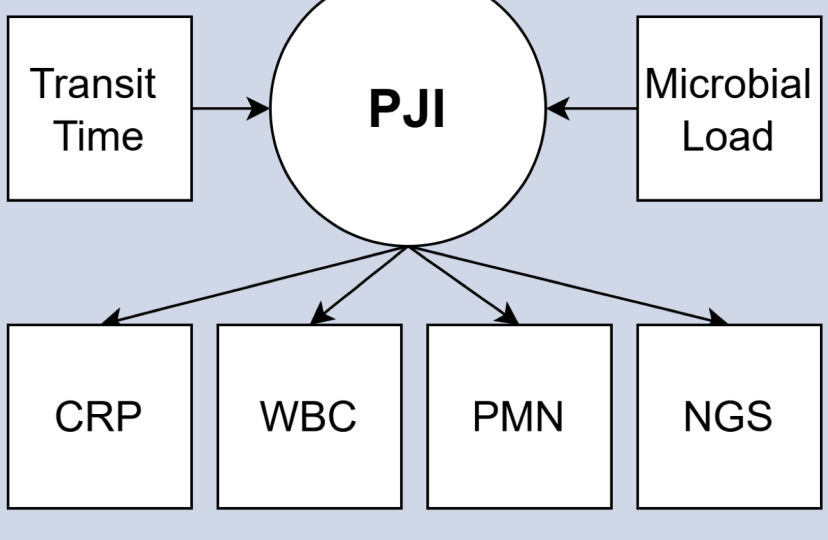
Table 2. Comparing diagnostic performance of individual synovial fluid test results to synovial fluid ICM-18 modified criteria for PJI diagnosis

Diagnostic	Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)	PPV (95%CI)	NPV (95%CI)
Microbial ID					
Fluid Culture	0.29 [0.194-0.408]	0.972 [0.928-0.989]	0.752 [0.697-0.8]	0.828 [0.673-0.919]	0.743 [0.685-0.793]
NGS (16s+ITS)	0.508 [0.408-0.608]	0.94 [0.899-0.966]	0.801 [0.761-0.837]	0.802 [0.705-0.872]	0.801 [0.752-0.843]
Host Response					
WBC	0.77 [0.692-0.834]	0.982 [0.963-0.991]	0.914 [0.883-0.937]	0.953 [0.907-0.977]	0.9 [0.857-0.931]
PMN%	0.951 [0.873-0.982]	0.948 [0.91-0.971]	0.949 [0.918-0.969]	0.897 [0.829-0.94]	0.976 [0.937-0.991]
Alpha Defensin	0.967 [0.907-0.989]	0.902 [0.82-0.948]	0.923 [0.871-0.955]	0.823 [0.719-0.894]	0.983 [0.95-0.994]
CRP	0.689 [0.572-0.785]	0.865 [0.804-0.909]	0.808 [0.764-0.846]	0.708 [0.615-0.786]	0.854 [0.795-0.898]

KEY Result 2: (See Table 3 below)

- SEM models incorporating microbial data (NGS & Culture) demonstrate improved performance compared to a biomarker-only model.
- **NGS SEM models outperform culture by every metric** regardless of optimization method.
- **Specificity optimization** demonstrated the best performance of all models improving NGS PPV to 94.1% and Specificity to 97.4%

Table 3. Comparing diagnostic performance of SEM models modified by inclusion of microbial results to ICM-18 synovial criteria diagnosis.

Model Type	Model Diagram	Method	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
Biomarkers CRP/WBC/PMN		Youden (Balanced)	0.812 [0.723, 0.878]	0.851 [0.793, 0.895]	0.736 [0.645, 0.81]	0.899 [0.846, 0.935]
		Specificity (Priority)	0.562 [0.463, 0.657]	0.957 [0.918, 0.978]	0.871 [0.766, 0.933]	0.811 [0.754, 0.857]
Culture		Youden (Balanced)	0.896 [0.819, 0.942]	0.941 [0.898, 0.967]	0.887 [0.808, 0.935]	0.947 [0.904, 0.971]
		Specificity (Priority)	0.844 [0.758, 0.903]	0.947 [0.905, 0.971]	0.89 [0.809, 0.939]	0.922 [0.876, 0.952]
NGS		Youden (Balanced)	0.977 [0.919, 0.994]	0.947 [0.906, 0.971]	0.894 [0.816, 0.941]	0.989 [0.961, 0.997]
		Specificity (Priority)	0.93 [0.856, 0.968]	0.974 [0.94, 0.989]	0.941 [0.87, 0.975]	0.969 [0.933, 0.986]

Discussion:

- **NGS** identifies infection at **nearly twice the rate of culture** without a meaningful loss of specificity, with further work needed to improve preoperative microbial identification.
- SEM provides a novel framework to better diagnose PJI and overcome harsh biomarker thresholds in previous diagnostic schemes.
- A positive **NGS** result **merits weighting comparable to a positive culture** in future PJI definitions.

Key Takeaways:

1. NGS shows improved preoperative microbial identification and sensitivity to PJI.
2. SEM will be a future method improving PJI diagnosis as it overcomes rigid thresholds.
3. A web based calculator using SEM criteria is being developed to define PJI

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