

382 **Figure Legends**

383 **Figure 1:** Flow chart detailing procurement of patient samples.

384 **Figure 2:** Comparison between NGS and Culture results based on MSIS criteria.

385 **Figure 3:** The plot shows the percent of bacteria present in the sample of culture positive
386 compared to culture negative patients. In patients that were clearly infected (with more than two
387 positive cultures), one or two organisms were predominant in the majority of cases and they
388 composed high percent of the sample.

Table 1

Table 1: Demographic characteristics and MSIS criteria for revision cases included in the study (n=65).

Data are presented as mean values (SD) or as numbers (%).

Characteristic	MSIS positive. (n=28) (range) or number (%).	MSIS negative. (n=37) (range) or number (%)	P value
Age (SD),yr	63.3±11.2	64.7±10.4	0.62
Sex			0.79
Male	19 (67.9%)	23 (62.2%)	
Female	9 (32.1%)	14 (37.8%)	
Body-mass index (kg/m ²)	32.6±7.0	30.1±6.2	0.14
Joint			0.45
Knee	15 (53.6%)	24 (64.9%)	
Hip	13 (46.4%)	13 (35.1%)	
MSIS criteria			
Major			
Sinus tract	4 (14.3%)	0	0.03
2 Culture positive	17 (60.7%)	0	<0.000
Minor			
Serum ESR (mm/hr)	66.0±29.6	20.5±15.3	<0.000
Serum CRP(mg/dL)	8.6±8.9	0.6±0.8	<0.000
Synovial fluid WBC count (cells/μL)	45201.2±68766.3	1379.3±1952.2	0.03
Synovial fluid PMN %	89.2±8.5	38.4±15.8	<0.000

Table 2

Table 2: Infection status of all patients at 6 months follow-up based according to NGS result.

	<u><i>Culture (+) infections</i></u> <u><i>(n=17)</i></u>		<u><i>Culture (-) infections</i></u> <u><i>(n=11)</i></u>		<u><i>Aseptic revisions</i></u> <u><i>(n=37)</i></u>		<u><i>Primaries</i></u> <u><i>(n=17)</i></u>	
	NGS (+)	NGS (-)	NGS (+)	NGS (-)	NGS (+)	NGS (-)	NGS (+)	NGS (-)
Infection free	7	0	5	1	8	24	6	11
Retained spacer	5	0	3	0	NA	NA	NA	NA
Reinfection	3	0	1	1	1	4	0	0
Lost to Follow-up	1	1	0	0	0	0	0	0

Table 3

Table 3: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of NGS according to the detection of any bacteria.

	Infected	Non-infected	Sensitivity (95% CI)	Specificity(95% CI)
Any bacteria	25	10	89.3% (71.77-97.73)	73.0% (55.88-86.21)
None	3	27		

Table 4

Table 4: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of NGS based on detection of $\geq 59.5\%$ of a single organism.

	Infected	Non-infected	Sensitivity (95% CI)	Specificity(95% CI)
$\geq 59.5\%$ bacteria present	20	2	71.4% (51.33-86.78)	94.6% (81.81-99.34)
None	8	35		

Table 5

Table 5: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of culture based on organism isolation.

	Infected	Non-infected	Sensitivity (95% CI)	Specificity(95% CI)
Culture positive	17	1	60.7% (40.58-78.50)	97.3% (85.84-99.93)
Culture negative	11	36		

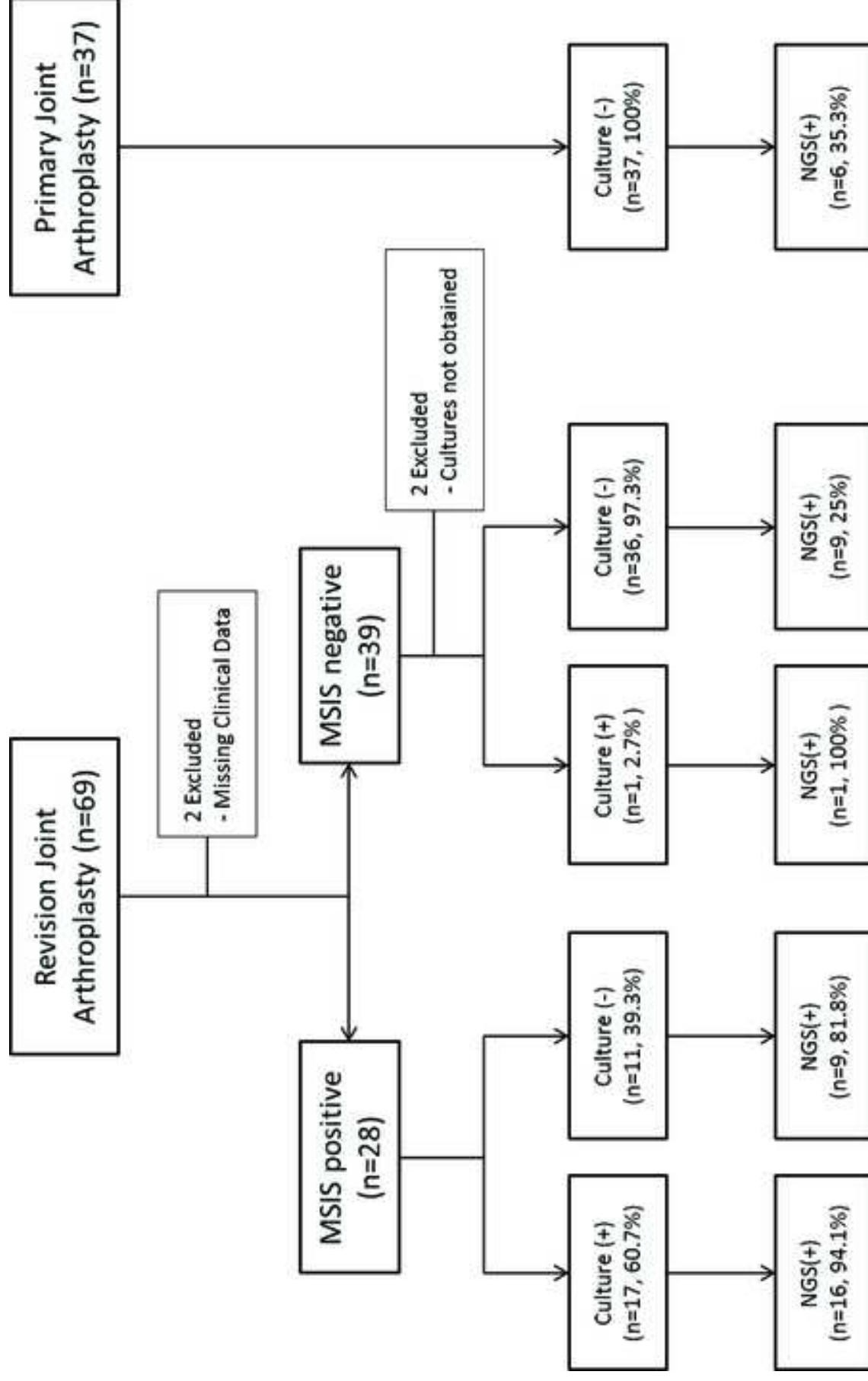


Figure 2

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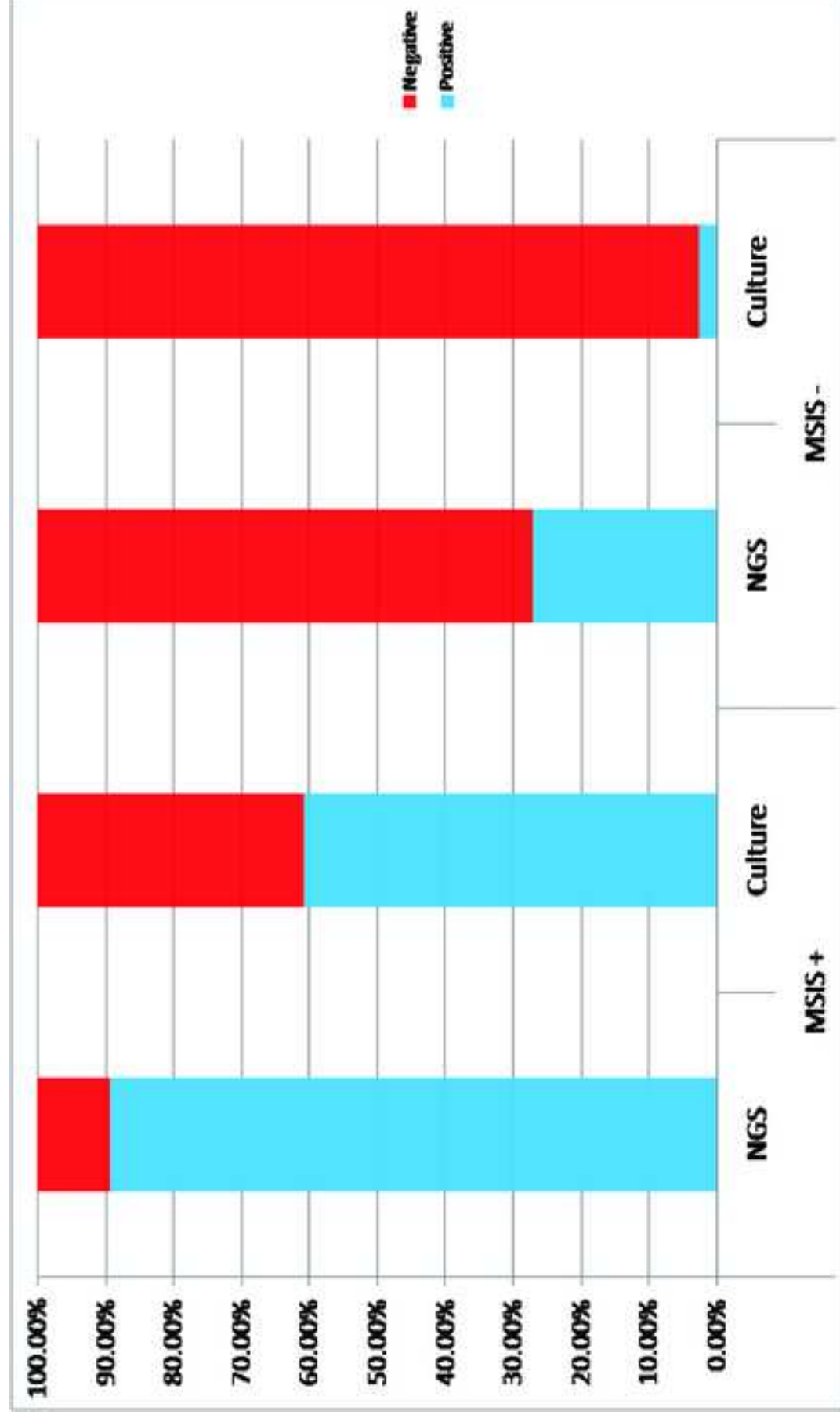
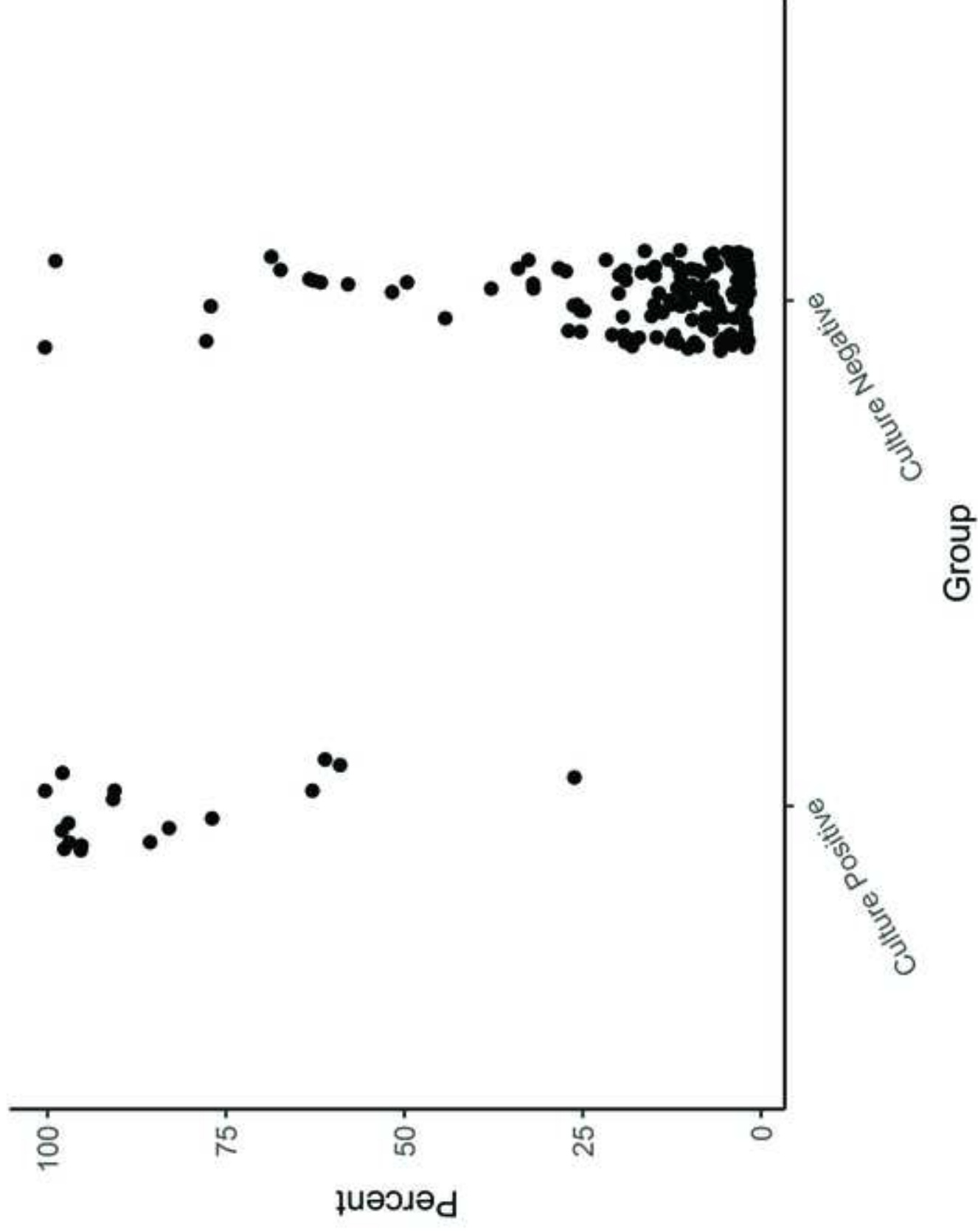


Figure 3

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Appendix A

DNA extraction and real-time PCR:

Upon arrival at the NGS lab, tissue samples were transferred to 2 mL screw cap tubes, while fluid samples are centrifuged for a period of 10 minutes. This was followed by DNA extraction. DNA extraction was performed using the Roche High Pure PCR Template Preparation Kit (Roche Diagnostics, Basel, Switzerland). The extraction process was modified by the inclusion of a beading step for tissue and cell disruption using 5mm steel beads, 5mm Zirconium Oxide beads and the use of the Qiagen tissuelyser II instrument (Qiagen, Hilden, Germany). The lysate generated from this step was then prepared using the Roche High Pure PCR Template Preparation Kit. The initial step in the performance of the assay was the performance of real-time polymerase chain reaction (real-time PCR), using the lifecycler 480 (Roche Diagnostics, Basel, Switzerland). This assay provides a quantitative assessment of the bacterial burden of the assay, and covers a range of organisms and antibiotic resistance genes including the following:

Enterococcus faecalis, *Klebsiella pneumoniae*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Candida albicans*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, methicillin resistance, vancomycin resistance.