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Appendix 1:

Methods: Using 2 fresh (1 aseptic and 1 septic) samples obtained during this study the synovial fluid calprotectin was measured by enzyme-linked immunosorbent assay (ELISA) and point of care (POC) testing after 3 freeze/thaw cycles. The samples were tested initially and after 3 freeze/thaw cycles. Measurements were obtained after each freeze/thaw cycle. At each time point duplicate tests were performed and the results were averaged when possible.

Results: The frozen and fresh samples demonstrated POC and ELISA agreement at every time point. In the aseptic sample, the calprotectin concentration varied from the baseline sample by <20%.

Table 1: Results of Calprotectin Frozen Stability Experiment

Sample	F/T	ELISA (mg/L)	% Bias from fresh	POC (mg/L)	POC Qualitative	Agreement
Aseptic	0	0.370	0	<14	Low Risk	-
	1	0.439	19	<14	Low Risk	Yes
	2	0.394	7	<14	Low Risk	Yes
	3	0.439	19	<14	Low Risk	Yes
Septic	0	>250	-	>300	High Risk	-
	1	>250	-	>300	High Risk	Yes
	2	>250	-	>300	High Risk	Yes
	3	>250	-	>300	High Risk	Yes

ELISA - enzyme-linked immunosorbent assay; F/T – Freeze/Thaw; POC – point of Care

Appendix 2:

Methods: Synovial fluid hemolysis index (HI) was measured on a c501 analyzer (Roche Diagnostic, Indianapolis, IN) on every sample enrolled in the study to evaluate for sample integrity. To evaluate the effect of blood contamination/hemolysis, 3 synovial fluid samples (S1-S3) were mixed with increasing ratios of whole blood hemolyzed by freezing. Calprotectin and the HI were measured in duplicate.

Results:

Addition of hemolyzed blood falsely increased calprotectin concentrations. The test result classification remained as low risk in all samples with up to 12.5% blood/hemolysate, corresponding to HI of 1,237 mg/dL. One of 3 samples with 25% blood/hemolysate (HI 2,763 mg/dL) changed classification from low to moderate risk in the ELISA test but the risk classification was not affected in the POC test. Risk classification changed to moderate or high risk in all samples with 50% blood/hemolysate on both the ELISA and POC tests.

% blood/ hemolysate	HI mean (SD), mg/dL	ELISA (mg/L)			ELISA Qualitative			POC (mg/L)			POC Qualitative		
		S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
0	60 (10)	0.1	0.4	0.5	Low	Low	Low	<14	<14	<14	Low	Low	Low
3.125	320 (20)	2.5	1.3	1.3	Low	Low	Low	<14	<14	<14	Low	Low	Low
6.25	603 (64)	6.7	3.4	3.9	Low	Low	Low	<14	<14	<14	Low	Low	Low
12.5	1273 (127)	13.1	6.1	7.3	Low	Low	Low	<14	<14	<14	Low	Low	Low
25	2763 (42)	25.0	11.9	13.1	Moderate	Low	Low	<14	<14	<14	Low	Low	Low
50	5850 (135)	64.2	20.6	24.7	High	Moderate	Moderate	80	22	28	High	Moderate	Moderate