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Supplementary Appendix

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Subject Inclusion/Exclusion Criteria for the Prospective Patient Cohort

Subject Inclusion Criteria

- Subject has undergone a total knee and/or hip joint arthroplasty.
- The subject will be evaluated for revision surgery.
- Operative samples are required for full Musculoskeletal Infection Society (MSIS) classification.
- Subject is ≥22 years of age.
- Subject has had no recent injections or surgeries of the joint (within past 6 weeks).
- Subject has or will have all of the medical tests required to allow MSIS classification.
- Subject signs informed consent form.

Subject Exclusion Criteria

- Subject has not undergone a total knee and/or hip joint arthroplasty.
- Healthy subjects without medical need for aspiration.
- Subject did not have a revision surgery.
- Subjects with a diagnostic synovial fluid specimen collection within the past 7 days.
- Subjects <22 years of age.
- Subject has had an injection, lavage, or surgery of the joint within the past 6 weeks.
- Subject does not have all of the medical tests required for MSIS classification.
- Subject does not sign informed consent form.

Subject Inclusion/Exclusion Criteria for the Supplemental Cohort of Synovial Fluid Samples

Sample Inclusion Criteria

- Results are available or will be available for medical tests required to perform MSIS classification.
- MSIS classification has been or will be assigned by the study's two Adjudication Panel main reviewers and the third adjudicator if there is discordant diagnosis between the two main reviewers.
- Sample was not obtained via lavage.
- If more than one sample was collected within the same joint during the same sample collection process (e.g., second sample collected from different location within the same joint), only the first sample is eligible for inclusion in the study.
- Collection of different samples from different joints, if both joints are scheduled for revision surgery.
- 1 mL is the preferred volume, with 250 uL as the minimum allowable volume, to be collected for the Synovasure® PJI ELISA Test and Synovasure® PJI Lateral Flow Test. If possible, the volume should be 1mL in order to ensure there is sufficient volume to perform the testing. If less than 1mL is available, please continue with testing, but notify the Sponsor via email of the reduced volume.

Sample Exclusion Criteria

- Results are not available for medical tests required to perform MSIS classification.
- MSIS classification has not been performed by the study's two Adjudication Panel main reviewers or by the third adjudicator if there is discordant diagnosis between the two main reviewers.
- Sample was obtained via lavage.
- Sample is from more than one collection within the same joint during the same sample collection process (e.g., second sample collected from different location within the same joint).
- Sample is from a follow-up collection performed less than 7 days from a previous diagnostic collection.
- Quantity Not Sufficient (QNS) for Synovasure® PJI ELISA Test and Synovasure® PJI Lateral Flow Test.

2013 Musculoskeletal Infection Society (MSIS) definition of Periprosthetic Joint Infection

Definition of Periprosthetic Joint Infection According to the International Consensus Group.

PJI Is Present When One of the Major Criteria Exists or Three Out of Five Minor Criteria Exist:

Major Criteria

- Two positive periprosthetic cultures with phenotypically identical organisms, OR
- A sinus tract communicating with the joint

Minor Criteria (three out of five)

1) Elevated serum C-reactive protein (CRP) AND erythrocyte sedimentation rate (ESR)
2) Elevated synovial fluid white blood cell (WBC) count OR ++ change on leukocyte esterase test strip
3) Elevated synovial fluid polymorphonuclear neutrophil percentage (PMN%)
4) Positive histological analysis of periprosthetic tissue
5) A single positive culture

Declaration: The consensus group wishes to state that PJI may be present without meeting these criteria, specifically in the case of less virulent organisms (e.g. Propionibacterium acnes). Thus, clinicians are urged to exercise their judgment and clinical acumen in reaching the diagnosis of PJI.

Reference: Parvizi J, Gehrke T. Definition of periprosthetic joint infection. The Journal of arthroplasty 2014;29:1331.

STARD 2015 Checklist

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	3,4
	4	Study objectives and hypotheses	4
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**6**	Eligibility criteria	6,7
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6,9
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	7
	10b	Reference standard, in sufficient detail to allow replication	7
	11	Rationale for choosing the reference standard (if alternatives exist)	7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	7
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	7
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	8
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	8
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8
	15	How indeterminate index test or reference standard results were handled	7,8
	16	How missing data on the index test and reference standard were handled	8
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	none
	18	Intended sample size and how it was determined	8
RESULTS			
Participants	**19**	Flow of participants, using a diagram	9
	20	Baseline demographic and clinical characteristics of participants	9
	21a	Distribution of severity of disease in those with the target condition	Not relevant,binary
	21b	Distribution of alternative diagnoses in those without the target condition	N/A
	22	Time interval and any clinical interventions between index test and reference standard	6, 8
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	9,10
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	9,10
	25	Any adverse events from performing the index test or the reference standard	9
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	12
	27	Implications for practice, including the intended use and clinical role of the index test	11,12
OTHER INFO			
	28	Registration number and name of registry	5
	29	Where the full study protocol can be accessed	Supplement
	30	Sources of funding and other support; role of funders	5

Adjudicator Results

Adjudicators were directed to strictly utilize the 2013 MSIS criteria in classifying samples.

Adjudicators received laboratory and clinical data for each case in the prospective cohort, but were blinded to the alpha-defensin results and to other adjudicators decisions.

Adjudicators could vote Yes, No, or Indeterminate for each sample.

N=291 samples received the same 2013 MSIS classification as having PJI or No PJI.

N=14 samples received a Yes or No vote from the first adjudicator and an Indeterminate vote from the second adjudicator, requiring a third adjudicator. The third adjudicator voted consistent with the first adjudicator in all 14 cases. These 14 samples requiring third adjudicator involvement resulted in 2 Lateral flow test false positives and 1 ELISA test false positive result.