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RESEARCH

Rapid detection of periprosthetic joint infection using a combination of 16s rDNA polymerase chain reaction and lateral flow immunoassay

A PILOT STUDY

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Objectives

The objective of this study was to develop a test for the rapid (within 25 minutes) intraoperative detection of bacteria from synovial fluid to diagnose periprosthetic joint infection (PJI).

Methods

The 16s rDNA test combines a polymerase chain reaction (PCR) for amplification of 16s rDNA with a lateral flow immunoassay in one fully automated system. The synovial fluid of 77 patients undergoing joint aspiration or primary or revision total hip or knee surgery was prospectively collected. The cohort was divided into a proof-of-principle cohort (n = 17) and a validation cohort (n = 60). Using the proof-of-principle cohort, an optimal cut-off for the discrimination between PJI and non-PJI samples was determined. PJI was defined as detection of the same bacterial species in a minimum of two microbiological samples, positive histology, and presence of a sinus tract or intra-articular pus.

Results

The 16s rDNA test proved to be very robust and was able to provide a result in 97% of all samples within 25 minutes. The 16s rDNA test was able to diagnose PJI with a sensitivity of 87.5% and 82%, and a specificity of 100% and 89%, in the proof-of-principle and validation cohorts, respectively. The microbiological culture of synovial fluid achieved a sensitivity of 80% and a specificity of 93% in the validation cohort.

Conclusion

The 16s rDNA test offers reliable intraoperative detection of all bacterial species within 25 minutes with a sensitivity and specificity comparable with those of conventional microbiological culture of synovial fluid for the detection of PJI. The 16s rDNA test performance is independent of possible blood contamination, culture time and bacterial species.

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Keywords: Periprosthetic joint infection, Rapid detection, 16s rDNA, Polymerase chain reaction, Lateral flow, Immunoassay

Article focus

- This study presents the initial results of a newly developed test for the rapid (within 25 minutes) intraoperative detection of bacteria from synovial fluid to detect periprosthetic joint infection (PJI). The 16s rDNA test combines a polymerase chain reaction (PCR) for amplification of 16s rDNA with a lateral flow immunoassay in one fully automated system.

Key messages

- The 16s rDNA test was able to provide a result in 97% of all samples within 25 minutes.
- The sensitivity and specificity of the 16s rDNA test were comparable with the

sensitivity and specificity of conventional synovial fluid culture.

Strengths and limitations

- This study is the first to develop a 16s rDNA based test within 25 minutes, allowing for a true intraoperative application.
- Initial results of a pilot study. Future prospective studies for intraoperative application and specialized surgical indications are planned.

Introduction

Before a revision of a total hip arthroplasty (THA) or total knee arthroplasty (TKA), it is mandatory to either confirm or exclude a periprosthetic joint infection (PJI), since the surgical strategies differ significantly between aseptic and septic revisions. In cases of an unclear preoperative diagnosis of PJI, the only other routinely utilized diagnostic option is the histological evaluation of an intraoperative frozen tissue section.[1] A previous meta-analysis by Tsaras et al[2] has reported convincing diagnostic evidence for the use of frozen tissue sections for the detection of culture-positive PJI in TKA and THA, with a diagnostic odds ratio of 54.7. However, currently there are no commonly utilized diagnostic alternatives for cases of unclear PJI status.

Although a *post hoc* differentiation between septic and aseptic cases is possible through other non-culture-based diagnostic methods, such as synovial cell count, leucocyte esterase, and α-defensin, none of these have been validated as a diagnostic tool to facilitate intraoperative decision-making.[3-8] Additionally, all of these diagnostic methods are dependent on the patient's immune response towards the presence of bacteria and therefore only allow for an indirect detection of PJI. It was the goal of this study to design a diagnostic test for an intraoperative discrimination between septic and aseptic cases through a direct detection of the causative bacterial species.

To enable an intraoperative diagnosis, a bacterial infection has to be directly detected in order to circumvent the delay due to microbiological culture. A direct detection of bacteria can be realized by multiplex polymerase chain reaction (PCR)-based amplification of 16s rDNA, which encodes highly conservative regions of the 16s ribosomal subunit and is common to all bacterial species. The detection of 16s rDNA, through multiplex PCR, can be performed from different sample materials, such as synovial fluid, periprosthetic tissue samples, and sonicate fluid.[9-13] Synovial fluid is the most promising material for such purposes due to the ease of acquisition at the beginning of revision surgery and the wide acceptance within the surgical community of its use to diagnose PJI.[13-16]

To our knowledge, there are currently only two other studies that were able to achieve a 'rapid' molecular diagnosis of PJI, within three hours and 4.5 hours, respectively.[12,17,18] While this represents a significant improvement over the standard diagnostic time of culture-based methods, this timeframe is still too great for a true intraoperative application to discriminate between septic and aseptic failures. The aim of this study was to develop a 16s rDNA PCR test system for the detection of PJI that would rapidly (within 25 minutes) facilitate intraoperative discrimination between septic and aseptic prosthetic joint failures.

Patients and Methods

Study design and patient cohort. A total of 77 patients were included in this prospective cohort study, between January 2014 and January 2015, and divided into a proof-of-principle cohort (n = 17, eight cases of PJI) and a validation cohort (n = 60, 23 cases of PJI). The sample size of the validation cohort was determined by power analysis based on the results from the proof-of-principle group (power = 0.80, α = 0.01; two-sided, minimum sample size = 21 per group). All patients provided written informed consent, and the study was approved by the local institutional review board. The proof-of-principle cohort was used to evaluate the test's functionality and to assess the optimal threshold for discrimination between septic and aseptic patients. The validation cohort was used to evaluate the test's diagnostic performance.

Synovial fluid samples were collected preoperatively for the THA and TKA revisions with a preoperative suspicion of PJI, or intraoperatively for THA and TKA revisions without preoperative suspicion of PJI. In addition, synovial aspirations of native joints were performed intraoperatively during primary THA and TKA surgery. These aspirations of native joints functioned exclusively as aseptic negative controls. Within one hour, the samples were transported to our research facility and frozen at -80°C prior to analysis. The proof-of-principle cohort was comprised of two primary TKAs, ten revision THAs or TKAs, and five THA aspirations (Table I). The validation cohort was comprised of seven primary THAs or TKAs, 32 revision THAs or TKAs, and 21 joint aspirations (Table II). Sample harvesting, patient/sample data collection, and documentation were performed in accordance with our institutional guidelines.[19] The physical properties of the samples were qualitatively assessed, including variances in synovial viscosity, optical clarity (blood contamination), and sample volumes.

PJI definition and intraoperative sample acquisition. PJI was defined according to the following criteria: intra-articular pus or presence of a sinus tract; histology indicative of infection (type II or III periprosthetic membrane); or positive microbiological culture of the same bacterial species in a minimum of two of the following samples: synovial fluid; intraoperative tissue sample; or sonicate fluid cultures (SFC).[20-23] The final diagnosis of PJI was

Table I. Patient and sample characterization for the proof-of-concept cohort (PC)

Sample ID	Gender	Age (yrs)	Preoperative suspicion*	Surgical procedure	Microbiological culture†	PJI‡	16s rDNA assay
PC_01	Male	19	Aseptic	Primary THA/TKA	N/A	Negative	Negative
PC_02	Female	84	Aseptic	Primary THA/TKA	N/A	Negative	Negative
PC_03	Male	55	Aseptic	Revision THA/TKA	Negative	Negative	Negative
PC_04	Female	52	Aseptic	Revision THA/TKA	Negative	Negative	Negative
PC_05	Female	60	Aseptic	Revision THA/TKA	Negative	Negative	Negative
PC_06	Female	71	Septic	Revision THA/TKA	Negative	Negative	Negative
PC_07	Female	47	Septic	Revision THA/TKA	Negative	Negative	Negative
PC_08	Male	87	Unclear	Joint aspiration	Negative	Negative	Negative
PC_09	Male	68	Unclear	Joint aspiration	Negative	Negative	Negative
PC_10	Male	37	Septic	Revision THA/TKA	Positive	Positive	Negative
PC_11	Female	76	Septic	Revision THA/TKA	Positive	Positive	Positive
PC_12	Male	60	Septic	Revision THA/TKA	Negative	Positive	Positive
PC_13	Female	90	Septic	Revision THA/TKA	Positive	Positive	Positive
PC_14	Male	85	Unclear	Joint aspiration	Positive	Positive	Positive
PC_15	Male	87	Unclear	Joint aspiration	Positive	Positive	Positive
PC_16	Male	74	Unclear	Joint aspiration	Positive	Positive	Positive
PC_17	Male	66	Unclear	Revision THA/TKA	Positive	Positive	Positive

*Based on the preoperative diagnostics
†Growth of the same bacterial species in at least two of the following samples: synovial fluid, intraoperative tissue sample, and sonicate fluid cultures (SFC)
‡Final diagnosis of PJI based on intraoperative samples and PJI definition
PJI, periprosthetic joint infection; THA, total hip arthroplasty; TKA, total knee arthroplasty; N/A, not available

made according to the results of the intraoperative microbiological and histological samples. The final diagnosis of PJI was the benchmark reference, against which the performance of the 16s rDNA test, as well as all calculations for sensitivity and specificity, were referenced.

Synovial fluid sampling was performed in an operating theatre with laminar air flow, utilizing a skin incision, and under fluoroscopic guidance, for all joint aspirations. Intraoperative synovial fluid aspiration was performed under direct visualization of the joint and prior to capsulotomy. Additionally, multiple periprosthetic tissue samples, a histological sampling of the periprosthetic membrane, and SFC were acquired for all cases of revision arthroplasty. The histological evaluation was performed according to the consensus classification of the periprosthetic interface membrane.[21] To optimize the microbiological culture methods, both synovial fluid and SFC were incubated in blood culture bottles.[24-26] Intraoperative tissue samples were cultured on standard agar plates. To allow for a detection of fastidious bacterial species, all microbiological cultures were incubated for 14 days.[27]

16s rDNA PCR test system. The 16s rDNA test is based on a targeted PCR and subsequent detection of the PCR products by lateral flow immunoassay. The 16s rDNA test was performed from intraoperatively acquired synovial fluid. The PCR primers target a highly conservative region of the 16s ribosomal subunit that is common to all bacterial species. The complete workflow is illustrated in Figure 1 and requires 25 minutes. Synovial fluid (total volume = 2 µl) was directly combined with the PCR master mix, containing differentially labelled forward (biotin) and reverse (Fluorescein isothiocyanate (FITC)) primers that are specific to a highly conserved 16s rDNA sequence (primers are available on request; Milenia Biotec GmbH, Gießen, Germany). Polymerase chain reaction (30 cycles; 15 minutes) was performed using the Labcycler 48s (SensoQuest GmbH, Göttingen, Germany). In the presence of 16s rDNA, the PCR produced double-labelled (biotin and FITC) DNA products. The PCR mixture was subsequently transferred to the lateral flow immunoassay test unit (Milenia Biotec), where the PCR fragments were captured via their biotin label by specific antibodies in a single-step procedure. The results were displayed as two bands on the test strip. The lower test band indicates the detection of the bacterial 16s rDNA product and the upper band serves as a control, confirming the correct function of the flow assay. The test results were evaluated by spectrometric measurement of the band intensity and quantified by ImageJ software (National Institutes of Health, Bethesda, Maryland; http://imagej.nih.gov).[28] The 16s rDNA assay score was calculated as the ratio between the intensity of the test and control bands and expressed in arbitrary units (AU).

Statistical analysis. All data are given as mean ± SD. The Mann–Whitney U test was used for group comparison and receiver operating characteristic (ROC) analysis was performed to determine the optimal cut-off for discrimination between the two patient groups. Assay sensitivity and specificity were calculated as previously described.[29] Sensitivity was defined as true positive (TP) / (TP + false negative (FN)), and specificity was defined as true negative (TN) / (TN + false positive (FP)), All statistical analyses were performed with SPSS software, version 18 (IBM Corp., Armonk, New York),

Table II. Patient and sample characterization for the validation cohort (VC)

Sample ID	Gender	Age (yrs)	Preoperative suspicion*	Surgical procedure	Microbiological culture†	PJI‡	16s rDNA assay
VC_01	Female	65	Aseptic	Primary THA/TKA	N/A	Negative	Negative
VC_02	Male	46	Aseptic	Primary THA/TKA	N/A	Negative	Negative
VC_03	Female	83	Aseptic	Primary THA/TKA	N/A	Negative	Negative
VC_04	Male	48	Aseptic	Primary THA/TKA	N/A	Negative	Negative
VC_05	Male	62	Aseptic	Primary THA/TKA	N/A	Negative	Negative
VC_06	Male	71	Aseptic	Primary THA/TKA	N/A	Negative	Negative
VC_07	Male	81	Aseptic	Primary THA/TKA	N/A	Negative	Negative
VC_08	Male	81	Aseptic	Revision THA/TKA	Negative	Negative	Positive
VC_09	Male	56	Aseptic	Revision THA/TKA	Negative	Negative	Negative
VC_10	Female	78	Aseptic	Revision THA/TKA	Negative	Negative	Negative
VC_11	Female	59	Aseptic	Revision THA/TKA	Negative	Negative	Negative
VC_12	Female	79	Aseptic	Revision THA/TKA	Negative	Negative	Negative
VC_13	Male	54	Aseptic	Revision THA/TKA	Negative	Negative	Negative
VC_14	Female	51	Aseptic	Revision THA/TKA	Negative	Negative	Negative
VC_15	Male	79	Septic	Revision THA/TKA	Negative	Negative	Negative
VC_16	Female	52	Septic	Revision THA/TKA	Negative	Negative	Positive
VC_17	Female	46	Septic	Revision THA/TKA	Negative	Negative	Negative
VC_18	Female	70	Septic	Revision THA/TKA	Negative	Negative	Negative
VC_19	Female	59	Septic	Revision THA/TKA	Negative	Negative	Negative
VC_20	Female	70	Septic	Revision THA/TKA	Negative	Negative	Negative
VC_21	Male	37	Unclear	Joint aspiration	Negative	Negative	Positive
VC_22	Male	62	Unclear	Joint aspiration	Negative	Negative	Negative
VC_23	Male	55	Unclear	Joint aspiration	Negative	Negative	Negative
VC_24	Male	67	Unclear	Joint aspiration	Negative	Negative	Negative
VC_25	Male	77	Unclear	Joint aspiration	Negative	Negative	Negative
VC_26	Female	77	Unclear	Joint aspiration	Positive	Negative	N/A
VC_27	Male	46	Unclear	Joint aspiration	Negative	Negative	Negative
VC_28	Male	60	Unclear	Joint aspiration	Negative	Negative	Positive
VC_29	Male	72	Unclear	Joint aspiration	Negative	Negative	Negative
VC_30	Male	73	Unclear	Joint aspiration	Negative	Negative	Negative
VC_31	Male	68	Unclear	Joint aspiration	Negative	Negative	Negative
VC_32	Male	86	Unclear	Joint aspiration	Negative	Negative	Negative
VC_33	Female	77	Unclear	Joint aspiration	Negative	Negative	Negative
VC_34	Female	71	Unclear	Revision THA/TKA	Negative	Negative	Negative
VC_35	Female	70	Unclear	Revision THA/TKA	Negative	Negative	Negative
VC_36	Male	85	Unclear	Revision THA/TKA	Negative	Negative	Negative
VC_37	Male	74	Unclear	Revision THA/TKA	Negative	Negative	Negative
VC_38	Male	61	Septic	Joint aspiration	Positive	Positive	Negative
VC_39	Female	89	Septic	Revision THA/TKA	Positive	Positive	Positive
VC_40	Female	76	Septic	Revision THA/TKA	Negative	Positive	Positive
VC_41	Female	80	Septic	Revision THA/TKA	Positive	Positive	Negative
VC_42	Female	78	Septic	Revision THA/TKA	Positive	Positive	Positive
VC_43	Female	70	Septic	Revision THA/TKA	Positive	Positive	Positive
VC_44	Male	66	Septic	Revision THA/TKA	Positive	Positive	Negative
VC_45	Male	60	Septic	Revision THA/TKA	Positive	Positive	Positive
VC_46	Male	66	Septic	Revision THA/TKA	Positive	Positive	Positive
VC_47	Male	63	Septic	Revision THA/TKA	Positive	Positive	Negative
VC_48	Female	75	Septic	Revision THA/TKA	Positive	Positive	Positive
VC_49	Male	59	Septic	Revision THA/TKA	Negative	Positive	Positive
VC_50	Male	70	Unclear	Revision THA/TKA	Negative	Positive	N/A
VC_51	Female	55	Unclear	Joint aspiration	Positive	Positive	Positive
VC_52	Female	55	Unclear	Joint aspiration	Positive	Positive	Positive
VC_53	Male	66	Unclear	Joint aspiration	Positive	Positive	Positive
VC_54	Male	84	Unclear	Joint aspiration	Positive	Positive	Positive
VC_55	Male	86	Unclear	Joint aspiration	Positive	Positive	Positive
VC_56	Female	64	Unclear	Joint aspiration	Positive	Positive	Positive
VC_57	Male	73	Unclear	Joint aspiration	Positive	Positive	Positive
VC_58	Female	67	Unclear	Revision THA/TKA	Negative	Positive	Positive
VC_59	Female	78	Unclear	Revision THA/TKA	Positive	Positive	Positive
VC_60	Male	65	Unclear	Revision THA/TKA	Positive	Positive	Positive

*Based on the preoperative diagnostics
†Growth of the same bacterial species in at least two of the following samples: synovial fluid, intraoperative tissue sample, and sonicate fluid cultures (SFC)
‡Final diagnosis of PJI based on intraoperative samples and PJI definition
PJI, periprosthetic joint infection; THA, total hip arthroplasty; TKA, total knee arthroplasty; N/A, not available

and statistical significance was defined at $p < 0.05$. The medical patient data and the results from the 16s rDNA PCR test system were evaluated in a double-blinded manner by two authors (SG and VJ).

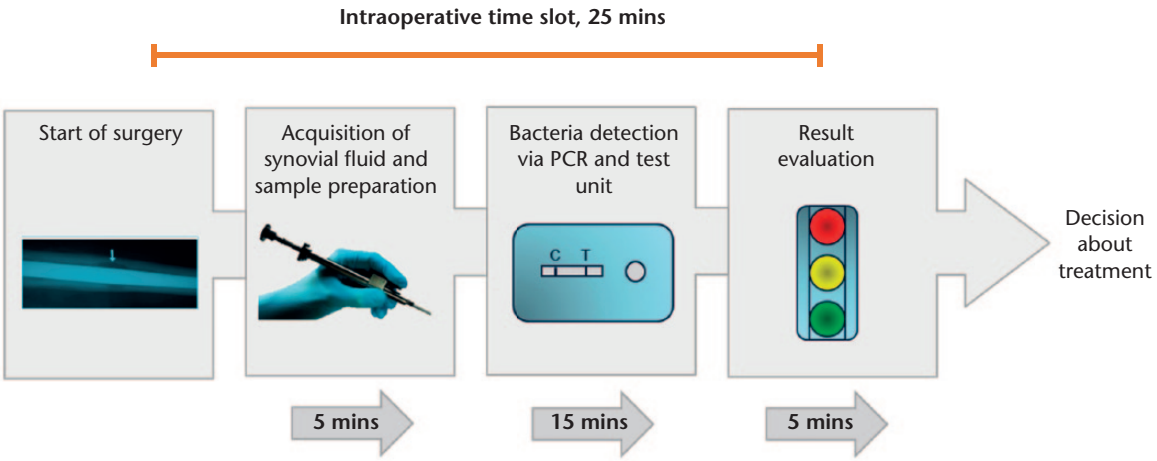

Fig. 1a

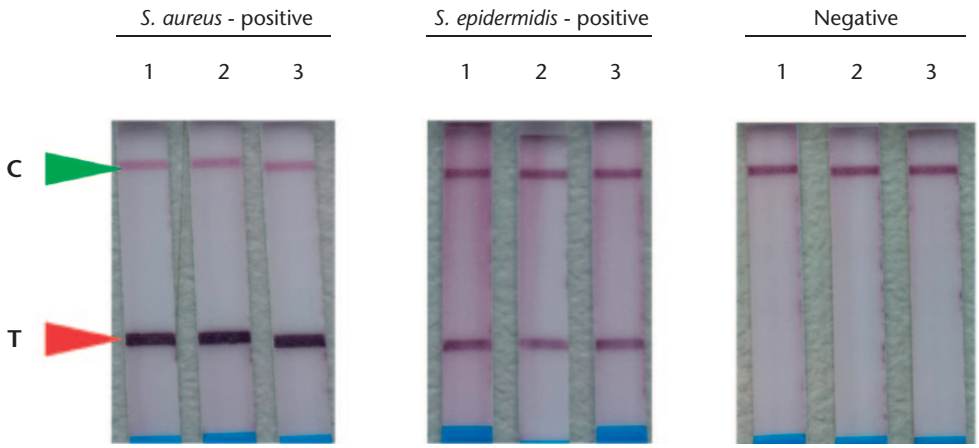

Fig. 1b

General workflow of the 16s rDNA test: a) chronological test principle, with polymerase chain reaction (PCR) followed by subsequent detection of the specific PCR products by lateral flow immunoassay; b) the results are displayed as one test band (T, detection of 16s rDNA); and c) a control band (C), confirming the correct function of assay).

Results

Test reliability. The 16s rDNA test system provided a diagnostic result within 25 minutes in 97% (75 of 77) of all patients. Two samples could not be evaluated due to massive protein precipitation from the synovial sample during the PCR. Other possible confounding factors for sample evaluation, such as variances in synovial viscosity, blood contamination, small sample volumes, or variances in transport times, did not negatively affect the test reliability.

Test performance. ROC analysis in the proof-of-principle cohort revealed an optimal cut-off level of 0.71 AU, between the test and control bands of the 16s rDNA test strip (Fig. 2). Utilizing this cut-off, the 16s rDNA test system was able to detect seven of eight PJI samples and all of the non-PJI samples correctly, achieving a sensitivity of 87.5% and a specificity of 100% (area under the curve (AUC) = 0.944, p = 0.001) in the proof-of-principle cohort (Fig. 2).

Validation of test performance. Using the predefined cut-off value in the validation cohort, the 16s rDNA test system achieved a sensitivity of 82% (true positive = 18, false negative = 4) and specificity of 89% (true negative = 32, false positive = 4) (AUC = 0.894, $p < 0.001$). Examination of the ROC curve of the validation cohort confirmed the predetermined cut-off of 0.71 AU as optimal to discriminate between PJI and non-PJI samples (Fig. 3). The performance of the 16s rDNA test was independent of the isolated bacterial species. The complete list of detected bacterial species grouped according to their detection by microbiological culture or 16s rDNA test is displayed in Table III.

Test performance in comparison with conventional microbiological methods. We directly compared the diagnostic performance of the 16s rDNA test with the individual performance of the conventional microbiological diagnostic methods, comprised of synovial fluid and periprosthetic tissue cultures, as well as the histological evaluation of the

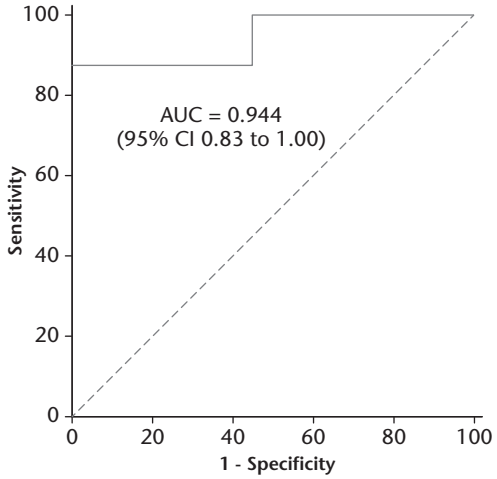

Fig. 2a

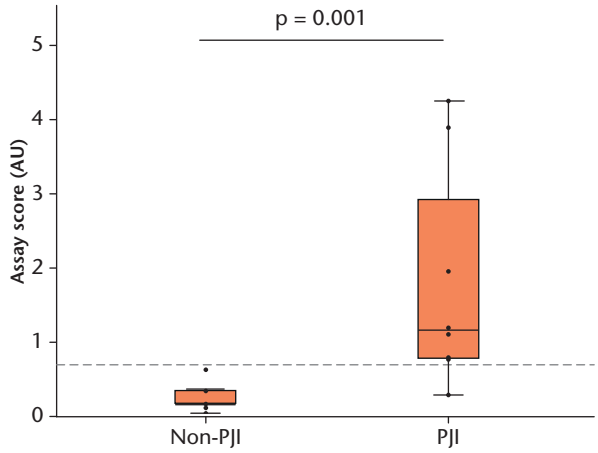

Fig. 2b

General test performance using the proof-of-principle cohort: a) receiver operating characteristic (ROC) curve for the 16s rDNA test based on the calculated ratio between test and control band to determine the optimal cut-off value to differentiate between periprosthetic joint infection (PJI) and non-PJI samples; and b) box-whisker plot displaying the performance of the 16s rDNA test to differentiate between PJI (n = 8) and non-PJI (n = 9) samples in the proof-of-principle cohort. Dashed lines indicate the optimal cut-off as determined by previous ROC analysis. The Mann–Whitney U test was used to obtain p-values. AUC, area under the curve; CI, confidence interval; AU, assay unit.

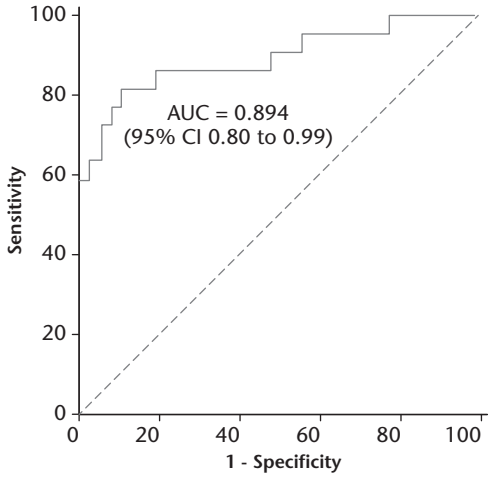

Fig. 3a

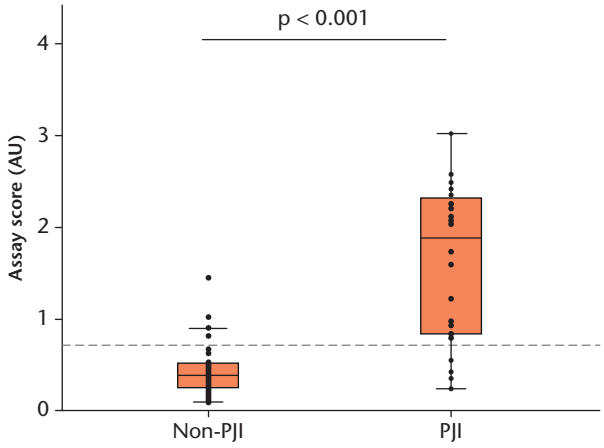

Fig. 3b

Validation of the test performance using the validation cohort: a) receiver operating characteristic (ROC) curve for the 16s rDNA test (ratio between test and control band on the test strip) to differentiate between periprosthetic joint infection (PJI) and non-PJI samples in the validation cohort; and b) box-whisker plot displaying the performance of the 16s rDNA test to differentiate between PJI (n = 23) and non-PJI (n = 37) samples in the validation cohort. Dashed lines indicate the predefined cut-off as determined using the proof-of-principle cohort. The Mann–Whitney U test was used to obtain p-values. AUC, area under the curve; CI, confidence interval; AU, assay unit.

periprosthetic membrane. The microbiological culture of synovial fluid achieved a lower sensitivity than that of the 16s rDNA test with 80%, and a specificity of 93%. The combination of synovial fluid and tissue sample cultures achieved a sensitivity of 86% and specificity of 86%.

Overall, the correlation between the 16s rDNA test and the microbiological cultures showed a concordance in 75% of all cases, with the 16s rDNA test and the microbiological cultures both being either positive or negative. The correlation between the 16s rDNA test and the histological evaluation of the periprosthetic tissue sample was slightly superior, with a concordance rate of 77%.

Discussion

Despite the longer time period associated with culture-based methods, which precludes an intraoperative application, the detection of PJI by microbiological culture remains the benchmark in PJI diagnostics. To avoid the disadvantages associated with microbiological culture, we developed a test for the rapid detection of bacterial 16s rDNA from synovial fluid (within 25 minutes). To our knowledge, the shortest reported times for the performance of a PCR-based 16s rDNA detection are, in the current literature, three hours and 4.5 hours.[12,17,18]

Table III. Detected bacterial species grouped according to their detection by microbiological culture or 16s rDNA test

Bacterial species	Culture positive	16s rDNA test positive
Staphylococcus epidermidis	+	+
Staphylococcus hominis	+	+
Staphylococcus caprae	+	+
Staphylococcus capitis	+	+
Staphylococcus warneri	+	+
Staphylococcus aureus	+	+
Streptococcus agalactiae	+	+
Propionibacterium acnes	+	+
Enterococcus faecalis	+	+
Enterococcus coli	+	-
Dermabacter hominis	+	-

A distinct advantage of the 16s rDNA test over other diagnostic methods, such as leucocyte esterase, is the high degree of reliability and resistance to contamination. The detection of leucocyte esterase from synovial fluid is very susceptible to blood contamination, making an evaluation of up to 17% of all samples impossible.[2] The high degree of reliability of the 16s rDNA test, with 97% of all samples providing a diagnostic result, and the execution within 25 minutes from only 2 µl of synovial fluid, allow for a true intraoperative application.

Since total joint arthroplasties release wear particles with heterogeneous physicochemical properties, these could theoretically interfere with our 16s rDNA test. To address this issue and to take the heterogeneity of potential patient cohorts into account, patients undergoing arthroplasty revision, as well as primary arthroplasty, were included in our patient collective. The high degree of correlation between the results of our 16s rDNA test and those of the microbiological culture shows that a reliable detection of PJI from synovial fluid is possible even in the presumed presence of wear particles. Although it was not the primary goal of this study to achieve a superior sensitivity over the standard intraoperative microbiological cultures, the 16s rDNA test achieved a slightly higher sensitivity than both the microbiological culture of synovial fluid and periprosthetic tissue cultures.[30] The differences in sensitivity and specificity of the 16s rDNA test in the proof-of-principle and validation cohorts could be attributed to the differences in sample size and PJI incidence between the cohorts.

In addition, the sensitivity of 82% achieved by our synovial fluid 16s rDNA test exceeded the reported sensitivity rates of other 16s rDNA tests which ranged from 64% to 76%.[9-11] The achieved correlation rate and sensitivity are independent of the bacterial species, since the utilized primer sequences match to a highly conservative region of bacterial rDNA encoding the 16s ribosomal subunit, which is identical in prokaryotes.[31]

The 16s rDNA test was able to detect all of the bacterial species isolated by microbiological culture, except in two cases (Table I). Only two isolations of *E. coli* and *Dermabacter hominis* were not detected. Both cases represent single positive bacterial isolations in two different patients. The isolation of *Dermabacter hominis* was only present in the SFC, with all other microbiological cultures remaining negative. The isolation of *E. coli* represented one of the two 16s rDNA tests which were not analyzable due to massive protein precipitation in the synovial fluid sample.

Our study also has a number of technical limitations. First, specialized equipment, such as a thermocycler, is necessary for an intraoperative application to perform the 16s rDNA test. Therefore, all samples were transported to our research facility for this study and proof of applicability in a true intraoperative scenario is pending. Nevertheless, the test was developed as a fully automated system with a focus on convenience, user friendliness, and rapid detection to allow for an intraoperative application, without further modifications. The translation of the test system into clinical application is the main goal in the continuation of this project. Second, the small patient cohort, to date, should be supplemented by a larger prospective cohort to confirm and validate our current findings. Third, specific indications, such as a prospective comparison between intraoperative frozen tissue sections and the current 16s rDNA test, should be investigated. Finally, our proposed test system has a restricted ability to distinguish between living and dead bacteria, which potentially limits its use to monitor the infection status after antibiotic treatment. Thus, further studies must be performed to validate our findings in synovial fluid from PJI patients after antibiotic treatment. Previous studies have shown that a pre-incubation of biological samples with the membrane-impermeant agent propidium monoazide prevents the amplification of the 16s rDNA from dead cells; such a modification to our 16s rDNA test could be a promising method to further maximize the clinical utility.[32,33] Furthermore, our 16s rDNA test rapidly detects the presence of bacteria, but does not detect the specific bacterial strain or, more importantly, a potential antibiotic resistance. Thus, we currently aim to extend our 16s rDNA test towards a multiplex approach, allowing for the simultaneous identification of clinically relevant bacterial strains, as well as specific antibiotic-resistant genes.

In conclusion, the current system can reliably and rapidly detect PJI, enabling an intraoperative application. The direct detection of bacterial 16s rDNA shows encouraging results, and warrants further evaluation in larger patient cohorts. The future addition of the detection of clinically relevant antibiotic resistance will be a focus of further research.

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Author Contribution

- V. Janz: Designing the study, Acquiring and evaluating the data, Preparing and revising the manuscript.
- J. Schoon: Acquiring and evaluating the data.
- C. Morgenstern: Acquiring and evaluating the data.
- B. Preininger: Designing the study, Acquiring and evaluating the data, Revising the manuscript.
- S. Reinke: Acquiring and evaluating the data.
- G. Duda: Designing and supervising the study, Revising the manuscript.
- A. Breitbach: Designing the study, Evaluating the data, Preparing and revising the manuscript.
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Conflicts of Interest Statement

- None declared