

New Perspectives for Prosthetic Valve Endocarditis: Impact of Molecular Imaging by FISHseq Diagnostics

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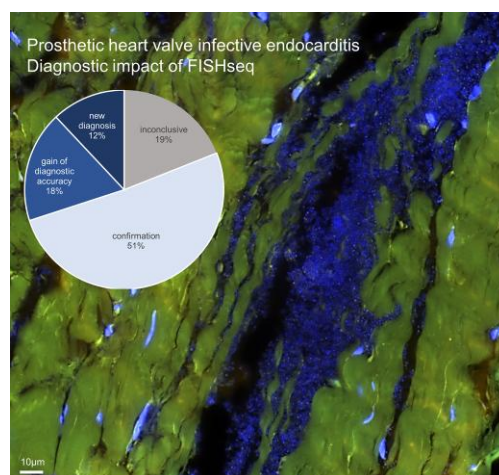
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Background. The microbial etiology of prosthetic valve infective endocarditis (PVE) can be difficult to identify. Our aim was to investigate the benefit of molecular imaging technique fluorescence in situ hybridization (FISH) combined with 16S rRNA-gene polymerase chain reaction (PCR) and sequencing (FISHseq) for the analysis of infected prosthetic heart valves.

Methods. We retrospectively evaluated the diagnostic outcome of 113 prosthetic valves from 105 patients with suspected PVE, treated in 2003–2013 in the Department of Cardiac Surgery, Charité University Medicine Berlin. Each prosthetic valve underwent cultural diagnostics and was routinely examined by FISH combined with 16S rRNA gene PCR and sequencing. We compared classical microbiological culture outcomes (blood and valve cultures) with FISHseq results and evaluated the diagnostic impact of the molecular imaging technique.

Results. Conventional microbiological diagnostic alone turned out to be insufficient, as 67% of preoperative blood cultures were noninformative (negative, inconclusive, or not obtained) and 67% of valve cultures remained negative. FISHseq improved the conventional cultural diagnostic methods in PVE in 30% of the cases and increased diagnostic accuracy. Of the valve culture–negative PVE cases, FISHseq succeeded in identifying the causative pathogen in 35%.

Conclusions. FISHseq improves PVE diagnostics, complementing conventional cultural methods. In addition to species identification, FISH provides information about the severity of PVE and state of the pathogens (eg, stage of biofilm formation, activity, and localization on and within the prosthetic material). As a molecular imaging technique, FISHseq enables the unambiguous discrimination of skin flora as contaminant or infectious agent.



Received 22 July 2022; editorial decision 25 October 2022; published online 1 November 2022

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Clinical Infectious Diseases® 2023;76(6):1050–8

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<https://doi.org/10.1093/cid/ciac860>

Keywords. infectious endocarditis; prosthetic valve endocarditis; fluorescence in situ hybridization; implant infections; molecular imaging.

Prosthetic valve endocarditis (PVE) is a severe complication of cardiac valve replacement surgery as it is associated with high mortality rates from 18% to 59% [1, 2]. With increasing frequency of valve replacement, PVE now accounts for up to 30% of all endocarditis cases [3–5]. The increasing number of transcatheter aortic valve implantations (TAVIs) aggravates this situation [6].

Despite recent advances in diagnosis and treatment, infectious endocarditis (IE) remains an incompletely understood disease with high morbidity and mortality [1, 4, 6, 7].

The diagnosis of IE is based on the modified Duke criteria [8]. Although these criteria remain useful as a diagnostic aid, they do not always provide a conclusive diagnosis of PVE; many PVE cases cannot even be confirmed intraoperatively [9, 10]. Thus, the final diagnosis of PVE, as well as the identification of the causative microorganisms, remain challenging [11]. This, however, is crucial for specific therapy and de-escalation of the antibiotic therapy, which is associated with improved patient outcome [12, 13].

The percentage of blood culture (BC)–negative PVE ranges between 3% and 50% of all PVE cases [14–17]. Negative cultures arise mostly because of prior antibiotic administration but may also be due to fastidious microorganisms with limited growth in conventional culture or requiring specialized tools for identification [18]. Moreover, many pathogens, like coagulase-negative staphylococci (CNS), are also part of skin flora; therefore, it is challenging to differentiate between contamination during sampling and infection.

In BC-negative PVE, the examination of the explanted prosthetic valve remains the last chance to obtain the diagnosis and to identify the causative pathogen. In the literature, data regarding prosthetic valve culture (VC) are sparse, with reported rates of positive results between 25% and 42% [19, 20].

In recent years, molecular techniques, among them fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR)–based techniques, have been gaining importance in IE diagnostics, particularly in culture-negative cases [15, 21–24]. The current European Society of Cardiology guidelines point out the necessity of performing molecular analysis in culture-negative cases [25].

In this paper, we investigate the benefit of the molecular imaging technique FISH combined with broad-range 16S rRNA gene PCR and sequencing (FISHseq) for the diagnosis of PVE.

METHODS

Patient Characteristics and Study Design

We retrospectively evaluated the microbiological diagnostic results of 113 prosthetic valves from 105 consecutive patients

with suspected PVE, treated in the years 2003–2013 in the Department of Cardiac Surgery, Charité–Universitätsmedizin Berlin (Table 1). Each prosthetic valve was considered in this study as an independent case. Although in most of the cases a single prosthetic valve was explanted during the procedure (111 out of 113), in 1 particular patient the explantation of 2 prosthetic valves during the same surgery was performed. Seven patients underwent surgery for PVE twice. In all cases, the explanted prosthetic valve was examined by both standard culture and FISHseq.

Prior to hospital admission, the majority of patients presented with fever (temperatures $<38.5^{\circ}\text{C}$) ($n=62$) or low-grade fever (temperatures $37.6\text{--}38.5^{\circ}\text{C}$) ($n=7$). Among the 44 cases, in which no fever was described, the patients experienced dyspnea ($n=29$), cardiac arrest ($n=2$), cardiogenic shock ($n=2$), severe respiratory failure ($n=3$), neurological symptoms ($n=3$), sternal wound infection ($n=1$), and stomach pain ($n=1$). Three cases were asymptomatic and admitted due to pathological echocardiography results.

The Ethics Committee of Charité–Universitätsmedizin Berlin approved this study (EA4/120/21).

Microbiological Culture Analysis

Blood cultures sets (aerobic and anaerobic) were inoculated with approximately 10 mL of blood and incubated 14 days in a continuously monitored blood culture system (BACT/ALERT; bioMérieux, Nürtingen, Germany).

Following an instrument-flagged positive event, the bottle was removed from the system and a Gram stain and subculture on solid media were performed, including Columbia agar with 5% sheep blood and chocolate agar under aerobic conditions with 5% CO_2 , Sabouraud and MacConkey agar incubated aerobically, as well as Schaedler agar incubated anaerobically (all culture media from Becton Dickinson, Heidelberg, Germany).

As defined in the major Duke criteria, we considered BC results positive if the same pathogen was found in at least 2 pairs of the preoperative BC. Consequently, we considered BC as negative when at least 2 BCs were negative. We considered BC as inconclusive when only 1 pair of BCs was positive or negative, or in cases with the growth of several pathogens.

Conventional VCs were performed in all explanted prosthetic valves, except in 2 cases. For this, heart-valve samples were processed in a flow cabinet and minced using sterile instruments. Gram stain and routine culture on solid media were performed as described above. In addition, thioglycolate broth was inoculated and incubated for 14 days. Culture media were examined for growth on day 1, 2, 7, and 14. In case of positive culture, microorganisms were identified by routine microbiological

techniques including an automated biochemical system and later MALDI-TOF (matrix-assisted laser desorption/ionization–time of flight) mass spectrometry (Vitek 2 and Vitek MS; bioMérieux, Nürtingen, Germany).

FISHseq

In the majority of the cases (n = 111), 1 part of the prosthetic valve was analyzed by conventional microbiological culture, whereas another part was fixated intraoperatively in FISHopt (MoKi Analytics, Berlin, Germany) and examined with FISHseq (see [Supplementary Figure A1](#)). In 2 cases, only FISHseq but no VC was performed.

Sections of each prosthetic valve were prepared and analyzed by FISH as described previously [22, 26]. Briefly, samples were fixed, embedded in cold polymerizing resin, sectioned, and submitted to hybridization. After incubation for 2 hours in a dark humid chamber at 50°C, slides were rinsed with water, air-dried, and mounted for microscopy with an epifluorescence microscope (AxioImagerZ2; Carl Zeiss, Jena, Germany) equipped with narrow-band filter sets (AHF-Analysentechnik, Tübingen, Germany). Sections were first screened with the pan-bacterial, 16S rRNA–directed probe EUB338 [27] and the nonsense probe (NON338) to exclude unspecific probe binding [28]. The nucleic acid stain DAPI (4',6-diamidino-2-phenylindole) was applied as a counterstain to visualize host cell nuclei and microorganisms that no longer contain ribosomes, or too few ribosomes to be visualized by microscopy. A fourth microscopic channel was left without fluorochrome to control autofluorescence of the prosthetic valve material.

Upon detection of microorganisms, a panel of FISH probes was applied for identification on a genus- or species-specific level [22]. Each hybridization experiment was controlled using positive reference strains and negative control strains with a minimum number of mismatches at the probe binding site. A *Candida*-specific FISH probe was included to detect *Candida* sp. in all cases where yeasts were suspected.

FISH targets the ribosomes, which are highly abundant in replicating and metabolically active microorganisms. Therefore, FISH-positive microorganisms were regarded as active, whereas FISH-negative microorganisms that did not contain enough ribosomes to elicit a FISH signal were classified as inactive [29, 30].

16S rRNA Gene Sequencing

Sample sections consecutive to those used for FISH were submitted to DNA extraction, PCR amplification, and sequencing of part of the 16S rRNA gene, as described [31]. Briefly, the first 500 base-pairs of the 16S rRNA gene including variable regions V1–V3 were amplified using pan-bacterial primers and Sanger sequencing performed at a commercial sequencing facility (Microsynth, Göttingen, Germany). Sequences were analyzed using the diagnostic-grade commercial Centroid database of the program SmartGene (SmartGene, Inc, Lausanne, Switzerland).

Table 1. Patient and Clinical Characteristics

	Data
Patient characteristics	
Duke criteria, n	
Definite infectious endocarditis	70
Possible infectious endocarditis	43
Total patients, n	105
Age, range, mean, years	28–83, 65
Male, n	78
Female, n	27
Prosthetic valve and clinical characteristics	
Total prosthetic valves, n	113
Valve position, n	
Aortic	69
Mitral	39
Pulmonary	5
Prosthetic valve type, n	
Bioprosthetic	92
Mechanical	18
Autograft	2
Homograft	1
Indication for surgery, n	
Endocarditis	87
Valve regurgitation	19
Valve stenosis	4
Valve thrombosis	2
Aortic dissection	1
Onset of suspected endocarditis after prosthetic valve implantation, n	
Early, <1 year	49
Late, >1 year	64
Time between valve implantation and explantation, days	
Mean	1416
Range	12–8488
Duration of antibiotic treatment before valve explantation, days	
Mean	16
Median	4
Range	0–217

Statistical Analysis

In order to assess the diagnostic accuracy of the FISHseq method, we calculated the positive and negative percentage agreement and their 95% confidence intervals (CIs), as previously described [24, 32] and in accordance with Food and Drug Administration (FDA) recommendations [33]. All statistical analyses were performed with R 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Conventional Microbiology (Blood Culture and Valve Culture) Results

Blood cultures in PVE were positive in 37 of 83 cases (44.6%), growing mainly streptococci, enterococci, and staphylococci ([Figure 1A](#) and [Supplementary Table A1](#)). In 30 cases, no BCs were obtained preoperatively; in 22 and 24 cases, they were inconclusive or remained negative, respectively (55.4%). Therefore, in 67% (n = 76) of the total 113 cases, preoperative

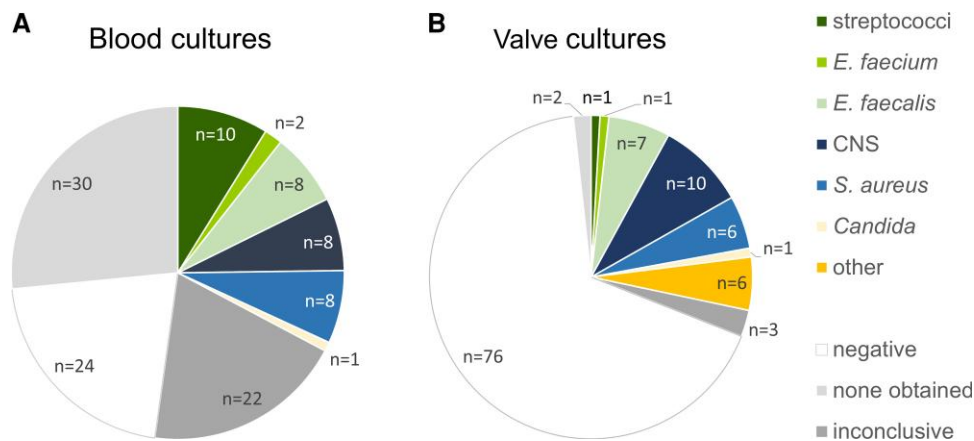


Figure 1. Culture results in PVE (N = 113). In over 50% of PVE cases, routine microbiology (BC and VC) was not informative regarding the etiologic pathogen: BC (A) had a high proportion of inconclusive results and missing samples; VC (B) had a high percentage of negative results. Note the difference in pathogen distribution: BC detected more streptococci, whereas VC identified a higher number of less typical PVE pathogens (marked as “other”). Abbreviations: BC, blood culture; CNS, coagulase-negative staphylococci; PVE, prosthetic valve endocarditis; VC, valve culture.

BCs alone were not available or not successful in the identification of the causative pathogen.

Valve cultures were positive in 32 of 111 cases (28.8%) (Figure 1B and Supplementary Table A1). Seventy-nine VCs (71.2%) remained negative or detected 2 or more pathogens. In 2 cases, no VC was performed. With regard to the valve position, there was no significant association with positive culture results (Supplementary Table A2).

FISHseq Results

FISHseq detected bacteria in 87 of 113 cases (77.0%), proving the diagnosis of PVE by direct visualization of microorganisms within the prosthetic material. Of these, the causative pathogen was identified in 51 cases (45.1%). In 26 of 113 cases (23.0%), no pathogen was detected either microscopically or by PCR (Supplementary Figure A2 and Supplementary Table A1).

Interestingly, the number of FISH-positive samples, indicating a high ribosome content and therefore activity, was significantly higher in patients with definite IE than in patients classified as possible IE according to the modified Duke criteria (chi-square test, $P < .01$) (Supplementary Table A3).

Valve Culture Results Compared With FISHseq Results

In 17 of 29 VC-positive cases (58.6% positive percent agreement), VC results were identical to FISHseq—that is, confirming the infection with the same pathogen (Figure 2). Neither method identified a pathogen in 49 of 76 cases (64.5% negative percent agreement). In 27 out of 76 cases with negative VCs, FISHseq succeeded in identifying the pathogen. This accounts for 35.5% of VC-negative cases. In 13 of these cases, FISHseq detected the same pathogen as the preoperative BC. FISHseq

identified different pathogens than VC in 3 cases. In 12 cases, FISHseq remained negative despite positive VC.

Spectrum of Identified Pathogens

Conventional cultures (BC plus VC) identified an etiologic PVE pathogen in 45 of 113 cases. When FISHseq complemented standard cultures, the number of cases in which a pathogen could be identified increased to 67 cases. Both diagnostic methods presented similar results with respect to pathogen distribution (Figure 3). However, FISHseq in combination with standard culture increased the detection of streptococci (9 vs 13), enterococci (10 vs 16), and rare PVE pathogens (3 vs 10). The latter included *Tropheryma whipplei*, *Listeria monocytogenes*, *Cutibacterium* (*Propionibacterium*) *acnes*, *Pseudomonas aeruginosa*, and *Corynebacterium* sp.

FISHseq: Diagnostic Impact

A case-by-case analysis showed that, in 33 of 113 cases (30%), the addition of FISHseq delivered new diagnostic information, either by identifying the IE pathogen that was missed by conventional culture or by adding information to the culture results (Figure 4). Within the latter group, most of the cases (15/19) were inconclusive in conventional culture, whereas FISHseq allowed pinpointing the IE pathogen (Figure 5). In 4 other cases (4/19), culture identified different pathogens than FISHseq (Figure 6).

Overall, the detection rates of the different techniques were 44.6% for BCs and 28.8% for VCs, and for FISHseq, 45.1% with species identification and 77.0% including past IE cases.

The clinical positive, negative, and overall percentages of agreement with 95% CIs were 74.2% (55.4–88.1%), 77.4%

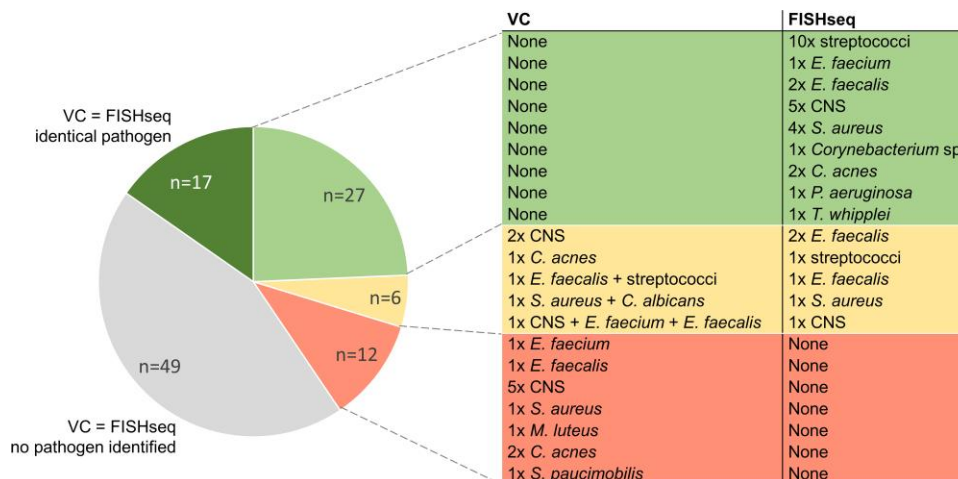


Figure 2. Pathogen detection: VC versus FISHseq (N = 111). In 59.5% (n = 66), VC and FISHseq results were identical, either identifying the same pathogen or detecting no pathogen on the prosthetic valve. VC remained negative in 24.3% (n = 12), whereas FISHseq detected typical prosthetic valve endocarditis pathogens, like streptococci, CNS, *Staphylococcus aureus*, or rare species like *Tropheryma whippelii*. Despite positive VC, FISHseq identified no pathogens in 12 cases. Interestingly, 9 of these cases grew species known as skin flora or contaminants. Abbreviations: CNS, coagulase negative staphylococci; FISHseq, fluorescence in situ hybridization combined with 16S rRNA gene polymerase chain reaction and sequencing; VC, valve culture.

(65.0–87.1%), and 76.3% (66.4–84.5%), respectively, between all culture and FISHseq results (Supplementary Table A4).

DISCUSSION

In recent years, the number of heart-valve surgeries has increased steadily [34], especially due to improved cardiovascular surgical techniques and the aging demographics in industrialized nations. Thus, PVE represents an increasing proportion of overall endocarditis cases [11, 30, 35]. Moreover, patients with PVE have an increased risk of a second IE episode [36].

Therefore, precise diagnostic procedures and treatment planning are of utmost importance [11, 35].

To our knowledge, this study is the first to evaluate a consecutive series of PVE cases by methods of molecular diagnostics (FISHseq) and compare them with conventional BC and VC results.

Blood Culture Is Important But Insufficient as a Single Diagnostic Tool in Prosthetic Valve Endocarditis

Conventional BC remains the most readily available and cost-effective diagnostic method, enabling not only identification of the pathogen but also antimicrobial susceptibility testing in IE without heart-valve surgery [26]. However, correct antibiotic treatment based on BC results is highly dependent on a meticulous workflow when collecting BC sets. As described elsewhere before, in this study a high proportion of inconclusive and missing BCs (22 and 30 of 113 cases; 19% and 27%, respectively) emphasizes a diagnostic loophole in everyday clinical practice.

Diagnostic Value of the Valve Culture Is Limited Due to a High Rate of Negative Results and Risk of Contamination

In our study, 32 of 111 VCs (28.8%) turned positive, with 14 cases confirming the pathogen found in BC. Three additional cases yielded polymicrobial VC and were therefore of limited value. In 3 BC-negative cases, VC grew *Sphingomonas paucimobilis*, *Staphylococcus epidermidis*, and *C. acnes*, which was hard to interpret without the microscopic evidence of the bacteria in FISHseq. However, in 5 cases, the pathogen diagnosed by VC was identical to the pathogen identified in inconclusive BCs (due to growth in 1 set only); and in 4 cases, BCs were not

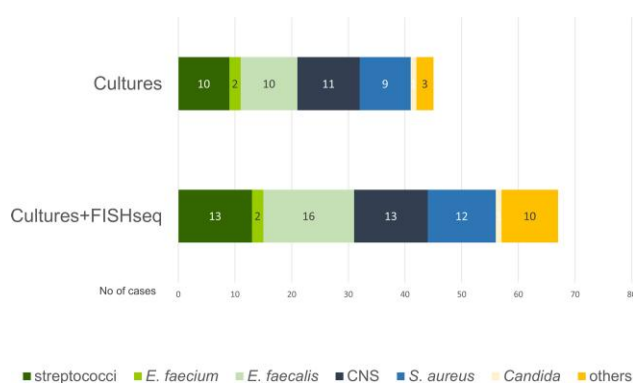


Figure 3. Pathogen distribution by conventional culture versus culture combined with FISHseq. FISHseq in combination with standard culture diagnostics (blood cultures and valve cultures) increased the number of detected pathogens from 45 to 67. In particular, streptococci, *Enterococcus faecalis*, and less typical PVE pathogens were detected more often. Abbreviations: CNS, coagulase negative staphylococci; FISHseq, fluorescence in situ hybridization combined with 16S rRNA gene polymerase chain reaction and sequencing; PVE, prosthetic valve endocarditis.

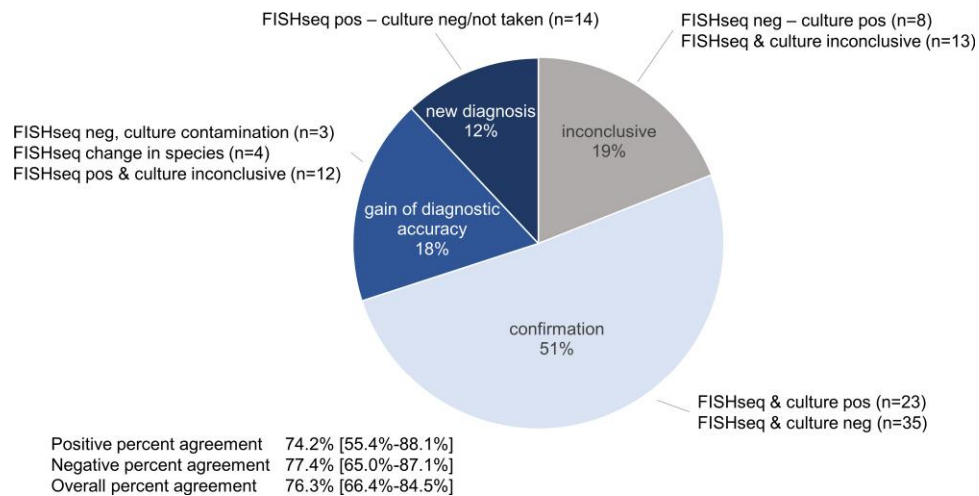


Figure 4. FISHseq: diagnostic impact (N = 113, culture = valve culture and blood culture). Between all culture and FISHseq results, the clinical positive, negative, and overall percentages of agreement with 95% confidence intervals are 74.2% (55.4–88.1%), 77.4% (65.0–87.1%), and 76.3% (66.4–84.5%), respectively (see also [Supplementary Table A4](#)). The light-blue color indicates congruent FISHseq and culture results, the light-gray color indicates the inconclusive cases where FISHseq remained negative despite plausible positive culture results (wrong piece of the heart valve investigated by FISHseq) or both FISHseq and culture remained inconclusive. The dark-blue and blue colors mark the cases where the addition of FISHseq delivered new diagnostic information, either by identifying the causative pathogen, which was missed by conventional culture, or by adding information to positive culture results, respectively. Abbreviations: FISHseq, fluorescence in situ hybridization combined with 16S rRNA gene polymerase chain reaction and sequencing; neg, negative; pos, positive.

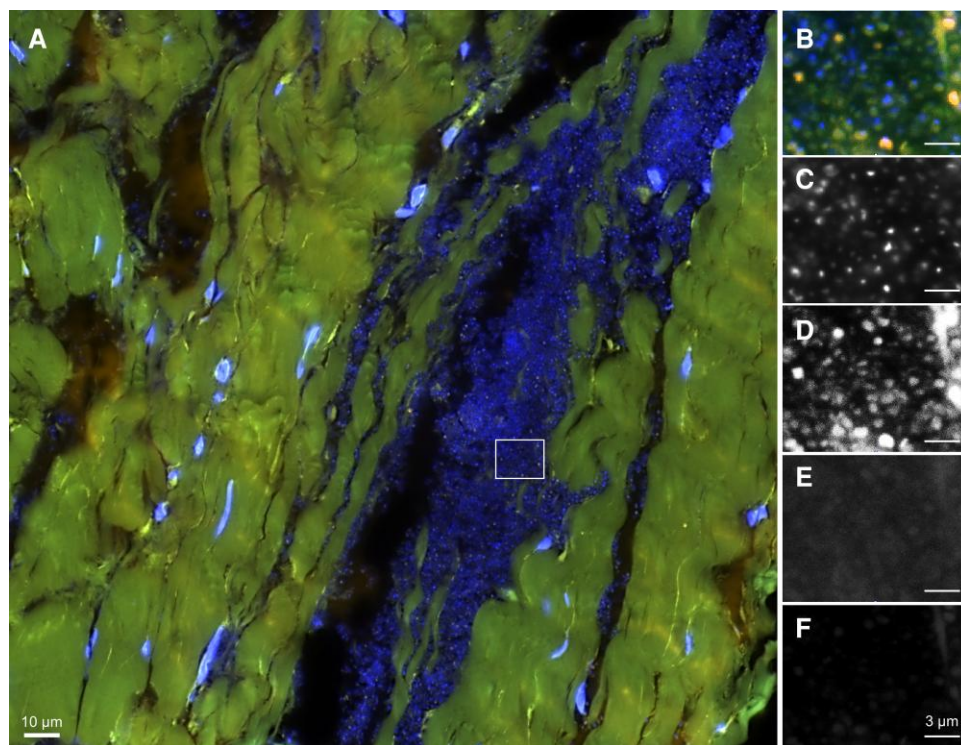


Figure 5. FISHseq in PVE allows clarification of inconclusive culture results. FISH analysis of a prosthetic heart valve with a single positive blood culture bottle with *S. epidermidis*, whereas the valve culture remained negative. FISHseq revealed *S. epidermidis* biofilms. **A**, Overview of the heart valve (green) with extensive biofilms (nucleic acid stain DAPI in blue and the staphylococci-specific probe STAPHY in orange). **B–F**, Magnification of the inset in panel **A**. **B**, All channels. **C**, DAPI in black-and-white. **D**, STAPHY in black-and-white. **E** and **F**, The *Staphylococcus aureus*-specific probe SAU and the nonsense control probe NON338 remained negative, respectively. Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; FISH, fluorescence in situ hybridization; FISHseq, fluorescence in situ hybridization combined with 16S rRNA gene polymerase chain reaction and sequencing; PVE, prosthetic valve endocarditis.

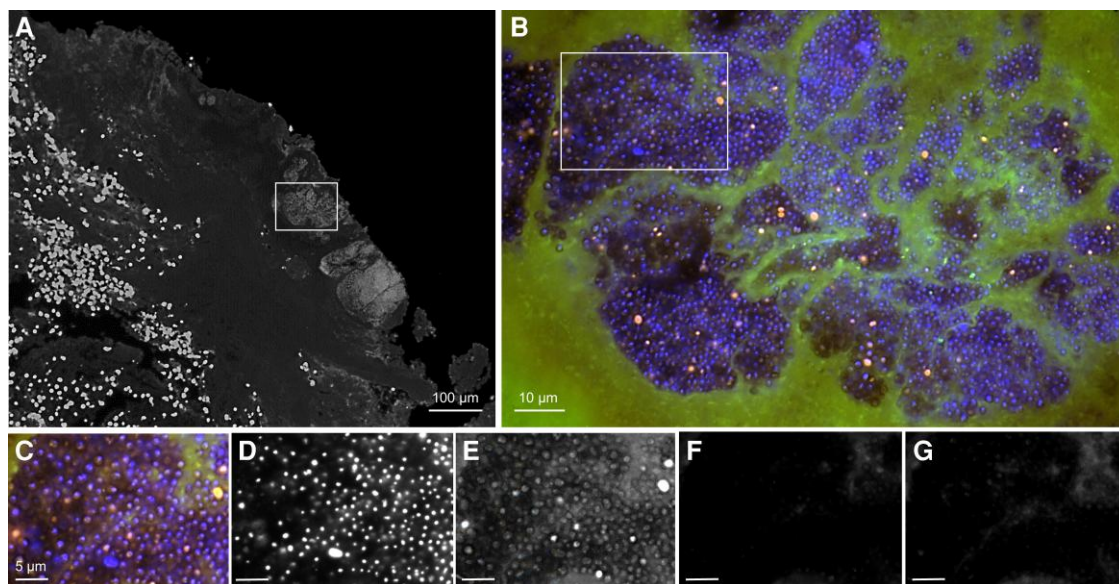


Figure 6. FISHseq analysis of a PVE case with mixed culture results. FISH analysis of a prosthetic heart valve where culture yielded both *Enterococcus faecalis* and *Staphylococcus aureus*. FISHseq revealed biofilms by *S. aureus* only. *A*, Overview of the heart valve (nucleic acid stain DAPI in black-and-white) with extensive biofilms. *B*, Magnification of the inset in panel *A*: heart valve (tissue background green) with *S. aureus* biofilms (nucleic acid stain DAPI in blue and *S. aureus*-specific FISH probe SAU in orange). *C–G*, Magnification of the inset in panel *B*. *C*, All channels. *D*, DAPI in black-and-white. *E*, SAU in black-and-white. *F* and *G*, The enterococci-specific probes EFAEC + FMDUR and the nonsense control probe NON338 remained negative, respectively. 16S rRNA gene PCR and sequencing resulted in a single-species chromatogram. Together with the clear mono-species FISH result of *S. aureus*, there was no indication of the presence of mixed PVE infection. Abbreviations: DAPI, 4',6-diamidino-2-p-henylindole; FISH, fluorescence in situ hybridization; FISHseq, fluorescence in situ hybridization combined with 16S rRNA gene polymerase chain reaction and sequencing; PCR, polymerase chain reaction; PVE, prosthetic valve endocarditis.

obtained and VC provided the only data available. Therefore, 9 of 111 VCs added diagnostic yield over BC.

Of 76 of 111 cases with negative VC, 41 cases received antibiotic therapy for more than 1 week prior to valve explantation. Only 17 cases received no antibiotic treatment preoperatively. This suggests that antibiotic treatment may have contributed to the high percentage of negative VCs in our analysis.

However, especially in cases with negative, inconclusive, or missing BCs, VC remains an important diagnostic tool since it is the “last chance” to identify the causative agent in PVE [25].

FISHseq as a Molecular Imaging Technique Can Prove Diagnosis of Prosthetic Valve Endocarditis

FISH is a molecular, culture-independent technique that allows direct visualization and identification of IE-causing organisms within the sample [22]. Broad-range PCR and sequencing may detect the etiology of infections [37]. However, the presence of bacterial DNA does not necessarily indicate viable bacteria, since DNA from dead bacteria also is amplified. Bacterial DNA may persist long after IE is cured, leading to false-positive PCR or next-generation sequencing (NGS) results [38]. Moreover, false-positive findings due to contamination with bacterial DNA from reagents or sampling contamination may occur [22].

The diagnosis of IE by FISH is highly probative as it has the potential to differentiate between contamination of the sample and true infection of the heart valve [22]. FISH can be considered as a bridging method between microbiology, pathology, and molecular diagnostics [26]. The strength of FISHseq is the molecular visualization of the microorganisms directly within the histological context, therefore proving their involvement in the infection. Thus, the combination of FISH and PCR enables not only an increased sensitivity but also a reduction in false-negative/-positive results.

In this study, FISHseq detected causative pathogens in 51 of 113 cases (45%), which suggests higher sensitivity as compared with BC (32%) and VC (28%). However, the cases that turned positive in cultural diagnostics were not always identical to those with positive FISHseq results. FISHseq identified PVE-causing pathogens in approximately one-third of the cases with noninformative (negative, inconclusive, or missing) BCs. In addition, in the study of Eichinger et al [39], FISHseq turned out to be the most effective diagnostic test for pathogen detection in native valve endocarditis. In this study, FISHseq identified microorganisms in 103 of 128 (80.4%) cases, whereas BCs were positive in 67 of 128 (52.3%) cases and VCs only in 34 of 128 (34%) cases.

In our study, out of 76 VC-negative cases, FISHseq proved the presence of a pathogen in 27 cases (35%). Mallmann et al

[22] delivered similar data for native valve IE with FISHseq-positive results in 11 out of 37 VC-negative cases.

In cases with inconclusive or polymicrobial culture results, FISHseq helped pinpoint the PVE-relevant pathogens and interpret the findings of single BC (Figures 5 and 6). Of 113 examined prosthetic valves in this study, in 3 cases VC detected 2 or more pathogens. FISHseq did not confirm any of these suspected polymicrobial infections and in each case identified a single agent associated with IE.

Culture results of typical skin flora may be difficult to interpret due to suspected contamination. An interesting example from this study is the case of a 69-year-old patient with IE of a prosthetic aortic valve, in which only in 1 BC was *S. epidermidis* detected. The VC remained negative. FISH visualized structured *S. epidermidis* biofilms and PCR confirmed the presence of *S. epidermidis*, detecting active IE with this pathogen (Figure 5). Without FISHseq, this case might have been treated as culture-negative PVE.

Prosthetic valve endocarditis caused by typical skin flora is gaining increasing attention. Lalani et al [40] observed that CNS PVE, when compared with *Staphylococcus aureus* or viridans group streptococcal PVE, was associated with a more aggressive course, higher rates of heart failure and surgery, as well as a trend toward higher rates of cardiac abscesses. As a possible explanation, the authors suggest a delay in diagnosis, given the tendency to consider CNS as a contaminant. *Cutibacterium acnes*, also a typical member of the skin flora, is known as an emerging pathogen among patients with PVE [41].

FISHseq turned especially helpful in the identification of less typical PVE-associated pathogens, like *T. whipplei*. Their relevance for PVE etiology could be proven by direct visualization of the microorganisms in situ. In previous studies, FISHseq has shown its usefulness in the diagnosis of fastidious bacteria, detecting *Bartonella quintana*, *T. whipplei*, *Lactobacillus paracasei*, or *Coxiella burnetii* on the examined heart valves [22, 26].

Study Limitations

A clear drawback of FISHseq is that prosthetic heart valves can be examined only after explantation. Furthermore, FISHseq does not provide antibiotic susceptibility testing of the identified pathogens. Therefore, the method cannot replace routine culture. However, it is a useful complement to routine culture both in culture-negative and questionable cases.

This study presents a retrospective evaluation of consecutive cases in a single-center approach. Thus, larger, prospective, multicenter studies are needed to analyze the impact of FISHseq on therapy guidance and on patients' short- and long-term outcome.

Diagnostic Impact of FISHseq

To sum up, the addition of FISHseq to the conventional culture delivered new information about PVE etiologic pathogens in

30% of cases, either by identifying the pathogen in culture-negative cases (12%) or by clarifying inconclusive positive culture results (18%) (Figure 4). This indicates that FISHseq is a useful diagnostic tool, improving the sensitivity of conventional culture in patients with PVE.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. M. M. H., F. R. K., A. M., and J. K. conceptualized the study and analyzed and interpreted the data. M. M. H., A. M., and J. K. wrote the original draft with input from all authors. All authors critically reviewed and approved the final version of the manuscript.

Acknowledgments. The authors thank Michael Schäffer for help with the database and Ernst Wellenhofer as well as Marie Gühmann for help with the statistical analysis.

Financial support. This work was supported by the European Regional Development Fund (grant number 10135571 to A. M.), the Bundesministerium für Wirtschaft und Energie, Germany (grant number 03EFFBE081 to J. K. and A. M.), and the Bundesministerium für Bildung und Forschung, Germany (grant numbers 13N14917 and 13N15820 to A. M.).

Potential conflicts of interest. A. M. and J. K. are employees of and hold shares in MoKi Analytics GmbH in addition to being employed by Charité, and A. M. is founder and head of the private practice Moter Diagnostics. D. v. S. reports support for attending meetings from Insmid Germany GmbH (participation fee NTM Summit Berlin 8.10.2022). A. M. reports German Federal Ministry of Research and Education (BMBF) grants 13N15824 (FIELD), 13GW0414A (PROCEED), and 13N15815 (TEAM) (to A. M. and J. K.; MoKi Analytics) and European Union ITN grant 814168 (GROWTH) (to A. M.; MoKi Analytics); honoraria for lectures by BioMérieux and Chiesi; support for invited talks at meetings by BÄMI, ECCMID, SGM, ERASMUS+, and REMMDI; patents planned, issued, or pending for "Control Preparation for Fish Methods in Microbiology" (PCT/EP2016/051630); a leadership or fiduciary role with Konsiliarlabor Tropheryma whipplei, appointed by Robert Koch-Institute; shares with MoKi Analytics GmbH, and private practice with Moter Diagnostics. J. K. reports German Federal Ministry of Research and Education (BMBF) grants 13N15824 (FIELD), 13GW0414A (PROCEED), and 13N15815 (TEAM) (to J. K.; MoKi Analytics) and European Union ITN grant 814168 (GROWTH) (to J. K.; MoKi Analytics); support for attending meetings and/or travel from the German Society of Hygiene and Microbiology (DGHM) (2022); patent I 07/137 (2008) Crystalline EGFR-matuzumab complex and matuzumab mimetics obtained thereof; a role as Vice Speaker of the Young German Society of Hygiene and Microbiology; and financial or nonfinancial interests as Chief Executive Officer of MoKi Analytics GmbH, a start-up of Charité, since 2018. V. F. is affiliated with the German Heart Center-Berlin, Charité-University Hospital Berlin, DZHK, ETH-Zürich (Department of Health, Science, and Technology and Transl. Cardiovascular Technology). He also declares institutional financial activities in relation to the following: educational grants (including travel support), fees for lectures and speeches, fees for professional consultation, and research and study funds with the following commercial entities: Medtronic GmbH, Biotronik SE & Co, Abiomed GmbH, Abbott GmbH & Co, KG, Boston Scientific, Edwards Lifesciences, Berlin Heart, Novartis Pharma GmbH, JOTEC/CryoLife GmbH, LivaNova, and Zurich Heart. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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