Polymerase Chain Reaction to Diagnose Infective Endocarditis
Will It Replace Blood Cultures?

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In this issue of Circulation, Breitkopf and colleagues report on a series of 52 excised heart valves on which they performed broad-range polymerase chain reaction (PCR) to identify microbes in valve tissues that were defined as having infective endocarditis (IE). Internal sequencing of amplicons, with specific “nested” primers to identify microbial subspecies, was performed after broad-range PCR. The authors report that on the basis of gross features and histopathology, 22 (42.3%) of the 52 valves, which otherwise had evidence of IE, also had microbial subspecies identified. Eight (44%) of 18 IE valves that were preceded by positive blood cultures, where blood culture data were available, were positive by broad-range PCR. von Reyn’s, Duke, and modified Duke criteria use blood cultures as a major clinical criterion to predict IE and histopathologic evidence of IE on cardiac valves as “definite” evidence of IE. Arguably, these two criteria together represent the most definite evidence of disease. Therefore, it may seem enigmatic that between and among major criteria used for the diagnosis of IE, >50% of the valves in the Breitkopf et al series did not yield a causative microorganism identified by broad-range PCR. At first glance, broad-range PCR may seem to be a highly sensitive method to detect the presence of microbes. Indeed, this method was ~3-fold more sensitive than the Gram stains and cultures performed on these tissues combined.

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Drawbacks of PCR: Tradeoffs in Sensitivity and Specificity

Broad-range PCR is used to target commonly shared bacterial 16S rRNA genes (via pan-bacterial primers), and subsequently, direct sequencing is used to detect and differentiate bacteria. Broad-range PCR should be an added benefit to blood cultures to screen for bacteria that may cause IE. In the past, contamination of PCR reactions with irrelevant bacteria had been a major drawback, but it may still occur today even in the face of rigorous techniques intended to prevent contamination of the specimen on which the broad-range PCR is to be performed. Specially designed PCR laboratories and carryover prevention techniques, the latter used by Breitkopf et al, also have been invoked to prevent contamination. Carryover prevention uses PCR reactions that substitute uracil for thymidine; subsequent reactions are initiated with uracil-N-glycosylase to degrade uracil containing amplified sequences that may have been carried over from previous reactions. The enzyme is then inactivated with heat before the test sample is added for amplification. Despite the use of preventive measures, wild (unamplified) background bacterial DNA that can attach itself to DNA polymerase may still be present in every reagent used in PCR reactions, including “sterile” water, primer preparations, and nucleic acid extraction reagents. Unfortunately, broad-range PCR still has the potential to inappropriately amplify and identify background sequences. This may confound results that yield bacterial species that could be contaminants, some of which (eg, Staphylococcus epidermidis and species of Streptococcus viridans) also can cause IE.

Reducing the problem of background contamination in broad-range PCR reactions can be approached by selecting primers with intrinsically low sensitivity, modifying thermal cycling parameters to obtain low amplification, or selecting for amplified sequences that exhibit a high signal (eg, via a postamplification colorimetric assay). Predigestion of the entire mixture of PCR reaction components with selected restriction enzyme(s) can render contaminating DNA unamplifiable by PCR. The enzyme(s) are then inactivated by heat before the addition of sample DNA. Nonetheless, small amounts of background bacterial DNA may remain, usually introduced during the processing of the specimen (eg, by plasticware, grinders), during DNA extraction (reagents and buffers), or bound to the DNA polymerase. Eighty-seven percent sensitivity and specificity versus blood culture was reported in one broad-range PCR approach that used decontamination strategies and tested blood (arguably the specimen of choice to diagnose IE) from 51 febrile intravenous drug users. All 8 patients who had definite IE (infected with S aureus, streptococci, or both) in this group also were positive by broad-range PCR. Despite the lower sensitivity reported by Breitkopf et al, specificity of the PCR as compared with culture and Gram stain was 100% in excised heart valves with IE. This indicates excellent control of contamination, with only 1 of 16 valves without suspected IE showing a possible contaminant with Aspergillus spp by culture but not by PCR that used fungal primers targeting 18S and 28S rDNA.

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A disadvantage of these approaches is that DNA from the causative agent(s) must be present at a relatively high concentration to be properly detected. The general result has been that sensitivity achieved by broad-range PCR strategies, particularly for the more common pathogenic agents of IE, often is no better than that with blood culture. Blood cultures were positive in 18 (40%) of 42 IE valves (excluding the single-valve specimen infected with *Bartonella quintana*) in the series reported by Breitkopf et al,1 in which available blood culture data indicated that the bacteria involved could have been readily cultivated.

### Highlights of PCR

There are 6 conserved areas in 16S rRNA genes of bacteria (=1600 bp) that are suitable for use as pan-bacterial primer targets. Successful primer selection must enable amplification of unique bacterial sequences to provide useful data to differentiate most or all of the causative agents involved at the DNA level. Long amplicons provide more information, but they are more difficult to generate, which results in loss of sensitivity. Nested primers that span unique sequences in the 16S rRNA gene in staphylococci and streptococci at the species level (species-specific PCR) also were used in the Breitkopf et al series;1 these primers were used in >75% of the PCR assays performed, and the authors believed their use was justified to identify the most commonly anticipated species. There is great advantage in being able to detect bacteria that are difficult to culture (or are uncultivable) by using PCR reactions that target not only 16S rRNA (broad-range PCR) but also other gene sequences that are uniquely species-specific (present in and out of 16S rRNA genes). One fastidious organism, *B quintana*, was encountered in the Breitkopf et al series, indicating that PCR can be an important adjunct to blood cultures in IE caused by organisms that may not be isolated by standard blood culture systems that do not typically use cell culture (eg, *Coxiella burnetii* [the agent of Q fever, diagnosed in 5% of cases of endocarditis in France],12 *Bartonella* spp, *Chlamydia* spp, *Tropheryma whippelii* [the Whipple’s disease bacterium], and other organisms such as the HACEK [Hemophilus aphrophilus, *H paraprophilus*, *H parainfluenzae*, Actinobacillus actinomycetemcomitans, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella* spp] group, *Legionella* spp, and *Mycobacterium* spp that are simply hard to grow or take a long time to grow in commonly used blood culture systems).

### Judicious Use of Blood Cultures

The low sensitivity of broad-range PCR (and even lower sensitivity of cultures and Gram stains) in the excised heart valves reported by Breitkopf et al1 may not be surprising if patients in this series, who were admitted mostly from other hospitals, had already received prolonged courses of antibiotics, after which they developed cardiac complications that resulted in operations to remove and replace infected valves. As expected, viable cultures and organisms visible on Gram stains of the valves may have disappeared even before the broad-range PCR (which does not require viable organisms, only remnant DNA) turned negative. PCR testing may revert to negative even before histopathologic (or gross) evidence of endocarditis clears. The importance of repeatedly positive blood cultures of common streptococci (and enterococci) or *S aureus* (echocardiographic findings notwithstanding) in this setting cannot be overemphasized. Positive blood cultures, although not absolutely required by Duke3 or modified Duke4 clinical criteria, were in fact present in 58% to 76% of definite cases reported in those series. Applying these blood culture criteria to the Breitkopf et al1 series should mean that 9 to 12 patients whose valves were infected with one of these organisms should have had positive blood cultures earlier; 7 did so (in addition, no blood culture data were available on 2 additional valves infected with these organisms). This suggests perhaps a low sensitivity of blood culture, which probably resulted from the use of antimicrobial therapy before blood cultures were taken. Of the remaining heart valves that were negative by molecular and conventional methods, 10 (43%) of 23 had positive blood cultures, a blood culture rate that is not different from the 8 (40%) of 20 whose valves were positive by PCR.

Although the diagnosis of the causative pathogen in endocarditis is useful and important in guiding therapy, the diagnosis by heart valve analysis is unlikely to be helpful in guiding initial therapy in all but those who undergo emergency surgery for complications that require immediate excision of the infected valve(s). Even then, most of these are caused by *S aureus*, which usually can be diagnosed easily by blood culture. Although negative blood culture results may often be attributable to fastidious organisms, modern blood culture systems have gone a long way toward overcoming this limitation as long as an adequate number of blood cultures have been obtained before instituting antimicrobial therapy and the microbiology laboratory is advised about the possible diagnosis of endocarditis. PCR is most beneficial in the identification of organisms that cannot be cultivated from blood culture systems routinely. In circumstances under which organisms are easier to grow (eg, most of the streptococcal species) or where they often grow even in the presence of antibiotics (eg, staphylococcal species), the most common cause of culture negativity is that treatment was begun before adequate blood culturing was performed.

Traditionally, it has been the opinion of many physicians caring for patients with endocarditis that at least 3 blood cultures should be taken during 1 hour in anyone suspected of having endocarditis before antimicrobial therapy is initiated. Defining who is suspected of having endocarditis and ensuring that all such patients are attended by physicians who share this point of view may be problematic and even impossible. Therefore, new and ingenious strategies to improve the sensitivity and specificity of PCR reactions to identify these patients are welcome. These strategies should include further reduction of background bacterial DNA (eg, at the time of DNA extraction); the use of long-range DNA polymerases to provide for more sequence information to minimize the number of PCR reactions (thereby diminishing the possibility of contamination); cleaner techniques for sample acquisition; and the application of these techniques to blood, the most relevant specimen in IE.

### References


**Key Words:** Editorials ■ endocarditis ■ polymerase chain reaction ■ valves ■ blood-borne pathogens
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