Apparent Culture-Negative Prosthetic Valve Endocarditis Caused by Peptostreptococcus magnus

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In two patients with prosthetic valve endocarditis due to Peptostreptococcus magnus, blood cultures in the BacT/Alert and BACTEC 9240 systems were signal negative. The capability of the BacT/Alert system to detect various Peptostreptococcus species was assessed. P. magnus and P. anaerobius could not be detected, and subcultures remained negative. The growth in conventional media of these two species and other Peptostreptococcus species was similar.

Gram-positive anaerobic cocci (GPAC) are commonly present in clinical specimens and are often mixed with other anaerobic or aerobic bacteria. GPAC can be involved in chronic infections of the upper respiratory tract, ears, mastoid, sinuses and teeth, intra-abdominal and female pelvic regions, subcutaneous and soft tissue abscesses, and diabetic foot ulcers, as well as in osteomyelitis and arthritis (2, 3, 7). Peptostreptococcus magnus, one of the GPAC species, is part of the normal flora of the human mucocutaneous surfaces. Although P. magnus is the most frequently isolated GPAC from clinical specimens, as well as in pure culture and from normally sterile sites, it has been scarcely reported as a cause of septicemia or native-or prosthetic-valve endocarditis (7, 8). Automated blood culture systems are developed in order to culture a broad variety of microorganisms. The anticipated pitfall of these systems is the inability to recover fastidious microorganisms with low counts, such as Brucella species (12). Recently, however, false-negative cultures in an automated blood system were also reported for Pseudomonas aeruginosa as well (5). We report here two cases of prosthetic-valve endocarditis due to P. magnus which was recovered after culturing the surgically removed infected valve, whereas BacT/Alert blood cultures, incubated for 14 days, remained negative. Therefore, we assessed the capability of the BacT/Alert system to detect GPAC bacteremia.

Case 1. The first case was a 65-year-old man who suffered from a mitral valve incompetence due to degenerative changes. The patient was scheduled for mitral valvuloplasty and a venous single graft coronary bypass on the posterior descending branch. Routine preoperative screening for infectious dental foci was negative. During the operation, valvuloplasty appeared to give insufficient results, so the mitral valve was excised and replaced with a prosthetic heart valve (Medtronic Hall prosthetic mitral valve was implanted. The removed valve was immediately transported to our laboratory in a sterile container. Several impression smears were made. Gram staining showed some destructed cells and no bacteria. Histopathologic preparations showed only degenerative changes and no bacteria. Valve parts were ground and cultured on Columbia blood agar (Oxoid, Basingstoke, England), on chocolate agar (GC Agar Base; Oxoid), and in BBL Thioglycollate Medium (Becton Dickinson, Cockeysville, Md.). Incubation was done at 5% CO₂ and anaerobically at 37°C for up to 7 days. The patient was treated with vancomycin (2,000 mg/24 h), gentamicin (240 mg/24 h), and rifampin (1,200 mg/24 h). Despite maximal support, the patient died 2 days later because of untreatable cardiogenic shock. On autopsy, a dilated hypertrophic left and right ventricle was found with signs of an old infarction in the posterior wall with recent extension to the lateral side of the septum. The grafts on the posterior descending branch and the descending anterior branch were patent. There was no dehiscence of the reimplemented prosthetic heart valve. In the BacT/Alert system no growth was detected after incubation for 14 days. Subcultures of the four BacT/Alert bottles on Columbia blood agar and chocolate agar were also negative. Anaerobic culture of the removed infected heart valve yielded a few colonies of large gram-positive cocci after 48 h of incubation on blood agar. Also, the Thioglycollate Medium showed growth after 48 h. The cocci were biochemically inert, sensitive for kanamycin, and resistant for sodium polyanethol sulfonate (SPS) and were identified as P. magnus. The identification was con-

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firmed by 16S rRNA sequencing (National Institute of Public Health, Bilthoven, The Netherlands).

Case 2. The second case was a 39-year-old male who underwent an aortic valve replacement because of severe aortic regurgitation. Routine preoperative screening for infectious dental foci was negative. Two months later, the aortic valve dehiscence and valvular vegetations, and aortic root abscesses could be detected by TEE. Blood cultures collected at local hospital B and incubated in the BACTEC 9240 remained negative, and no antibiotic therapy was started. The patient was referred to our hospital for a second aortic valve (Sorin 29) replacement. On admission, the CRP level was elevated (104 U/liter), and blood cultures were obtained. The removed valve was incubated according to standard operating procedures as described above (case 1). The Gram staining of impression smears showed some leukocytes and sporadic gram-positive cocci. Empirical treatment was started with vancomycin (2,000 mg/24 h) and gentamicin (240 mg/24 h). Seven blood culture sets incubated for 14 days in the BacT/Alert system remained negative. 
P. magnus grew after 96 h of culture of the valve vegetations on blood agar and in Thioglycollate Medium. The patient was started on penicillin G (12,000,000 U/24 h) and metronidazole (1,500 mg/24 h). A few days later, a third operation was necessary because of the presence of abscesses detected by TEE. A bioprostheses (Freestyle) was inserted. The postoperative period was without complications, and the patient recovered completely. After 6 weeks of treatment, the CRP level was normal (<3 U/liter), and the antibiotics were discontinued.

The findings of these two cases prompted us to the question whether 
P. magnus could be detected in the BACTEC and BacT/Alert automated blood culture systems. Since we use the BacT/Alert system, assessment was done in this system. We compared the growth of 
P. magnus and other GPAC, including a more fastidious species ( 
P. anaerobius ) in blood culture media. The isolates from the patients (G9g1, G11a2), other isolates from our own collection, and strains from the American Type Culture Collection (ATCC) were used (Table 1). After 48 h of anaerobic growth a colony of GPAC was taken from a subculture, digested in phosphate-buffered saline (Merck), and then further diluted in horse blood. Aerobic (FA) and anaerobic (FN) BacT/Alert FAN bottles were seeded with 5 ml of horse blood containing 10³ to 10⁴ GPAC CFU/ml and incubated as recommended by the manufacturer (Organon Teknika, Durham, N.C.). Simultaneously (for quality control), 10 μl of the blood sample was plated on blood agar, and colonies were counted after 48 h of anaerobic growth. Bacterial growth in these bottles was compared with that in Thioglycollate Medium and Columbia Broth (Becton Dickinson, Cockeysville, Md.), daily spotted by eye. Subcultures from culture-negative bottles were made on days 3, 7, and 14 on blood agar and incubated anaerobically for up to 96 h.

P. micros , P. asaccharolyticus , and P. productus (recently renamed as Ruminococcus productus ) grew readily in the anaerobic BacT/Alert FAN bottles and were routinely detected via CO₂ production. Some P. micros and P. asaccharolyticus isolates also grew in the vented aerobic BacT/Alert FAN bottle (two of six isolates automatically detected and one of six isolates after subculture; Table 1). 
P. magnus and P. anaerobius were not detected by the BacT/Alert FAN system, and subcultures remained negative (Table 1). 
P. magnus grew readily in Thioglycollate Medium and Columbia Broth. Repeated seeding experiments in BacT/Alert FAN bottles (FN) with inocula of 
P. magnus isolates ranging from 10³ to 10⁴ CFU/ml remained signal negative. Subcultures only revealed growth for the G9g1 isolate at an inoculum of 10⁵ CFU/ml. There was no increase in CFU after incubation for 3 days, and subcultures after days 7 and 14 were negative. Thus, survival in the BacT/Alert system seems possible at high inocula for a limited time. The lack of growth of 
P. magnus in the BacT/Alert system has not been described previously to our knowledge. SPS cannot account for this finding, because the 
P. magnus isolates were tested for susceptibility to SPS and proved to be resistant. The reason for the inability to grow in the BacT/Alert system for 
P. magnus , remains unclear. Strains of P. anaerobius are known to be sensitive for SPS. Their inability to grow in the BacT/Alert system is probably due to SPS. However, some P. anaerobius strains have been detected in the routine use of the BacT/Alert system (11).

At local hospital A, where patient 1 was originally treated, 28 blood culture bottles had been taken. No growth was detected in the routine setting by their BACTEC system. When the cultures of the valve became positive, 16 bottles were still available for prolonged incubation (up to 28 days) and subculture. In only two bottles was 
P. magnus detected after subculture. Thus, in the BACTEC system 
P. magnus also does not grow readily and at least cannot be automatically detected, probably due to insufficient CO₂ production.

Endocarditis caused by GPAC remains a rare event. P. micros , P. magnus , and P. anaerobius are the species that have been described in case reports so far (6, 7, 8, 10). Anaerobic bacteria are recovered from 3 to 8% of the positive blood cultures in comparative studies of various automated blood culture systems (1, 4, 9, 11, 13). Clostridium and Bacteroides species are the predominant clinical relevant anaerobic species isolated. P. micros is the most common GPAC isolate cultured from automated blood systems, probably because of its ability to produce CO₂. The frequency of 
P. magnus in such systems may be underestimated, based on our findings. Since we were able to culture the organism from the excised valve in a case of apparent culture-negative endocarditis, 
P. magnus may be the etiological agent in other cases of culture-negative endocarditis.

![Table 1. Growth of peptostreptococci in BacT/Alert FAN anaerobic and aerobic blood culture media, Thioglycollate Medium, and Columbia Broth supplemented with 5 ml of horse blood](image-url)
and has to be taken into account when selecting empirical treatment. Remarkably, in an earlier report of prosthetic valve endocarditis, \textit{P. magnus} also was only detected after culture of the infected valve (8).

In cases of bacteremia and native- or prosthetic-valve endocarditis, automated blood culture sets may be not sensitive enough to detect microorganisms such as \textit{P. magnus}. Therefore, we recommend the use of additional media, such as conventional Thioglycollate Medium, for blood cultures from patients suspected for such infections.

REFERENCES