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# Laboratory diagnosis of infective endocarditis

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*The diagnosis of infective endocarditis is based on positive blood cultures. Modern microbiological techniques can isolate the aetiological agent in 90–95% of cases. The rapidity of detection has been improved by inoculation of 10 ml of blood, adequate dilution and media and systematic subcultures. Lysis-centrifugation has greatly improved the detection of fungi in blood.*

## Introduction

Infective endocarditis may be suspected by the skilful clinician in patients who present a fever, a changing or newly detected heart murmur, petechiae, haematuria or splenomegaly. Various ancillary diagnostic parameters such as circulating immune complexes, rheumatoid factor, teichoic antibodies for *S. aureus* antigenemia, echocardiography and radiology have been shown to be useful for establishing a diagnosis. However, infective endocarditis can only be confirmed by persistent positive blood cultures.

During the last three decades the spectrum of organisms causing infective endocarditis has not changed particularly; but the frequency of endocarditis has increased and the proportions have changed. Increasing age and prosthetic valve favour infections with diphteroids and *S. epidermidis*, enterobacteriaceae and fungi are encountered in immunocompromised hosts[1–4] finally *S. aureus* and *Pseudomonas* species are more frequently isolated in drug addicts[5,6]. New techniques have improved the detection of micro-organisms in blood over the past decade and have recently been reviewed[7]. Because of the diagnostic importance of blood cultures in infective endocarditis, certain aspects should be considered here.

## Diagnosis of bacteraemia

Bacteraemias can be classified according the time micro-organisms are detected in the bloodstream. First transient bacteremias occur after manipulation of infected tissue, instrumentation of contaminated mucosal surfaces, surgery in contaminated areas, and initially in a variety of systemic and localized infections. Second, intermittent bacteraemia points to undrained abscesses, often associated with fever of undetermined origin. Finally, continuous bacteraemia is the hallmark of acute or subacute bacterial endocarditis due to discharge from endocardial vegetation or any other intravascular focus. The amount of circulating micro-organisms is not only stable in these conditions as one would expect by definition, but also low: 54% of bacteraemias have colony counts of only 1–30 cfu $ml^{-1}$ in streptococcal and 41% 1–10 cfu $ml^{-1}$ in staphylococcal endocarditis[8]. Both conditions, continuity and low order of magnitude dictate number of cultures and volume of blood required for the detection of intravascular infection. In subacute endocarditis three cultures should be collected over a period of 24 h and repeated if they remain negative after 24–48 h of incubation. In acute situations three blood cultures can be collected within 1–2 h before starting therapy. Each culture should be obtained by separate venipuncture and considered as such even if multiple bottles are filled[7]. Increasing volume of blood is related to increased recovery of clinically significant organisms[9,10], but for establishing the diagnosis of infective endocarditis two culture sets are in general sufficient[8]: collection of additional blood cultures as well as prolonged incubation over 14 days may be necessary in previously treated patients or those with endocarditis due to fastidious micro-organisms[8,11]. Ninety eight percent of cases containing streptococci are isolated in the first two blood cultures.

The frequency of culture-negative infective endocarditis varies from 3 to 64%[12]. Negative cultures

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can be caused by right-side endocarditis in drug addicts, or marantic endocarditis and prior antimicrobial therapy as well as by particular organisms with fastidious growth: *Haemophilus* sp., *Actinobacillus* sp., *Cardiobacterium hominis*, *Eikenella corrodens*, *Brucella* sp. and growth-deficient streptococci belong to this group. Also, mycobacteria, *Coxiella burnettii*, Chlamydiae, fungi and viruses are not routinely cultured in laboratories[12]. Finally technical errors and the way of processing blood cultures in the laboratory can produce false-negative results.

**Culture media**

Most proposed enriched media as tryptic or Trypticase soy, supplemented peptone, brain heart infusion, Columbia and brucella are adequate for the detection of aerobic and because of their low redox-potential not only facultatively but also strictly anaerobic organisms. However, the generic equivalence of blood culture media can never be presumed. Various supplementation, the amount of vacuum and the composition of the gases in the bottle varies widely for each manufacturer. Only one additive is regularly found in blood culture media: 0·025 to 0·05% sulfopolyanetholsulfonate (SPS), a polyanionic anticoagulant, inhibits the complement and lysosyme activity of blood, interferes with phagocytosis and inactivates clinically achievable concentrations of aminoglycosides. Clearly the addition of SPS in blood culture media improves the speed of growth and recovery of most clinically relevant organisms. Its inhibitory effect on *N. gonorrhoeae* could be detrimental in the diagnosis of infective endocarditis, however this diagnosis is rare[13]. Blood culture media made hypertonic by the addition of 10–30% sucrose or sorbitol have been advocated to enable the isolation of cell wall-defective bacteria from blood. The true value is controversial and appears to be medium dependent[14]. Also, the dilution in the culture medium is essential to prevent the bactericidal effect of fresh human serum or circulating antimicrobial agents. A dilution of at least 1 : 5 to 1 : 10 has been shown to be necessary[15].

**Neutralization of antibiotics**

Administration of antibiotics within 2 weeks before obtaining blood cultures reduces the proportion of positive blood cultures and delays growth[8]. The mechanism of this inhibitory effect is unclear. Because of the short half life of most antibiotics no antimicrobial activity in the circulating blood can be expected after this period. Also, the postantibiotic suppression of bacterial growth quantitated in *in vitro* studies lasts only for 1–3 h[17]. As already mentioned, dilution of blood or addition of SPS can inactivate circulating antibiotics. In patients treated with betalactam antibiotics, penicillinase is often added to the broth medium. However, the efficiency and need for penicillinase is not clear and has only been evaluated in a small number of patients[18,19]. More recently another approach has been used: processing the blood in an antimicrobial removal device before or during culturing adsorbs the remaining antibiotic on polymere resins, increases the detection of bacteraemia and decreases the time required for bacterial identification in clinically septic patients receiving antimicrobial therapy[20,21]. It is possible that these systems have no advantage over the more classical use of biphasic medium blood culture bottles[22]. It can be expected that this might also be the case when using a modification of the standard biphasic system developed by Roche Diagnostics. This system consists of a routine blood culture bottle onto which a cylinder is attached containing an agar-coated slide. The blood culture broth medium can then be subcultured onto the agar slide by tipping the bottle at the time of macroscopic examination[23]. Another approach to remove bacteria from the exposure of antibiotics could be the recently commercialized lysis-centrifugation system (Isolator System, Du Pont de Nemours and Co, Inc., Wilmington, DE[24]). In this technique the blood specimen is anticoagulated, the erythrocytes and leukocytes are lysed and the sample centrifuged in a special tube. After disgarding the supernatant the concentrate containing micro-organisms is plated on appropriate medias.

**Fastidious organisms**

As already mentioned various fastidious organisms as *Haemophilus* sp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis* and *Eikenella corrodens* are encountered in infective endocarditis. Routine subcultures on enriched media and prolonged incubation over 2 weeks should be sufficient to detect these organisms. Nutritionally variant, vitamin B6-dependent or satelliting streptococci, have been responsible for 5–6% of endocarditis[25]. They grow well in the presence of fresh human blood

but to obtain growth on subculture the media must be supplemented with either L-cysteine, pyridoxal hydrochloride or the blood agar media get a cross-streak of *S. aureus*. Fungal endocarditis is increasingly encountered in patients with prosthetic valves, drug addiction, severe underlying disease or prolonged parenteral nutrition. *Candida* sp. are the most frequently isolated. As an obligatory aerobic organism yeast cannot be detected in unvented broth bottles and in vented bottles the growth is slow. Until recently vented biphasic brain heart infusion medium was recommended to shorten the time for recovery and to increase the detection yield of fungi[26]. The lysis-centrifugation system described earlier has clearly been shown to be superior to conventional systems for rapidity and yield in fungal blood cultures[27].

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