

An evaluation of extended incubation time with blind subculture of blood cultures in patients with suspected endocarditis

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BACKGROUND: In a small proportion of patients, bacterial endocarditis is due to organisms that grow slowly and may not be recovered in conventional blood cultures incubated for five days. This has led to recommendations for prolonged incubation and routine subculture of negative cultures.

OBJECTIVE: The above-mentioned approach is evaluated.

METHOD: The microbiology of all blood cultures subjected to prolonged incubation and the charts of individuals who had organisms recovered after five days were evaluated to determine their clinical significance.

RESULTS: In all, 507 blood cultures were handled using an extended incubation and blind subculture protocol. Fifty-three blood cultures in 27 patients were positive. Blood cultures were positive after five days in only five cases; patient outcomes were not affected by the results in any of these cases, although several fastidious organisms (ie, *Haemophilus paraphrophilus* and *Haemophilus parainfluenzae*) were recovered in the first five days of incubation.

CONCLUSION: Prolonged incubation and blood subcultures in patients with suspected endocarditis or infections due to fastidious organisms do not represent a wise use of increasingly scarce resources.

Key Words: Blood cultures; Diagnosis; Endocarditis

The diagnosis of infective endocarditis (IE) continues to rely heavily on the isolation of the causative organism from blood cultures (1,2). While conventional blood cultures are usually positive within several days, a small proportion are negative (3). There are many reasons why patients may have 'culture-negative' endocarditis. Some agents may be fastidious, slow growing or noncultureable, patients may have received prior antimicrobial therapy, and low-grade bacteremia may result in a very small inoculum. The failure to culture an adequate volume of blood is perhaps the most important reason for falsely negative blood cultures (4). Traditionally, many laboratories have followed the practice of extending the incubation of blood culture bottles from 14 to 21 days to improve recovery rates (2). Expanded protocols require additional resources, occupy valuable space on blood culture instruments, may increase contamination rates and lead to a delayed final report. I evaluated our experience with consecutive blood cultures for suspected endocarditis collected between June 2002 and August 2005 at the Queen Elizabeth II Health Sciences Centre

Une évaluation du temps d'incubation prolongée de sous-cultures à l'aveugle d'analyses sanguines chez des patients atteints d'une endocardite présumée

HISTORIQUE : Dans une faible proportion de patients, l'endocardite bactérienne est causée par des organismes qui croissent lentement et risquent de ne pas être dépistés dans des cultures sanguines incubées pendant cinq jours. Cette constatation a donné lieu à la recommandation de prolonger le temps d'incubation et de procéder à des sous-cultures systématiques des cultures négatives.

OBJECTIF : La démarche précédente est évaluée.

MÉTHODOLOGIE : On a évalué la microbiologie de toutes les analyses sanguines soumises à une incubation prolongée et le dossier des personnes chez qui on avait dépisté des organismes après la période habituelle de cinq jours, afin de déterminer leur signification clinique.

RÉSULTATS : Dans l'ensemble, 507 analyses sanguines ont été manipulées au moyen d'un protocole d'incubation prolongée et de sous-cultures à l'aveugle. Cinquante-trois analyses sanguines prélevées chez 27 patients étaient positives. Les analyses sanguines étaient positives après la période de cinq jours dans seulement cinq cas. L'issue de ces patients ne changeait en rien par suite de ces résultats, même si quelques organismes pernicieux (p. ex., *Haemophilus paraphrophilus* et *H parainfluenzae*) ont été prélevés dans les cinq premiers jours suivant l'incubation.

CONCLUSIONS : Une incubation prolongée et des sous-cultures sanguines des patients atteints d'une endocardite présumée ou d'une infection causée par des organismes pernicieux ne constituent pas une utilisation judicieuse de ressources de plus en plus rares.

('the Centre') laboratory (Halifax, Nova Scotia). The Centre services the Capital District Health Authority, which has a population of 395,000. All regional blood cultures, with the exception of those from the Izaak Walton Killam Health Centre (a children's and women's hospital in Halifax, Nova Scotia), are processed in our laboratory.

METHODS

Specimens were received in BACTEC vials (Becton Dickinson, Canada), either as a single aerobic vial (BACTEC Plus Aerobic/F) or a set (with the addition a BACTEC Standard Anaerobic/F vial). Cultures were continuously monitored on a BACTEC 9240 instrument (Becton Dickinson, Canada). When the IE protocol was requested, the incubation time was extended to 24 days from five days. On day 10, three to five drops of media from each vial were subcultured onto a chocolate agar slope, loosely capped, incubated in 5% CO₂ at 35°C and examined daily for 14 days. The vial was returned to the instrument and monitored for a further 14 days. The final report, if negative, was issued on the 24th day.

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TABLE 1
Organisms recovered from blood cultures and subjected to the prolonged incubation protocol

	Isolates, n	Patients, n
<i>Staphylococcus</i> species (not <i>Staphylococcus aureus</i>)	24	16
<i>Escherichia coli</i>	9	4
<i>S aureus</i>	8	5
<i>Propionibacterium acnes</i>	2	2
<i>Haemophilus parainfluenzae</i>	2	1
<i>Haemophilus paraphrophilus</i>	2	1
<i>Enterococcus</i> species	4	3
<i>Peptococcus anaerobius</i>	3	1
Viridans streptococci	1	1
<i>Streptococcus mutans</i>	2	1
<i>Klebsiella pneumoniae</i>	1	1
<i>Burkholderia cepacia</i>	1	1
Total	59	37

RESULTS

During the 38-month period, 507 blood cultures were handled using the IE protocol used at the Centre. Fifty-three blood cultures from 27 patients were positive – 47 were positive with single isolates, and six had two isolates. The frequency and distribution of the organisms recovered are shown in Table 1.

In five cases, positive blood cultures would not have been recognized if our endocarditis protocol had not been applied. In the remainder, vials were flagged by the instrument as positive within the first five days. Table 2 summarizes the results of blood cultures positive during the extended incubation period. In two cases, single colonies of coagulase-negative staphylococci were recognized on the chocolate agar subculture at 14 and 22 days. One patient had *Propionibacterium acnes* detected by the instrument on day 14. One patient had coagulase-negative staphylococci seen on a Gram stain from a vial flagged by the instrument on day 11. In one case, *Enterococcus faecalis* was detected on day 6. The same patient had *E faecalis* in a blood culture collected the day before, which was positive by day 3.

The majority of isolates were nonfastidious, including 24 coagulase-negative staphylococci, nine *Escherichia coli* and eight *Staphylococcus aureus* strains. Several patients had organisms that may be considered more fastidious. One patient had *Haemophilus paraphrophilus* in two cultures, each of which was flagged positive by the BACTEC instrument on day 1. Another patient had two blood cultures positive on days 1 and 2 for *Haemophilus parainfluenzae*. A third patient had all three cultures positive on day 3 for *Peptostreptococcus anaerobius*. A fourth patient had *Streptococcus mutans* detected on days 2 and 3, and another had a viridans streptococcus recovered after one day of incubation.

DISCUSSION

In our experience, the prolonged incubation of blood cultures did not positively impact the care of any patient, because no additional cases of IE were diagnosed and no fastidious organisms were detected after day 5. All but one organism detected after day 5 were deemed contaminants. The variety of organisms seen in the study did not reflect the

TABLE 2
Summary of circumstances in which isolates were recognized after the fifth day of incubation using the extended incubation protocol

Patient	Isolate	Day detected	Detected by instrument?	Observations
1	<i>Staphylococcus</i> species (not <i>Staphylococcus aureus</i>)	14	No	Single colony on agar subculture, contaminant
2	<i>Staphylococcus</i> species (not <i>S aureus</i>)	22	No	Single colony on agar subculture, contaminant
3	<i>Staphylococcus</i> species (not <i>S aureus</i>)	11	Yes	Flagged by instrument, contaminant
4	<i>Enterococcus faecalis</i>	6	Yes	Accompanying second culture positive on day 3
5	<i>Propionibacterium acnes</i>	14	Yes	Flagged by instrument, contaminant

expected distribution of patients with endocarditis. Instead, it appeared to reflect that many patients initially assessed with fever did not have endocarditis, when endocarditis was considered possible, but rather had bacteremia from a variety of sources.

It was not possible to determine the extent to which delayed reporting of negative cultures may have altered decisions relating to the duration of antibiotic treatment or the search for the correct diagnoses. Our experience is similar to that of Varetas et al (5), who also concluded that extended incubation was not productive. Towns and Reller (6) recommended blind subculture of automated IE blood cultures at five days, rather than extending the instrument incubation for two or three weeks. My findings suggest that even this step is not a valuable use of resources. My findings are also consistent with those of Cockerill et al (4) and Baron et al (7). Baron et al (7) found that an extensive protocol (including extended incubation) of blood cultures for endocarditis yielded only three clinically relevant results. Cockerill et al (4) found that all 51 patients with endocarditis who had blood cultures incubated for up to 168 h were detected within the first five days.

A small proportion of patients with IE have negative blood cultures, despite best efforts to isolate the causative organism. In recent years, there has been increasing success diagnosing culture-negative endocarditis using molecular means, particularly via broad-range polymerase chain reaction assay with primers directed against conserved sequences in the bacterial 16S ribosomal RNA gene (8-12). The 16S ribosomal polymerase chain reaction assays with subsequent sequencing of the amplicon has been successful in a number of cases of IE due to *Haemophilus* species (*H parainfluenzae*, *H aphrophilus*, *H paraphrophilus*), *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella* species (the HACEK group), *Tropheryma whipplei* and *Bartonella* species (3). The judicious use of serology in IE cases due to *Coxiella burnetii*, *Bartonella* species, *Mycoplasma* species or *Legionella* species is often diagnostic (3,13).

These observations suggest that the prolonged incubation and blind subculture of blood cultures in suspected cases of

IE are unlikely to contribute to patient care. It is suggested that laboratories apply routine practices with much shorter incubation times, and, when three blood cultures are negative, focus on other nonculture-based strategies for the diagnosis of IE.

REFERENCES

1. Washington JA. The microbiological diagnosis of infective endocarditis. *J Antimicrob Chemother* 1987;20(Suppl A):29-39.
 2. Fowler VG, Sheld WM, Bayer AS. Endocarditis and endovascular infections. In: Gerald L, Mandell JE, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases*, 6th edn. Philadelphia: Elsevier Churchill Livingstone, 1995:975-1022.
 3. Brouqui P, Raoult D. Endocarditis due to rare and fastidious bacteria. *Clin Microbiol Rev* 2001;14:177-207.
 4. Cockerill FR III, Wilson JW, Vetter EA, et al. Optimal testing parameters for blood cultures. *Clin Infect Dis* 2004;38:1724-30.
 5. Varetas K, Taylor PC, Mukerjee C. Determination of the optimum incubation period of continuously monitored blood cultures from patients with suspected endocarditis or fungaemia. *Pathology* 2002;34:167-9.
 6. Towns ML, Reller LB. Diagnostic methods current best practices and guidelines for isolation of bacteria and fungi in infective endocarditis. *Infect Dis Clin North Am* 2002;16:363-76.
 7. Baron EJ, Scott JD, Tompkins LS. Prolonged incubation and extensive subculturing do not increase recovery of clinically significant microorganisms from standard automated blood cultures. *Clin Infect Dis* 2005;41:1677-80.
 8. Hryniewiecki T, Gzyl A, Augustynowicz E, Rawczynska-Englert I. [Amplification reaction to bacterial DNA for diagnosing infective endocarditis.] *Med Dosw Mikrobiol* 2002;54:265-72.
 9. Rothman RE, Majmudar MD, Kelen DG, et al. Detection of bacteremia in emergency department patients at risk for infective endocarditis using universal 16S rRNA primers in a decontaminated polymerase chain reaction assay. *J Infect Dis* 2002;186:1677-81.
 10. Millar B, Moore J, Mallon P, et al. Molecular diagnosis of infective endocarditis – a new Duke's criterion. *Scand J Infect Dis* 2001;33:673-80.
 11. Lisby G, Gutschik E, Durack DT. Molecular methods for diagnosis of infective endocarditis. *Infect Dis Clin North Am* 2002;16:393-412.
 12. Lang S, Watkin RW, Lambert PA, Bonser RS, Littler WA, Elliott TS. Evaluation of PCR in the molecular diagnosis of endocarditis. *J Infect* 2004;48:269-75.
 13. Houpiikian P, Raoult D. Diagnostic methods. Current best practices and guidelines for identification of difficult-to-culture pathogens in infective endocarditis. *Cardiol Clin* 2003;21:207-17.
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