J Clin Pathol 1984;37:568–570

Effect of volume of blood cultured on detection of *Streptococcus viridans* bacteraemia

DC SHANSON, F THOMAS, D WILSON

From the Departments of Clinical Microbiology and Oral Surgery, St Stephen's Hospital, London SW10

SUMMARY Fifty eight patients undergoing dental extraction each had 45 ml blood collected. This was divided into 30 ml and 15 ml blood samples for culture. The 30 ml sample was inoculated into 120 ml nutrient broth with 0·05% liquoid and the 15 ml sample into 60 ml of identical broth so that the final dilution of blood in broth was always 1/5.

Bacteraemia due to viridans streptococci was found in 27 and 15 patients by culturing the 30 ml and 15 ml blood samples respectively. Only one further case of streptococcal bacteraemia was detected by culture of the total volume of blood collected (45 ml) rather than culture of the 30 ml blood sample alone. These findings suggest that the culture of 30 ml blood results in the detection of up to 80% more blood cultures yielding *Streptococcus viridans* than the culture of only 15 ml blood. The collection of more than 30 ml blood for each culture is unlikely to prove worthwhile. It is suggested that 30 ml rather than 15 ml blood is probably the optimal volume of blood for each culture of *S viridans* when patients with suspected infective endocarditis are investigated.

Only a few reported studies have determined the independent effect of volume of blood cultures on the detection of bacteraemia where other variables such as the ratio of blood to broth, the medium, atmosphere of incubation, and processing methods were identical for all blood cultures.[1–3] A recent study at the Mayo Clinic of bacteraemia indicated that the yield of 15 ml samples is 25% greater than that of 5 ml samples.[4] In the Association of Clinical Pathologists Broadsheet a 15 ml blood sample is recommended for culture.[5] None of the reports published so far have commented on bacteraemia due to viridans streptococci. *Streptococcus viridans* is still the most common cause of endocarditis, and patients often have a low grade bacteraemia. Although the culture of a large volume of blood would be expected to yield more streptococci than the culture of a small volume, there are no reports indicating the optimal volume of blood for the isolation of viridans streptococci. There are too few patients with streptococcal endocarditis to allow worthwhile studies to be carried out. Patients undergoing dental extractions, however, may also have predictable *S viridans* bacteraemias. We report the results of a study on patients undergoing dental extraction, where the results of culture of 30 ml and 15 ml blood samples were compared and all the other blood culture factors were controlled so that blood volume was the only variable.

Accepted for publication 2 February 1984

Material and methods

COLLECTION OF BLOOD SAMPLES AND INOCULATION OF BLOOD CULTURE MEDIA

A total of 45 ml venous blood was collected from the antecubital fossa of each patient into a 50 ml syringe within 2 min of dental extraction. The extractions were carried out under general anaesthesia, and patients who had received recent antibiotic therapy were excluded from the study. Thus 58 patients were entered into the study. The blood was immediately injected through the rubber caps of the two bottles as two blood samples—30 ml and 15 ml. The volumes of broth inoculated were 120 ml and 60 ml nutrient broth respectively so that there was always a 1/5 final dilution of blood. The order of inoculation of the two blood samples varied between patients so that in about half the cultures the 15 ml sample was inoculated before the 30 ml sample. The nutrient broth used contained 0·05% liquoid, supplied by Southern Group laboratories, and was identical for both volumes of broth.

INCUBATION AND SUBCULTURES

The bottles were incubated at 37°C with the caps loose in a 10% carbon dioxide aerobic atmosphere

Results of culturing 15 ml and 30 ml blood samples from 28 patients with S viridans bacteraemia

Patient no	*Organism*	*Result of subculture of blood broths on*									
		Day 1		*Day 2 to 4**		*Day 5*		*Day 7*		*Day 10*	
		30 ml	*15 ml*	*30 ml*	*15 ml*	*30 ml*	*15 ml*	*30 ml*	*15 ml*	*30 ml*	*15 ml*
1	*S sanguis*	−	−	+(4)	−	+	−	+	−	+	−
2	*S mitis*	−	−	−	−	−	−	+	−	+	−
3	*S milleri*	−	−	−	−	−	−	+	−	+	−
4	*S sanguis*	−	−	+(4)	−	+	−	+	−	+	−
5	*S mitis*	+	+	+	+	+	+	+	+	+	+
6	*S mitis*	+	+	+	+	+	+	+	+	+	+
7	*S mitis*	+	−	+	+(3)	+	+	+	+	+	+
8	(a) *S sanguis*	+	−	+	−	+	+	+	+	+	+
	(b) *S milleri*	+	−	+	−	+	+	+	+	+	+
9	*S mitis*	+	+	+	+	+	+	+	+	+	+
10	*S sanguis*	+	−	+	−	+	−	+	−	+	−
11	*S milleri*	+	+	+	+	+	+	+	+	+	+
12	*S sanguis*	−	−	−	−	+	−	+	−	+	−
13	*S mitis*	+	−	+	−	+	−	+	−	+	−
14	*S sanguis*	−	−	+(4)	−	+	−	+	−	+	−
15	*S salivarius*	+	−	+	−	+	−	+	−	+	−
16	*S sanguis*	+	+	+	+	+	+	+	+	+	+
17	*S sanguis*	−	+	−	+	−	+	−	+	−	+
18	*S mitis*	+	−	+	−	+	−	+	−	+	−
19	*S sanguis*	+	+	+	+	+	+	+	+	+	+
20	*S mitis*	+	+	+	+	+	+	+	+	+	+
21	*S salivarius*	+	+	+	+	+	+	+	+	+	+
22	*S sanguis*	+	−	+	−	+	−	+	−	+	−
23	(a) *S sanguis*	+	+	+	+	+	+	+	+	+	+
	(b) *S salivarius*	−	−	−	−	+	−	+	−	+	−
24	*S mitis*	+	+	+	+	+	+	+	+	+	+
25	*S mitis*	+	+	+	+	+	+	+	+	+	+
26	*S sanguis*	−	−	+(4)	−	+	−	+	−	+	−
27	*S sanguis*	−	−	+(3)	−	+	−	+	−	+	−
28	*S mitis*	−	−	−	−	+	+	+	+	+	+
Total no strains of streptococci isolated		19	12	24	13	27	16	29	16	29	16
Total no patients with bacteraemia detected		18	12	23	13	25	15	27	15	27	15

*Subcultured when bottle first appeared turbid (on day indicated).

for up to 10 days. The broths were inspected daily and subcultured whenever turbidity or other signs on macroscopic inspection suggested a positive broth. In addition, routine subcultures were carried out after 1, 5, 7, and 10 days' incubation. Subcultures were performed using a syringe and needle to transfer about 0·1 ml of mixed broth to fresh blood agar plates, which were incubated for 48 h in a carbon dioxide incubator.

IDENTIFICATION OF ISOLATES

Alpha or non-haemolytic Gram-positive cocci that were resistant to optochin, catalase negative and coagulase negative, and failed to hydrolyse aesculin in a medium containing 40% bile were identified as viridans streptococci unless Lancefield grouping by the Streptex test (Oxoid) suggested the presence of a Lancefield group D streptococcus. The viridans streptococci were further identified by the API 20 streptococcal identification test (API system).

Results

Viridans streptococci were isolated from the blood of 28 of the 58 patients when the culture results of both 30 ml and 15 ml blood samples were combined.

Comparison of the 30 ml and 15 ml blood culture results showed consistently higher yields of streptococci from the 30 ml samples for all subculture times (Table). After one day's incubation seven more strains of streptococci were isolated and six more patients with bacteraemia were detected by the culture of 30 ml rather than 15 ml blood. After seven days' incubation 27 and 15 patients with *S viridans* bacteraemia were detected by the culture of 30 ml and 15 ml blood volumes respectively, a significant difference (sign test, $p = 0{\cdot}001$). Streptococci were found in both blood broths from 15 patients, and for two of these patients the blood cultures were first positive with the 30 ml sample (patients 7 and 8).

Culture of the total blood sample collected (that is, 45 ml), yielded growth of only one more streptococcal strain (from patient 17) compared with the results of culture of the 30 ml sample alone.

Discussion

Previous reports comparing 5 ml with 2 ml,[2,3] 10 ml with 5 ml,[1] and 15 ml with 5 ml[4] blood volumes have

found greater yields of organisms isolated from patients when the larger volumes of blood were cultured. The greatest volume of blood previously investigated was 20 ml, where duplicate 10 ml blood cultures yielded significantly more isolates from patients with bacteraemia than single 10 ml blood cultures.[4] As a result of this finding a minimum of 20 ml blood has been suggested for each blood culture from adult patients in the USA.[4] In the present study culture of a 30 ml blood sample resulted in the detection of 80% more patients with *S viridans* bacteraemia than the culture of a 15 ml blood sample. The collection of 45 ml blood for culture was of doubtful value, however, since only one further case of bacteraemia was detected when the culture results for the 15 ml and 30 ml samples were combined. The optimal volume of blood for detecting *S viridans* bacteraemia is therefore 30 ml.

Infective endocarditis with negative blood cultures might occasionally be due to the use of suboptimal blood culture techniques to detect low grade streptococcal bacteraemia. We suggest that 30 ml rather than 15 ml blood should be collected for each culture from adult patients with suspected endocarditis for the optimal detection of *S viridans* bacteraemia.

We are grateful to Miss M Tadayon for excellent technical assistance and to members of the anaesthetic department who assisted with the collection of blood cultures.

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Requests for reprints to: Dr DC Shanson, Department of Clinical Microbiology, St Stephen's Hospital, Chelsea, London SW10 9TH, England.