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An Evaluation of the Effect of one-day Blind Subculture in the Initial Detection of *Salmonella typhi* Positive Blood Cultures

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During the 8-month period of May to December, 1978, a total of 3,529 blood cultures were taken from Yonsei Medical Center patients and the effect of blind subculture in the initial detection of *Salmonella typhi* positive culture was analyzed. The blind subculture at the end of 1-day incubation (1-d BS) detected 35.0% of *S. typhi* positive specimens. All of the *S. typhi* positive specimens by 1-d BS were also macroscopically positive. However, by doing slide agglutination with the growth on subculture plate *S. typhi* was identifiable tentatively. This saved a day compared to macroscopic examination alone. Therefore the 1-d BS is concluded to be a valuable procedure for the isolation of this organism from blood. For the isolation of *S. typhi* 7-day incubation was concluded adequete based on the fact that there was only 1 specimen which bacame positive after over 1-week incubation.

Blood culture is one of the most important etiologic diagnostic tools because bacteremia results in a wide variety of infections and clinical conditions (Gotoff and Behrman, 1970; Sullivan *et al.*, 1972; Isenberg and Paster, 1974). Almost all species of bacteria can invade the blood stream (Watt and Okubadejo, 1967; Chong and Lee, 1970; Crowley, 1970; Hermans and Washington, 1970; Chong *et al.*, 1973; Chong and Kim, 1975; Chong *et al.*, 1975a; Chong *et al.*, 1975b; William *et al.*, 1975) and a blood culuure method should be able to support the growth of even the most demanding bacteria (Bartlett *et al.*, 1974). In some species the growth is slow even with a good culture method or the growth does not give a macroscopically obvious change to the media (Blazevic *et al.*, 1974; Hall *et al.*, 1974). Therefore daily macroscopic observation alone of the culture is considered an inadequete procedure. It is for this reason that blind subcultures are recommanded. Blind subcultures at the end of one-day incubation (1-d BS) are done for the detection of growth and that at the end of 7-days (7-d BS) for the proof of no growth before the termination of the culture (Bartlett *et al.*, 1974).

In contrast to that found in most western countries, typhoid fever is not erradicated in Korea as yet, hence blood culture is used most frequently for the diagnosis of this infection (Chong and Kim, 1975). Growth of *S. typhi* gives a definite turbidity to the media which could hardly be missed macrosco-

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pically, but the growth requires a somewhat longer incubation time than most other enteric bacilli (Chong, 1974). Apparently the general recommandation of the blined subculture scheme is not for the isolation of *S. tyhpi* primarily. Therefore it is quite questionable if the 1-d BS and 7-d BS scheme are any help in satisfying our primary aim of blood culture. This study was conducted to clarify that point.

MATERIALS AND METHODS

Blood for culture was taken from patients in the Yonsei Medical Center during the period from May to December, 1978. The procedure of blood culture was to draw 10 ml of blood and inoculate the evenly divided samples to a 50 ml tryptic soy broth (TSB) with 0.05% sodium polyanetholsulfonate (Ellner and Stoessel, 1966; Chong, 1974) and a 50 ml of Brewer thioglycollate medium (BTM). However, less than 10 ml of blood was taken from some patients. These media were prepared from dehydrated products of Difco and dispensed in bottles with screw caps and rubber diaphragms. Inoculated bottles were incubated at 35 C.

Besides daily macroscopic observations for 7 days, blind subcultures were done at the end of 1- and 7-day incubation. It was our working timetable that all specimens inoculated during the previovs day before 8:30 P.M. were examined macroscopically between 9:30 to 11:30 A.M. The macroscopically growth negative TSB at the end of 1-day incubation were subjected to 1-d BS at around 13:30 to 15:30 P.M. Therefore, a 1-day incubation actually meant about 14 to 38 hours in the case of gross observation and 18 to 42 hours in the case of blind subculture.

The procedure of blind subculture was as follows: using a disposable syringe, a small amount of well mixed TSB culture was drawn, one drop spread onto a blood agar plate and incubated in a CO_2 incubator. The original TSB bottles were returned to an incubator and examinations were done regardless of the 1-d BS result. The bacteriological procedure was performed by one of three workers on a monthly rotation basis.

The effect of blind subculture in the initial detection of growth done primarily for *S. typhi* but also for *S. paratyphi-A* were analyzed by comparing the results of blind subculture to that of macroscopic examination. The data were also compared with the effect in the initial detection of growth of *Escherichia coli* and *Staphylococcus aureus*.

RESULTS

During the 8-month study period, there were a total of 3,529 blood culture specimens processed. Among these, there were a considerable number of specimens for which the 1-d BS or 1-d macroscopic observation was missed because of the administrative difficulty of Sunday or holiday coverage. Excluding positive cultures from such specimens, there were 183 *S. typhi* and 31 *S. paratyphi-A* positive cultures which data were usable for the analysis. There were also 28 *E. coli* and 40 *S. aureus* positive cultures and the data of which were usable for the purpose of comparisons.

Among the 183 *S. typhi* positive specimens, 47 showed growth grossly at the end of 1-day incubation and no subculture was necessary. From all the rest of the specimens, which were subjected to 1-d BS, 64 yielded positive results. Among these 64 positives, 60 showed growth macroscopically at the end of 2-days

Table 1. Comparison of frequencies of initial detection of positive blood cultures by different procedures

Initial detection of growth by	No. of specimen (%)			
	S. typhi	*S. paratyphi-A*	*E. coli*	*S. aureus*
Macroscopically (1-day)	47 (25.7)	9 (29.0)	16 (57.1)	10 (26.3)
Blind subculture (1-day)	64 (35.0)	11 (35.5)	6 (21.4)	14 (36.8)
Macroscopically (2- to 7-day)	72 (39.3)	11 (35.5)	6 (21.4)	14 (36.8)
Total	183 (100)	31 (100)	28 (100)	38 (100)

Table 2. Comparison of 1-day blind subculture and macroscopic examination in the initial detection of positive blood culture

Bacteria	Initial detection of growth by	No. of macroscopically positive specimen (%)							
		incubation time (day)							Total
		1*	2	3	4	5	6	7	
S. typhi	Macroscopically (1-day)	47 (25.7)	NA**	NA	NA	NA	NA	NA	47 (25.7)
	Blind subculture (1-day)	NA	60 (32.8)	4 (2.2)	0	0	0	0	64 (35.0)
	Macroscopically (2- to 7-day)	NA	37 (20.2)	27 (14.8)	5 (2.7)	3 (1.6)	0	0	72 (39.3)
	Total	47 (52.7)	97 (53.0)	31 (16.9)	5 (2.7)	3 (1.6)	0	0	183*** (100)
S. paratyphi-A	Macroscopically (1-day)	9 (29.0)	NA	NA	NA	NA	NA	NA	9 (29.0)
	Blind subculture (1-day)	NA	9 (29.0)	2 (6.5)	0	0	0	0	11 (35.5)
	Macroscopically (2- to 7-day)	NA	8 (25.8)	2 (6.5)	0	0	0	1 (3.2)	11 (35.5)
	Total	9 (29.0)	17 (54.8)	4 (12.9)	0	0	0	1 (3.2)	31*** (100)
E. coli	Macroscopically (1-day)	16 (57.1)	NA	NA	NA	NA	NA	NA	16 (57.1)
	Blind subculture (1-day)	NA	5 (17.9)	1 (3.6)	0	0	0	0	6 (21.4)
	Macroscopically (2- to 7-day)	NA	4 (14.3)	2 (7.1)	0	0	0	0	6 (21.4)
	Total	16 (57.1)	9 (32.1)	3 (10.7)	0	0	0	0	28*** (100)
S. aureus	Macroscopically (1-day)	10 (26.3)	NA	NA	NA	NA	NA	NA	10 (26.3)
	Blind subculture (1-day)	NA	14 (36.8)	0	0	0	0	0	14 (36.8)
	Macroscopically (2- to 7-day)	NA	10 (26.3)	3 (7.8)	1 (2.6)	0	0	0	14 (36.8)
	Total	10 (26.3)	24 (63.2)	3 (7.8)	1 (2.6)	0	0	0	38*** (100)

* 1-day incubation was actually 14-38 hours in macroscopic examination and 18-42 hours in blind subculture.

** NA, not applicable.

*** Mean incubation time (day) for the macroscopic detection of growth: *S. paratyphi-A*, 2.0; *S. typhi*, 2.0; *E. coli*, 1.5; *S. aureus*, 1.9.

Table 3. Actual incubation time of the macroscopically 1-day positive specimens

Actual incubation time (hour)	Number of specimen (%)			
	S. typhi	*S. paratyphi-A*	*E. coli*	*S. aureus*
33.5~38	34 (72.3)	6 (66.7)	4 (25.0)	4 (40.0)
14~33.5	13 (27.7)	3 (33.3)	12 (75.0)	6 (60.0)
Total	47 (100)	9 (100)	16 (100)	10 (100)

and the remaing 4 at the end of 3-days of incubation.

Among the 1-d BS negative specimens, 72 subsequently became positive macroscopically sometime during the end of 2-7 day incubation. No growth was detected by the 7-d BS (Table 1, Table 2). Macroscopic detection of growth was most frequently done at the end of 2-day incubation and the mean incubation time was 2.0 days.

Among the 31 specimens with positive *S. paratyphi-A*, 9 were macroscopically positive at the end of 1-day incubation, 11 were 1-d BS positive and the remaining 11 were macroscopically positive after 2- to 7-day incubation (Table 1). Macroscopic examination detected the growth most frequently at the end of 2-day incubation and the mean incubation time was 2.0 days (Table 2). Among the 28 *E. coli* positive specimens, 16 were initially detected macroscopically at the end of 1-day incubation. The 1-d BS detected 6 positives. Macroscopic examination after 2- to 7-day incubation detected 6 positives. Overall macroscopic detection of growth was most frequently found at the end of 1-day incubation and the mean incubation time was 1.5 days (Table 2).

A total of 38 specimens yielded *S. aureus*. Of these 10 were detected macroscopically at the end of 1-day incubation. The procedure of 1-d BS initially detected 14 positives. Further incubation and daily macroscopic examination detected 14 positives. Overall macroscopic detection of growth was made most frequently at the end of 2-day incudation, and the mean incubation time was 1.9 days (Table 2).

DISCUSSION

Blazevic *et al.* (1973) reported the importance of blind subculture and gram stain for the initial detection of positive blood cultures in some species of bacteria such as *Haemophilus*. Hall *et al.* (1974) also stressed the importance of blind subculture, but not that of gram staining. However, as far as *S. typhi* is concerned, one could raise questions if it is of any value to improve the initial detction of positive cultures. Growth of *S. typhi* generally imparts the medium a definite turbidity which is readily detectable by macroscopic examination. Also the timing of blind subculture at the end of 1-day incubation may not be optimum, because the rapidity of growth of *S. typhi* may be different from other bacteria.

It has been our routine procedure that when the growth of bacteria is detected macroscopically in the broth, tests for identification are done directly from the broth. And most of the time, the identification can be completed by the following day. When we have growth on blind subculture plates we proceed to the identification from these.

As shown in table 1, 1-d BS detected 64(35.0%) *S. typhi*. The rates were similar in *S. paratyphi-A* (35.5%) and in *S. aureus* (36.8%), and lower in *E. coli* (21.4%). Although the macroscopic examination at the end of 2-day incubation(which is the time the result of 1-d BS is read), detected majority of 1-d BS positive specimens (60 of 64) the identification of the growth can not be completed until the next day (Table 2). However, 1-d BS positive 64 *S. typhi* were tentatively identifiable by doing slide agglutination. This saved a day. Because about one half of our isolates were *S. typhi*, it was not waste but a worthy procedure to do.

As to the time for doing blind subculture, the day after receiving the specimen was considered optium. If the blind subculture had been done at the end of 2-day incudation, at most 39 could have been detected by this procedure. Namely by that time, 144 specimens had already been detected by macroscopic observation alone (Table 2).

In the detection of growth of *S. paratyphi-A* the effect of 1-d BS was quite similar to that of *S. typhi*(Table 1, 2).

Growth of *E. coli* was faster; 57.1% of which were macroscopically positive at the end of 1-day incubation. Therefore only 21.4% of *E. coli* positive specimens were initially detected by 1-d BS (Tadle 1, 2).

In *S. aureus*, 1-d BS detected 36.8% of positive specimens. This rate was similar to that of *S. typhi*(Tadle 1, 2). However, in *S. aureus* 1-d BS did not appreciably shorten the reporting time, because, as it was with *E. coli*, the species identification depended on biochemical reactions.

Although it has been a common practice to keep blood cultures for at least 2 weeks before it is reported negative (Bauer et al., 1977), the recent trend is to hold it for a week only (Bartlett et al., 1974). During this study, there was only one specimen which became positive after over a week incubation. The organism was *S. typhi*. If the specimen was held for only a week then it could have been reported negative. However, as the common practice is to collect 3 specimens of blood from a patient, little benefit in the isolation of *Salmonella* can be expected by holding over a week.

In this study, the mean incubation time repuired to detect growth of *S. typhi* macroscopically was somewhat shorter comared to the previous study (Chong, 1974). However close analysis of the specimens which were macroscopically positive at the end of 1-day incubation, revealed that 72.3% of them with *S. typhi* and 66.7% with *S. paratyphi-A* were actually incubated for 33.5 to 38 hours because they were received between 8:30 P.M. and 12:00 P.M. of the previous day (Table 3).

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