

# The effect of temperature of the culture medium on the outcome of blood culture

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**SYNOPSIS.** Small numbers of seven species of bacteria commonly causing bacteraemia in man were inoculated into fresh human blood, and then cultured by a standard method used for diagnostic blood culture. Prior warming of the medium did not improve the recovery of the inoculated bacteria and it is suggested that such warming is not required when performing diagnostic blood culture.

In many hospitals bottles of sterile media used for blood culture are stored and used at room temperature, in other hospitals they are kept in and used direct from a refrigerator, whilst in a few hospitals the medium is warmed to 37°C before use. In this paper we report experiments performed to find out whether the result of blood culture is affected by the initial temperature of medium in which blood cultures are made. For this purpose fresh sterile blood was infected with known organisms in such numbers as to resemble blood from patients with bacteraemia. Aliquots of this infected blood were then placed in bottles of blood culture medium at six different initial temperatures and incubated at 37°C in the usual way.

## Materials and Methods

The culture medium used throughout the experiments was digest broth with 0.1% glucose, as supplied by Southern Group Laboratories for blood culture.

The organisms used were *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus viridans*, *Streptococcus faecalis*, *Streptococcus pneumoniae*, and *Neisseria gonorrhoeae*. *Neisseria gonorrhoeae* was used rather than *Neisseria meningitidis* because it was thought that their growth characteristics were similar and gonococci were more readily available in our laboratory. All the organisms used were isolated originally from clinical material and were considered to be pathogenic. Each species was grown overnight in broth at 37°C before inoculation. The number of infective particles in

each culture were estimated using a previously calibrated EEL nephelometer. Working dilutions of the cultures were then prepared in broth. The streptococci and neisseriae were submitted to light ultrasonification (Electrosonic (H60, PZT) for 15 seconds) to separate pairs and chains so facilitating the nephelometry. Surface viable counts using horse blood agar (Oxoid Columbia agar) were performed to confirm the nephelometry.

Media for the experiments were held for at least three hours before use at each of the following temperatures: 5, 20, 37, 45, 56°C. At each temperature 12 bottles of medium were used, six for controls and six for the definitive investigations.

Two 60 ml amounts of fresh venous blood were taken from one of the authors for each experiment. As soon as the blood had been taken a sufficient amount of the bacterial suspension to give a final concentration of approximately 1 infective particle per ml was added to the syringe full of blood and mixed by inversion. Four ml of this infected blood, estimated to contain 4 infective particles, was then added to each of 15 bottles of medium. This procedure was repeated until six blood cultures at each of the different initial temperatures were made. These blood cultures were then incubated immediately at 37°C for 24 hours.

At each initial temperature controls were made by adding organisms without blood to six bottles of medium then incubating at 37°C. The organisms were from working dilutions and the numbers added to each of the bottles in the control experiment were the same as the number added in the definitive experiments in which blood was used, that is, an estimated 4 infective particles. In the control experiment involving the more fastidious organisms, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*,

it was necessary to add 8% of sterile horse serum to the culture medium in order to obtain growth.

## Results

### CULTURES WITH BLOOD

The accompanying table of results shows that with the medium initially at 5, 20, and 37°C five or six of the six bottles in each of the groups yielded positive results, ie, the bacteria grew as well in an initially cold medium as in the media initially at 20 and 37°C. At 45°C the yield of positive cultures of *Neisseria gonorrhoeae* was reduced, and at 56°C the yield of both *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* was reduced.

### Controls

There was a fall in yield of positive cultures of *Neisseria gonorrhoeae* when the initial temperature of the medium was 20°C or above, and a less clearly marked fall in the yield of positive cultures of *Staphylococcus aureus* and *Streptococcus viridans* as the initial temperature of the medium increased.

A trend test applied to the results of the *Neisseria gonorrhoeae* cultures showed that as the initial temperature of the medium was increased the fall in the yield of positive cultures was significant,  $\chi^2_1 = 17.8152$  ( $P < 0.01$ ).

### Discussion

It is well known that sudden chilling can cause a loss of viability of organisms such as *Escherichia coli* (Meynell, 1958) and it is common knowledge that pathogenic neisseria are very sensitive to temperature. No doubt it is for this reason that when making blood cultures before heating of the medium to 37°C has been recommended (Stokes, 1968). Nonetheless the results of the present experiments suggest that

between 5 and 37°C the initial temperature of the culture medium does not effect the outcome of the blood culture. Some organisms such as *Staphylococcus aureus* appear resistant to cold shock (Gorrill and McNeil, 1960). The length of time that an organism is held at a low temperature is also critical (Strange and Dark, 1962) but there is little loss of viability if after rapid cooling the suspension is immediately warmed. Our findings are in accordance with this observation since nearly all the bottles which had been at 5°C yielded organisms after incubation. For this reason we suggest that if blood cultures are made in cold media they should be incubated at once.

Theoretically bacteria which have never previously been grown artificially may be more susceptible to cold shock than those accustomed to artificial media. This point would be conclusively proved if over a period, one extra bottle at 5°C was inoculated in parallel with warm medium for routine blood cultures and no difference was observed.

Bottles of medium at temperatures above 37°C were included in the experiment because phagocytosis is reduced or inhibited at low temperatures (Brumfitt, Glynn, and Percival, 1965) and probably also at higher temperatures. If rapid heating or cooling of the blood when it was added to the culture medium had a greater lethal effect on the phagocytes than the bacteria then there might be an advantage in using either hot or cold media. This idea was not supported by our results.

Growth did not occur in every bottle to which an inoculation was added. This was probably due to the random nature of the distribution of the organisms in the blood and the broth. It is likely in situations such as this, where very small numbers of organisms are used, that some of the inocula do not contain any organisms. This is probably also true of the clinical situation for which reason more than one blood culture should be made when bacteraemia is suspected.

Temperature (°C)	<i>S. typhi</i>	<i>E. coli</i>	<i>Str. aureus</i>	<i>Str. viridans</i>	<i>Str. faecalis</i>	<i>Str. pneumoniae</i>	<i>N. gonorrhoeae</i>	Totals
5 Test	6	6	5	6	6	6	5	40
5 Control	6	6	6	4	6	6	5	39
20 Test	5	6	5	6	6	5	6	39
20 Control	6	6	3	6	6	5	3	35
37 Test	6	6	5	6	5	5	6	39
37 Control	6	6	5	6	6	6	2	37
45 Test	6	6	5	6	6	6	4	39
45 Control	6	6	3	5	6	6	2	34
56 Test	5	6	6	6	6	1	0	30
56 Control	6	6	3	4	6	6	0	31

Table Number of cultures out of six showing growth after incubation

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