

Growth of staphylococci on nutrient agar at low temperature (12°C).

It is well recognised that staphylococci will grow over a wide range of temperatures and that the minimum of the range is relatively low. There appears to be no documented evidence on the subject, however, particularly with reference to any species differences that might exist within the genus. Routine bench experience in hospital laboratories commonly gives the impression that *Staphylococcus aureus* grows much more readily at bench temperatures than coagulase negative species. Some preliminary experiments designed to provide objective data on this matter showed this to be correct. At a temperature of 15°C all of a small batch of *S aureus* isolates grew abundantly on nutrient agar within five days, whereas non-pigmented coagulase negative staphylococci grew either slowly or not at all within that time. Several pigmented isolates of this category did, however, grow as well as the coagulase positive organisms. It was then established, again with small batches, that all coagulase positive organisms also grew well at 12°C, whereas none of the non-pigmented coagulase negative isolates showed visible growth within 20 days. Again, several of the pigmented isolates grew well.

More extensive trials were then set up at this temperature (12°C), with all pigmented coagulase negative organisms excluded. Testing for coagulase was done by a tube method using human plasma. Colony appearance, coccoid morphology, Gram stain positivity, and catalase positivity were the only other characteristics observed in the selection. Single colony isolates were selected randomly from routine cultures mainly of wounds, skin, and nares and cultured in nutrient broth. Early stationary phase growth was used for inoculations and the broth cultures were diluted 1/200. About 0.2 ml of each suspension was inoculated on to a nutrient agar slope for low temperature incubation and on to a nutrient agar plate to check for growth at 25°C. The agar medium was blood agar base no 2 (Oxoid). Slopes were used in preference to plates for the low temperature studies to reduce contamination, which was inevitable on plates incubated for such long periods. The slopes were placed in sealed containers to reduce evaporation and then incubated in a cabinet maintained at 12°C. They were examined initially at weekly intervals and later more irregularly over at least 60 days. The container of inoculated tubes was

Growth of staphylococci on nutrient agar slopes at 12°C

Staphylococci	Growth at 12°C	
	No of isolates	% Showing growth
Coagulase positive	150	100
Coagulase negative	300	7

given a preliminary cooling at 4°C for about 30 minutes before placing it in the cabinet in order to reduce the period of exposure to bench temperature and to effect a more rapid equilibration with the experimental temperature.

The table summarises the results of the study. All of the coagulase positive organisms tested yielded heavy growth within seven days, whereas only 7% of the coagulase negative organisms yielded visible growth during incubation periods ranging from 60 to 150 days. Most of the organisms in the latter category which grew did so in about the same time as that taken by the isolates of *S aureus*. A few, however, grew slowly or poorly. A large proportion of the coagulase negative isolates which failed to grow at 12°C also remained negative when transferred to a temperature of 37°C, indicating that the low temperature incubation had been lethal.

Using API methods some of the coagulase negative low temperature positive isolates were speciated and included: *S warneri*, *S hyicus*, *S cohnii*, and *S xylosum*. Coagulase negative, low temperature negative isolates included: *S epidermidis*, *S simulans*, and *S haemolyticus*.

This work, although essentially exploratory, nevertheless showed unequivocally that sharp distinctions existed among the staphylococci with respect to their minimum growth temperatures on nutrient agar slopes. Obviously, the culture medium and other growth conditions such as aeration would be expected to modify the results. While no special measures were employed for critical temperature control, random mixing of the slopes in the containers ensured that no serious bias was introduced in this aspect of the experimental design.

The finding that the coagulase positive staphylococci all grew well at 12°C was not unexpected, but we were surprised that such a large fraction of the coagulase negative organisms failed to grow and, in fact, died at that temperature. With this latter group minimum temperature for growth obviously covers a fairly wide range, with a probable upper limit of about 15°C.

Pigmented coagulase negative staphy-

lococci appear to resemble *S aureus* in their growth temperature patterns but both groups need further investigation at temperatures lower than those used here to provide this data.

The taxonomy of the coagulase negative staphylococci is a highly controversial subject. Low temperature growth studies may provide some useful data for this purpose. Obviously, no useful conclusions can be drawn from the few examples of speciation recorded here.

CP XANTHIS
DI ANNEAR
Microbiology Department,
Royal Perth Hospital,
Perth, Western Australia 6001

Is the ratio of total cholesterol to albumin useful as a predictor for the risk of coronary heart disease?

In an attempt to diminish the costs of public health care, Nanji and Reddy¹ suggest the use of the total cholesterol to albumin ratio (TC:Alb) for predicting whether an individual has a total cholesterol to high density lipoprotein cholesterol ratio (TC:HDL-C) below or above the discrimination value of 5—that is, normal or diseased, respectively. TC:Alb might then be used to predict the risk of coronary heart disease. We have several criticisms of their paper.

I We tried to produce the results of Nanji and Reddy. Our population consisted of arbitrarily chosen outpatients, without liver or kidney disease, attending a general hospital. The Table compares the correlation coefficients obtained in our study with those of Nanji and Reddy. We found no significant correlation (with a level of significance of 5%) between albumin and high density lipoprotein cholesterol. TC:Alb v TC:HDL-C gave a correlation coefficient much lower than that of Nanji and Reddy and of the same magnitude as the correlation coefficient between TC:HDL-C and total cholesterol. So