

Bacterial contamination of platelet concentrates stored at 20°C

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SYNOPSIS Qualitative and quantitative data on the bacterial contamination of 1000 platelet concentrates prepared and stored at 20°C are presented. Using cooked meat medium the incidence of contamination was 6.3%. However, nutrient agar platelet counts showed the concentration of bacteria to be low. The clinical significance of these results is discussed.

In a series of important studies Murphy and his colleagues demonstrated that the preparation and storage of platelet-rich plasma and platelet concentrates at 22°C rather than 4°C improved their life span *in vivo* (Murphy and Gardner, 1969; Murphy, Sayar, and Gardner, 1970). The potential clinical relevance of this finding has been confirmed by Handin and Valeri (1971), and it is likely that this approach to platelet procurement and storage will be widely instituted.

Although Murphy and Gardner (1969) drew attention to the possible danger of bacterial proliferation if a storage temperature of 22°C was introduced, they concluded that in practice it was likely to be minimal. This conclusion has been supported by the findings of Silver, Sonnenwirth, and Beisser (1970) and of Katz and Tilton (1970) who found no evidence of bacterial contamination in 140 platelet concentrates stored at room temperature for up to 96 hours. Other studies by Buchholtz, Young, Friedman, Reilly, and Mardiney (1971) indicated an estimated minimal contamination rate of 2.4% in individual platelet concentrates stored at room temperature. However, the magnitude of bacterial contamination was not recorded as quantitative techniques were not used, but it was assumed to be low as the overall frequency of clinically significant infections or reactions appeared to be quite small.

In view of the significant discrepancies between these three studies, the clinical importance of the administration of contaminated blood products to a large proportion of patients who are likely to be immunosuppressed, and the knowledge that certain bacteria cause aggregation of platelets (Clawson and White, 1971), a study of the bacterial contamination of platelet concentrates prepared and stored at room

temperature (20°C) was instituted in our own centre. The timing of this study was particularly significant as it coincided with the introduction of a modified double bag system for the collection of blood and preparation of components. This system has two additional outlet lines and could represent an increased hazard to contamination in its own right (Cash and Allan, 1972).

Materials and Methods

One thousand individual platelet concentrates of approximately 20 ml were studied. They were not specially prepared for this investigation: the first 500 units were studied without the knowledge of the teams responsible for the withdrawal and subsequent processing. All units were obtained from the routine platelet bank which had not been used after storage for 24 hours at room temperature (20°C).

The platelet concentrates were prepared within 12 hours of blood withdrawal at 20°C in a specially designed double bag (Tuta Limited). The significant feature of this design is the introduction of two outlet lines sited at the top edge of the transfer pack and terminating in male luer fittings (Cash and Allan, 1973). These outlet lines facilitate ready plasma and platelet concentrate pooling, and also provide easy access for bacteriological culture (figs 1 and 2).

After storage at 20°, 1 ml aliquots of resuspended platelet concentrates were transferred to sterile Macartney bottles containing 10 ml cooked meat medium (Oxoid Oxo Limited, London). These were incubated at 37° and 20°C respectively for 48 hours and then subcultured onto four nutrient agar plates which were maintained, anaerobically and aerobically, at 37° and 20°C, respectively, for 48 hours. If bacterial growth was present on the nutrient agar

INITIAL PLATELET CONCENTRATE CULTURE

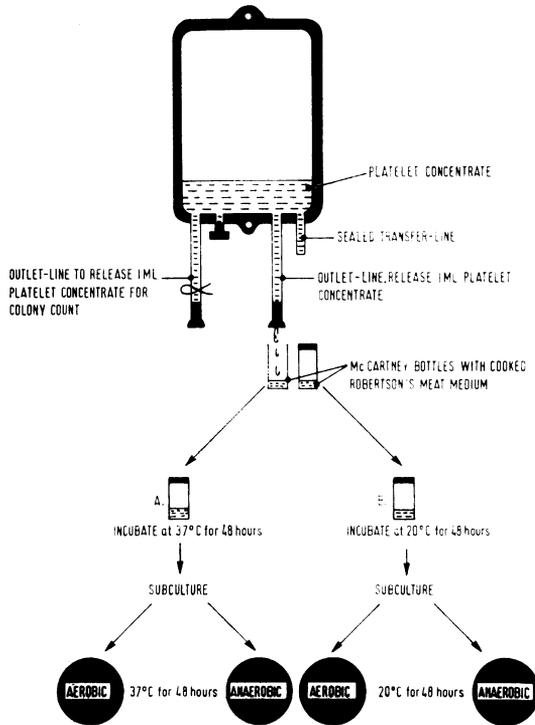


Fig 1 Bacteriological procedure adopted for the initial culture of platelet concentrates.

PROCEDURE WITH POSITIVE SUBCULTURE

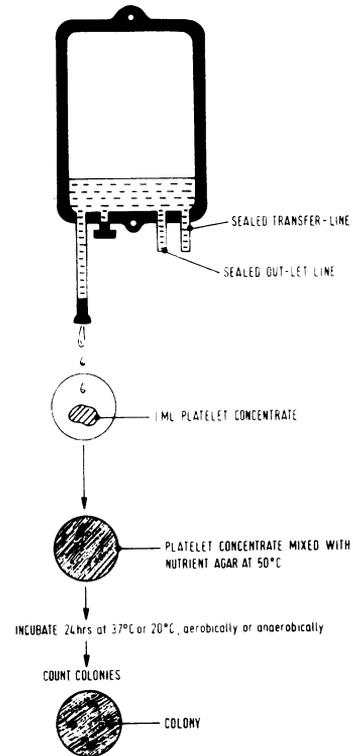


Fig 2 The plate-count procedure used to quantitate contaminated platelet concentrates.

plates then the original platelet concentrates, which had remained at room temperature for this period (96 hours), were retrieved and late counts were performed on nutrient agar, using 1 ml aliquots. Sampling for this check culture was performed through the unused outlet line. The experimental procedures are summarized in figures 1 and 2.

Results

The duration at which the platelets were maintained at 20°C is shown in table I. The mean storage period was 60 ± 12 hours. The results of the initial culture studies revealed a contamination rate of 6.3% from

20 packs	0-24 hours
299 packs	25-48 hours
681 packs	49-74 hours
1000							60 ± 12 hours

Table I Duration at 20°C from time of withdrawal to time of initial culture

1000 platelet concentrates. However, only 44% of these positive initial cultures were confirmed by the less sensitive plate-count technique, despite a further 96 hours' storage at 20°C. The plate-count studies further revealed that the bacterial content in 78% of the contaminated platelet concentrates was less than 10 organisms per ml, and the maximum recorded, in one only, was 585 organisms per ml. The types of organism grown in the initial cultures are shown in table II. Eighty-three per cent were *Staphylococcus albus*, and there were significant differences in the cultural characteristics of these staphylococci (table III). Thus 32% grew only at 20°C.

<i>Staphylococcus albus</i>	52
Coliform	6
<i>Clostridium welchii</i>	3
<i>Staphylococcus aureus</i>	1
Micrococcus	1

Table II Organisms isolated from initial cultures (63 cultures)

Arbitrary Types	37°C		20°C	
	Aerobic	Anaerobic	Aerobic	Anaerobic
I (30%)	+	+	+	+
II (30%)	+	+	-	-
III (28%)	-	-	+	+
IV (4%)	-	-	+	-
V (4%)	-	+	-	-
VI (4%)	+	-	+	+

Table III Pattern of cultural characteristics of *Staphylococcus albus* in platelet concentrates

A detailed retrospective analysis of the case records of 175 successive patients who, during the period September 1969 to September 1971 had received 1800 platelet concentrates, which had been stored for up to 24 hours at 20°C, revealed no clinical evidence of sudden septicaemia related to platelet concentrate administration.

Discussion

The existence of bacterial contamination in platelet concentrates stored at ambient temperature, reported in this investigation, supports the findings of Buchholtz *et al* (1971), although the frequency in our study appears to be significantly higher. However, the figure of 2.4% reported by these authors was a calculated one, based on the culture of platelet concentrate pools. In this calculation it was assumed that only one of the donations in a contaminated pool had been the source of the organisms recovered. Moreover, it seems possible that a significant dilution factor could have arisen: if one of an eight-unit pool contained less than 5 organisms per ml, it is probable that the culture of such a pool would be falsely negative (Walter, Kundsinn, and Button, 1957). This feature may explain the relative difference between the two studies as 52% of our individual platelet concentrates appeared to have less than 5 organisms per ml.

We were unable to pinpoint the specific source of contamination. Although it is possible that exogenous sources arose during the culturing procedures it seemed unlikely that this could be the complete explanation. Such a conclusion is supported by the absence of a trend, either up or down, in the incidence of contamination, as the study proceeded over a nine-month period. We have assumed that it arose for the most part during blood withdrawal, but efforts made to investigate this feature proved to be disappointing. The first 500 units were obtained during a period when the blood withdrawal teams were unaware that bacterial contamination studies were in progress. At the end of this period the incidence of contamination was discussed with the

senior staff responsible for the withdrawal of blood. Despite increased efforts on their part the level of contamination remained identical in the second 500 units.

Although it was recognized that the newly designed Tuta double bag represented a potentially more 'open' system than the more conventional double-bag unit, this did not appear to be the decisive factor. Cultures of 100 separate platelet concentrates prepared and stored at 20°C in standard Fenwal double-bags (JD-2) revealed a contamination rate of 6%. Buchholtz *et al* (1971) found no difference in the contamination between 'open' and 'closed' collection systems.

Buchholtz and his colleagues (1971) emphasized that there appeared to be a highly significant increase in the incidence of contamination between units stored for 24 hours or longer and those which were fresh, so that bacterial recovery increased with storage time. Our results did not confirm this observation: 10% of the units which had been stored for under 24 hours were contaminated, whereas the level fell to 5.0% and 6.7% in the 25-48-hour and 49-74-hour groups respectively. This apparent fall was not statistically significant ($\chi^2 = 0.014$, $p < 0.1$).

One of the important new features of this study was the provision of quantitative data on the magnitude of bacterial contamination in the positive platelet concentrates. Thus it was demonstrated that although the frequency of contamination was as high as 6.3% the number of organisms per unit volume of platelet concentrate was exceedingly low. The question then arises as to the clinical significance of this level of contamination. Do these 63 platelet concentrates represent a hazard to the recipients? At the present time there is no ready answer to this crucial question. The absence of clinical evidence of post-infusion septic shock in the retrospective study of 175 patients who had received over 1800 individual platelet concentrates would suggest that the real risk appears to be small, particularly when one considers that 62% of these infusions were given to immunosuppressed patients. It is possible that even in these patients there is a critical number of infused organisms required to produce clinical evidence of septicaemia and that the highest recorded level of 585/ml, which represents a total of 12×10^8 organisms, is well below this threshold. Such a conclusion is supported by the information available from published reports of post-transfusion bacterial shock, in which bacterial counts in the transfused products have ranged from 1×10^6 to 1×10^9 organisms per ml (Borden and Hall, 1951; Braude, Sanford, Bartlett, and Mallery, 1962; Braude, Williams, Siemienski, and Murphy, 1953; Stevens,

Legg, Henry, Dille, Kirby, and Finch, 1953). This may have represented a total infusion of between 5×10^8 and 5×10^{11} organisms.

This study does not prove that the storage of platelet concentrates at 20°C is bacteriologically safe. It suggests, however, that the risk is low. There is an urgent requirement for more quantitative data from immunosuppressed patients, and this will best be achieved by detailed bacteriological follow up on all platelet transfusions associated with any type of reaction.

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