Report of Routine Tests for Psychrophilic and Mesophilic Contaminants in Banked Blood

Hugh Chaplin, Jr., Estelle Chang and Robert W. Kolb

Blood Bank, Clinical Center and Laboratory of Biologics Control, National Microbiological Institute, National Institutes of Health, Bethesda, Maryland

Received for publication January 24, 1955

Emphasis has recently been directed upon the clinical importance of “cold growing” (psychrophilic) as well as “warm growing” (mesophilic) bacteria as a source of contamination in banked blood (Stevens et al., 1953; Pittman, 1953; Wetterlow et al., 1954). These authors stressed that certain psychrophilic organisms are killed at an incubation temperature of 37 C and that it is therefore important in bacteriological studies on stored blood that a temperature range be selected to insure detection of such organisms. The present authors are not aware of any published results of the use of the newly recommended temperature conditions for routine sterility tests carried on in the course of a normal blood bank operation.

This report is a brief summary of the results of bacteriological studies on 213 unselected outdated bloods; the techniques for culturing were designed to detect both mesophilic and psychrophilic organisms. Similar studies on the residual contents in 155 blood bottles returned from the hospital wards following transfusion are included.

Materials and Methods

Transfusion equipment. Bottles for gravity bleeding and containing 75 ml of acid-citrate-dextrose anticoagulant solution (NIH Sol. A) were obtained from commercial sources, as were disposable plastic donor sets with attached 15-gauge needles and disposable recipient sets. Representative samples from each lot purchased were checked for sterility and nonpyrogenicity before use and found to be satisfactory.

Blood collection. A uniform technique was employed in preparing the donor’s skin at the site for phlebotomy. A circular area of skin not less than 3 inches in diameter was scrubbed vigorously for 30 to 45 seconds with a sterile 2 x 2” gauze sponge soaked in sterile soft soap solution (20 per cent). The soap was removed with sterile 70 per cent alcohol. Two per cent aqueous iodine was applied and allowed to dry for 30 to 60 seconds. Immediately after application of the iodine, sterile 2 per cent novocaine solution was injected intracutaneously at the site for phlebotomy. Immediately prior to phlebotomy the iodine was removed with sterile 70 per cent alcohol.

During the first part of this study, the preparation of the blood bottle stopper was as follows: after removal of the tear-off retaining ring and underlying dustcap, the exposed surface of the rubber stopper was painted with 2 per cent aqueous iodine, which was allowed to dry prior to insertion of the 15-gauge donor set needle and the 15-gauge airway needle with cotton filter. However, after information was obtained from the manufacturers which indicated that the stopper surface was sterile prior to removal of the dust cap, the application of iodine was discontinued.

During collection of 500 ml of blood by gravity, the contents were gently swirled several times to insure mixing. On completion of bleeding, the donor tubing was clamped within 0.5 inches of the hub of the bottle needle, which was then removed. The air vent needle was removed. The exposed surface of the stopper was wiped clean with 0.1 per cent benzalkonium chloride solution. The bottle was then gently inverted 5 or 6 times and placed in the blood storage refrigerator. Within 6 to 24 hours, a clean dust cap was placed over the stopper and secured by a tamper-proof seal. Bloods were stored at 4–6 C in refrigerators equipped with air-circulating fans and continuous temperature recording devices which were checked daily by visual reading of a mercury thermometer. Blood was considered to have outdated after the elapse of 21 days from the date of drawing.¹

Bacteriological studies. The technique for culturing outdated bloods was as follows: the contents of the bottle were thoroughly mixed by repeated inversion, the clean dust cap was removed, and the stopper was painted with 2 per cent aqueous iodine. After 30–60 seconds the iodine was removed with sterile 70 per cent alcohol which was then burned off in a Bunsen flame. With a sterile needle and syringe, 5 ml of the blood was removed and planted into 5 tubes of fluid thioglycollate medium (1 ml of blood per tube of 15 ml of thioglycollate medium). Three of the inoculated tubes were incubated at 32 C and the remainder at room temperature.

¹ As described in the Minimum Requirements: Citrated Whole Blood (Human), National Institutes of Health, dated March 15, 1955.

¹ Fluid thioglycollate medium is formula indicated in National Institutes of Health Memorandum: Culture Media for the Sterility Test, dated August 11, 1954.
(22 to 25 C). These temperatures have proved satisfactory in the recovery of mesophilic and psychrophilic contaminants in stored blood (Pittman, 1953). After a period of 48 to 72 hours incubation, the cultures were shaken and subcultured, that is, 0.05- to 0.1-ml amounts of inoculum per 15-ml tube thioglycollate medium. The parent cultures were reincubated for an additional 7 days. The subcultures were incubated at temperatures corresponding to their parent cultures for 7 days. When bacteria were recovered, smears were made and examined for morphology and gram staining reactions.

These studies were subdivided into three groups:

**Series 1. Outdated bloods cultured directly from storage at 4 to 6 C.** A group of 111 bottles was handled with particular emphasis on detecting cold-growing organisms. The bloods were cultured immediately upon removal from the 4 to 6 C storage refrigerator.

**Series 2. Outdated bloods incubated 48 to 72 hours at 32 C.** A group of 102 outdated bloods, after gentle mixing, was placed at 32 C to promote growth of any mesophilic organisms whose multiplication had been prevented at 4 to 6 C and which might not have been present in sufficient numbers to be detected in a 5-ml sample. Culturing was done after 48 to 72 hours incubation.

**Series 3. Bottles returned to the blood bank after completion of transfusion.** A group of 155 bottles was studied after their return to the blood bank following use in the operating rooms or on the hospital wards. The bottles in this series had been subjected to a variety of conditions: room temperature for 1 to 6 hours during the transfusion and an additional period of 0 to 18 hours of storage at room temperature or refrigerator temperature (2 to 8 C) prior to being cultured.

The administration of transfusions is performed by ward physicians with the following recommendations for technique. The clean dust cap is removed, the exposed surface of the stopper wiped with 70 per cent alcohol, and the spike tip of the disposable recipient set immediately inserted through the site marked “outlet.” The recipient set tubing is clamped off, the bottle hung inverted on the transfusion stand, and a plain sterile 18- to 20-gauge hypodermic needle inserted at the site marked “air.” The tubing is then unclamped, and allowed to fill with blood, prior to the transfusion. Although the majority of transfusions are administered according to these directions, considerable variation in technique undoubtedly occurs since strict control of these measures is not attempted by the blood bank staff. The majority of the bottles were returned to the bank with the recipient set and airway needles removed; however, not infrequently, these were still in place. Soiling of the surface of the stopper with small amounts of blood which had oozed out at the site of insertion of the recipient set was frequently observed.

The exposed surface of the rubber stopper in each bottle was rinsed with 95 per cent ethyl alcohol and flamed with a Bunsen burner. Five ml of 0.85 per cent sterile saline was aseptically added with a sterile needle and syringe and the bottle shaken so that all inner surfaces of the bottle and stopper were thoroughly rinsed with the diluent. Five ml of the rinse was then planted into 5 thioglycollate tubes and the technique thereafter was exactly as described above.

### Results

The results of the studies on outdated bloods are summarized in table 1. Growth indicating contamination was not obtained from any of the 213 banked bloods examined. In three instances in the second series, 1 out of 5 tubes cultured showed growth considered to be contamination picked up during sterility testing.

Table 2 summarizes the results of studies on the residual contents in 155 blood bottles returned to the blood bank following transfusion. Evidence for contamination was found in six bottles (4 per cent). The organisms were all gram positive and from their morphological characteristics resembled staphylococci (2), streptococci (1), diphtheroids (1), sarcina (1), and mixed staphylococci and bacilli (1). These organisms are typical of air contaminants and probably were introduced at the time of transfusion. They are in contrast to the psychrophilic gram negative bacilli reported in the literature (Stevens et al., 1953; Pittman, 1953) as having multiplied in banked blood during storage at 4 to 6 C and as having been responsible for severe reactions in recipients.

Among the six bottles from which organisms were recovered there was no apparent correlation between the presence of contamination and the age of the stored bloods.

### Table 1. Routine culturing of 213 unselected outdated banked bloods

<table>
<thead>
<tr>
<th>Outdated Bloods Cultured from:</th>
<th>Age in Days</th>
<th>No. of Bottles</th>
<th>Growth at 32 C</th>
<th>Growth at 22-25 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series 1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage at 4 to 6 C</td>
<td>22-26</td>
<td>111</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Series 2:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 to 72 hr incubation at 32 C</td>
<td>24-27</td>
<td>102</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2. Routine culturing of the residual contents in blood bottles after transfusion

<table>
<thead>
<tr>
<th>Results of Sterility Tests</th>
<th>No. of Bottles</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth at:</td>
<td></td>
</tr>
<tr>
<td>32 and 22 to 25 C</td>
<td>149</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
</tr>
<tr>
<td>both 32 and 22 to 25 C</td>
<td>3</td>
</tr>
<tr>
<td>32 C alone</td>
<td>1</td>
</tr>
<tr>
<td>22 to 25 C alone</td>
<td>2</td>
</tr>
<tr>
<td>Total bloods examined</td>
<td>155</td>
</tr>
</tbody>
</table>
DISCUSSION

The 368 bottles of blood which form the basis of this report were selected from among approximately 1000 units of blood processed by the blood bank during the time covered by the study.

No signs of transfusion reaction were observed in any of the recipients of the blood described in table 2 with the exception of a febrile episode occurring 12 hours after transfusion in one patient with chronic lymphocytic leukemia. This patient, who received one of the 149 bloods which showed no growth on culture, regularly develops delayed febrile reactions to transfusion, for which no specific cause is evident.

The number of bottles tested in the present study is too small to give a reliable indication of the incidence of contamination which can be expected in blood drawn and processed as described under Materials and Methods. It is reassuring that no organisms were recovered from any of the 213 outdated bottles tested. In Braude's (1952) much larger series of 1,697 bloods examined, the incidence of contamination was 2.3 per cent. However, it is impossible to compare that author's results with those of the present study because a) cultures were incubated at 37 C and b) the blood was cultured after only 24 hours of storage (possibly too early to reflect the maximum bactericidal activity of the fresh blood, and also too early to permit significant multiplication of any psychrophilic organisms which might have been present in small numbers).

It might be expected that during transfusion organisms would gain access to the interior of the bottle via the air vent needle (which is not equipped with a cotton filter) and at the site of penetration of the stopper by the recipient set. It is interesting that detectable contamination of the bottle contents by these means, under the conditions prevailing on the wards of this hospital, occurred infrequently as shown in table 2.

The availability of commercially produced sterile disposable blood bottles and bleeding equipment, such as were used during the course of these investigations, provides blood banks with an added safeguard against contamination of their stored blood. With the increasing use of banked blood in modern medical practice it is to be expected that there will be continued efforts toward improvements in bleeding equipment and technique of phlebotomy directed towards insuring the sterility of the blood. The efficacy of these measures can be assessed only by the results of carefully conducted routine bacteriological studies. The authors wish to re-emphasize the importance of conducting such studies at incubation temperatures which will insure the detection of both psychrophilic and mesophilic organisms.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. Margaret Pittman and Dr. J. T. Tripp, both of the Laboratory of Biologies Control, for their encouragement and counsel throughout the course of these investigations.

SUMMARY

1) Routine sterility tests designed to detect both psychrophilic and mesophilic bacteria have been performed on 213 unselected outdated bloods. No organisms were recovered. 2) Similar studies on the residual contents in 155 bottles returned from the hospital wards after transfusion revealed an incidence of contamination of 4 per cent. 3) The importance of performing the sterility tests at proper incubation temperatures was re-emphasized.

REFERENCES


