STERILIZATION OF BLOOD BANK BOTTLES

PRACTICAL HINTS

BY

G. E. O. WILLIAMS, M.D.

Merseyside War Blood Bank

The present increase in the use of stored plasma for the treatment of shock has drawn attention to the necessity for the most rigorous aseptic technique in handling and storing this product. Stored blood, by reason of its shorter "life," the need for its careful storage at low temperatures, and the phagocytic action of its leucocytes, is less liable to contamination. Plasma, on the other hand, has not only been deprived of the defences afforded by its cellular content but it is also apt to be stored for longer periods, and often at temperatures more conducive to bacterial growth. Moreover, the frequent presence of turbidity due to lipoids in unfiltered plasma and the gradual formation of granular deposits even in clear filtered plasma combine to make the detection of even gross contamination a difficult matter without resort to laboratory procedures, and consequently often lead to rejection.

Routine bacterial examination of the plasma prepared by the Merseyside War Blood Bank revealed in a number of the earlier samples the presence of contamination, particularly by spore-bearing bacilli of the *subtilis* group. This necessitated a careful review of the arrangements for the sterilization of bottles and other apparatus employed in the processing of plasma.

The Apparatus

The bottle used for storage of blood or plasma is of 560 c.cm. capacity, and is fitted with rubber diaphragm and perforated metal screw cap. As an internal filter a quantity of glass shot is included in each bottle. The plasma, which on standing separates from the cellular elements of whole blood, is withdrawn into its sterilized bottle by the "closed" method, using a short piece of rubber tubing fitted with needles, of suitable length and diameter, which are pushed through the holes in the metal cap and pierce the rubber diaphragm of each bottle. These decanting sets of rubber tubing with needles attached are sterilized by boiling immediately before use, and there is thus little opportunity of infection taking place during the process of plasma withdrawal, particularly as all the air admitted into the system passes through sterile filters of cotton-wool. It was therefore concluded that the source of the contamination observed was to be sought in the process of sterilizing the bottles.

The bottles are sterilized in a steam-operated hospital apparatus ordinarily used for the sterilization of dressings. The articles to be sterilized are exposed for twenty minutes to steam under positive pressure, and drying is effected by the application of a partial vacuum for a further period. Before the bottles are placed in the sterilizer their screw caps are covered by two thicknesses of grease-proof paper secured by an encircling rubber band, and the caps are screwed back one complete turn so as to break the airtight joint. The grease-proof paper is intended to prevent contamination of the cap of the bottle after sterilization, and after the bottle is removed from the sterilizer the screw is finally tightened. This is done while the bottle is still warm, and the vacuum produced on cooling is subsequently used in the filling process.

Objects Aimed At

The investigation of the efficiency of this method of sterilizing the bottles aimed at determining whether: (1) the temperature inside the bottle during sterilization was raised to that regarded as adequate; (2) the rise in temperature was maintained long enough; and (3) the condition produced actually destroyed both vegetative organisms and spores.

The first two questions were easily and satisfactorily answered by the use of sterilizer control tubes which were suspended inside the bottle and examined after the completion of the process. The control tubes used by us were of a well-known make, containing a red fluid which turned green after exposure to a temperature of 115° C. for twenty-five minutes or its bactericidal equivalent in time at higher or slightly lower temperatures.

The most satisfactory method of answering the third question was by observing the effect of our sterilizing process on bottles known to be contaminated. Bottles were therefore deliberately inoculated with various bacterial cultures: (1) with 5 c.cm. of a broth culture of *B. subtilis*; (2) with a dry smear from a slope culture of the same strain of organism; and (3) with a dry smear of *Staphylococcus citreus*. Bottles 1 and 3 were found to be efficiently sterilized, but the *B. subtilis* in Bottle 2 survived the sterilizing process. It was therefore concluded that the spores of this organism had escaped destruction because of the absence of sufficient moisture. The entrance of actual steam into the bottles is obviously not free or certain under the conditions of the experiment, as the rubber diaphragm inside the screw cap may sometimes be presumed to act as a valve.

Effect of Increased Alkalinity

To overcome this difficulty the citrate solution used as anticoagulant for the blood may be included in the bottle at the time of sterilization. We had, however, previously discontinued this practice on account of the increased rate of haemolysis resulting in blood collected in bottles so sterilized, and because the tests had shown that this haemolysis was due to the greatly increased alkalinity developing in the citrate autoclaved in the presence of the glass shot included as an internal filter. As it is the steam generated from the citrate solution within the bottle which kills the spores, recourse was had to including a small quantity of distilled water with the glass shot.

Experiment has shown that the optimum sterilizing effect is produced with as little as 4 c.cm. of water, and since the adoption of this procedure we have not again been troubled by contamination of the plasma withdrawn. The water added is practically all evaporated, and the small amount left is not sufficient to affect adversely the alkalinity or the concentration of the anticoagulant added at the time the blood is taken from the donor.

A further demonstration of the ill effects of the alkalinity produced by the heating of citrate solution in soft glass has recently been noted. Suspensions of red cells for grouping are collected in tubes containing citrate-saline solution. These tubes, some of which are required for the transport by post or by rail of cell suspensions from outlying centres, were formerly autoclaved to prevent bacterial growth. It was found, however, that red-cell suspensions in these alkaline tubes very soon haemolysed at room temperatures. To overcome this objection it has become necessary to omit the preliminary sterilization of the tubes and to risk the possibility of bacterial growth.

Summary

These simple observations on the sterilization of bottles, etc., used in blood banks are presented in the hope that they will particularly benefit those who, even as we did, have been taking for granted the efficiency of their autoclaves in producing bactericidal and spore-destroying conditions *inside* the numerous pint bottles necessary in a large blood bank, and who may have been experiencing similar difficulties in the preservation of blood.

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Clinical Memoranda

Staphylococcal Septicaemia simulating Left Perinephric Abscess

The following case is interesting enough to be placed on record.

CASE REPORT

A soldier aged 24 was admitted to this hospital on the evening of July 1, 1940, with "acute left lumbago." Apart from enteric fever five years previously he had had no illnesses of note. His present disorder began twelve days before admission with acute pain in the left loin, radiating to the left thigh. There were no associated symptoms of trouble with micturition.

On admission he looked flushed and extremely ill. His temperature was 99.4°, pulse 104, and respirations 24. He was of good physique, his heart and chest were normal, and examination of the central nervous system disclosed nothing unusual. There was extreme tenderness over the left lumbar region behind, but no swelling. The left kidney was not palpable. A freshly voided specimen of urine was crystal-clear, with no abnormal constituents. A catheter specimen had a specific gravity of 1015, with neutral reaction, and gave no growth on culture. The patient was treated expectantly, and 1 oz. of mist. sod. sal. was given four-hourly for six doses, and then three times a day, with copious fluids, glucose, and fruit juices, etc. Pyrexia continued, and he had a severe attack of pain simulating left renal colic on July 3, for which 1/3 grain of morphine was necessary, and anti-phlogistine was applied to the left loin and renewed every twelve hours.

On July 4 1 gramme of sulphanilamide four-hourly, for six doses, was given empirically. This did not seem to ease the pain or to make much difference to the pyrexia. He had a further attack of what appeared to be severe left renal colic on the 5th, for which morphine was again given. The pain became easier on the 6th, and the temperature showed signs of settling on the 7th, but rose again on the 8th, with aggravation of the loin pain. On July 9 the left perinephric region was carefully explored under local anaesthesia, but no pus was found. He was radiographed on the same day for ? perinephric abscess, ? renal calculus. The symptoms were very suggestive of perinephric abscess. A "straight" radiograph revealed no signs of calculus and there did not appear to be a psoas shadow on the left side: this suggested perinephric abscess, but there was no swelling at the back, and exploration was negative.

At the suggestion of Mr. J. B. Ferguson Wilson, consulting surgeon to this hospital, blood was taken for culture. Meanwhile the patient was given 1 gramme of sulphanilamide four-hourly for six doses and then thrice daily for four days. The temperature now began to subside, his general condition became much better, his appetite was improved, and he slept without the use of the usual soporific drugs.

Blood culture gave an abundant growth of *Staph. albus*. Progress was maintained, and on July 22 the patient's general

condition was so satisfactory that he was allowed to get up daily. He was given the usual tonic treatment with intramuscular liver extract, intensive doses of inorganic iron by mouth and intramuscularly, and malt and oil. On August 15 he was discharged from hospital, and recommended for three weeks' leave before going back on active duty.

ISAAC ROSE, M.B., Ch.B., D.R.C.O.G.,
Medical Officer in Charge of St. Helen
Municipal Hospital, Barnsley.

Retroperitoneal Haemorrhage from Angioma of Kidney

The following is presented as an unusual case of acute abdomen.

CASE REPORT

A married woman aged 39 was admitted to hospital on June 29, 1940, complaining of an intermittent abdominal pain which first appeared about seven days previously, but which had become very much worse in the last twenty-four hours. The pain was generalized in the abdomen, with maximal intensity in the right upper part, whence it passed into the back. There was no history of any preceding illness, accident, or even undue exertion.

The bowels, which were usually regular, had not acted for two days. There was no frequency of micturition, but the patient had noticed that her intermittent abdominal pain sometimes came on after passing urine. She had never noticed blood in her urine. There had been no vomiting. She had two children, the younger being 8 years old. Her periods were regular, the last being fourteen days before admission. There was nothing else of any importance in the history.

The clinical findings as noted at the time were as follows. The temperature was 99°, pulse 80, and respirations 20. The patient was strikingly pale—suggesting internal haemorrhage. The abdomen showed limited respiratory excursion and slight general fullness. There was normal liver dullness; the spleen was not felt. In the right upper quadrant of the abdomen was a vaguely defined, smooth, rounded, very tender swelling, resonant anteriorly and not moving on respiration. It extended from the costal margin above (where it could not be felt as separate from the liver) to the level of the umbilicus below, and from the midline of the abdomen medially to the mid-axillary line laterally. Apparently the lump did not originate from the loins. Pelvic examination revealed no abnormality. The lungs were clinically normal. The urine was normal to chemical tests.

No definite diagnosis could be made, but there was a bias towards empyema of the gall-bladder. We could not, however, reconcile the signs of internal haemorrhage with any pre-operative diagnosis, and operation was undertaken for "acute abdomen."

A right upper paramedian incision was made and the hepatic flexure of the colon presented on a mass of retroperitoneal blood clot. The peritoneum was divided in the right paracolic gutter and reflected medially, so that the right kidney could be delivered and removed. The kidney proper was normal in size and shape, but its enlarged pelvis was filled by a solid tumour which had ruptured through the anterior wall of the pelvis. There were about two pints of coagulated blood behind the peritoneum, extending as far down as the pelvic brim. Most of this blood was removed, and drainage was established through the loin from the affected area.

Convalescence was complicated by bilateral femoral phlebitis plus infection of the residual blood clot, but the drainage proved adequate and a slow recovery followed. Microscopical examination of the specimen by Dr. W. A. Robb showed a simple angioma of the kidney pelvis.

My thanks are due to Mr. A. L. Candler, senior surgeon to the Royal Devon and Exeter Hospital, for allowing me to treat and to report this case.

C. W. R. PRICE, M.B., B.S., F.R.C.S. Ed.,
Resident Surgical Officer, Royal Devon
and Exeter Hospital, Exeter.