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Autoclaves and their dangers and safety in laboratories

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SUMMARY

Using a laboratory downward displacement vertical autoclave with the help of thermocouples recorded on a 12 point multichannel strip recorder, the risk of failing to sterilize laboratory discard buckets has been demonstrated. The use of proper temperature and time controls can prevent this risk.

A load in a bucket with perforated sides is more easily sterilized than in a solid bucket. Wire baskets, where appropriate, facilitate the sterilizing practice. The addition of water to a bucket does not reduce the time of heating up.

It is desirable that sealed bottles of media should not be sterilized in simple downward displacement autoclaves, but if used, strict monitoring of temperatures and times is essential both in the heating up stage and especially in the cooling stage. The temperatures in bottles are slow to rise and very slow to fall. Bottles at high temperature 80–105° C. or over have a high internal pressure which can allow the bottles to explode when subjected to thermal shock if removed too early.

It is suggested that all laboratory autoclaves should have a load temperature simulator or similar device to control the temperature of the load during the cycle automatically. For the sterilization of fluid media, it is suggested that, in addition to a simulator there should be accelerated cooling to reduce damage to the media and, what is more important, to rapidly bring down the temperature and thus the internal pressure in the bottles to a safe level. The opening of the sterilizer door or lid should be automatically controlled by the load temperature simulator.

INTRODUCTION

For years past pathologists have been content to use the same vertical autoclaves for disinfecting cultures and for preparing media. At one time gas was used to heat water in the bottom of the chamber. The steam so generated displaced the air and this could be detected by placing a tube from the air vent into a pail of water. When the air had been removed the valve was tightly closed and the temperature and pressure rose. There was usually one dial which indicated pressure only and one assumed that the proper temperature appropriate to the recorded pressure had been reached. One used to sterilize at 'so many pounds per square inch', e.g. 15 lb. per square inch for 30 min. If all the air was driven off one did get the necessary temperature, but very often some air was left in the chamber and the temperature did not rise according to the temperature/pressure relationship of pure steam. Later autoclaves had an electric element or a steam coil to heat the water, but the conditions obtained in the chamber were still far from satisfactory.

Most present-day models use live steam from an independent steam supply which comes into the chamber at the side as near to the top as possible. There is usually a thermometer in the chamber drain indicating the temperature in the lowest part of the chamber, but in some the thermometer probe is inside the chamber about 6 in. from the bottom. At the base is an exhaust valve which allows the steam to flush out the air rapidly in 3–5 min. After it is closed a 'near to steam' trap in the chamber drain allows air and water of condensation to escape during the rest of the cycle. When the temperature determined by the probe registers the desired temperature the sterilizing stage commences for the appropriate time, after which the steam is turned off. The temperature and pressure fall slowly and when the temperature reaches a so-called 'safe level', about 80° C. as determined by the chamber probe, the lid is opened.

If bottles, tubes and Petri dishes are held in wire baskets, air can escape from the basket so that adequate temperature may be reached. Nowadays solid sided buckets or boxes made of metal or rubber are employed. Their use can result in air being trapped in the container so that the temperature may not rise to an adequate sterilizing level at all levels within the load. If a simple downward displacement autoclave is used to sterilize fluid media in bottles the temperature of the fluid in the bottles may never reach sterilizing temperatures. Also on cooling, the temperature and pressure inside the bottles, especially if they are tightly screw-capped, may remain high long after the chamber temperature registers below 80° C. and the chamber pressure is well below atmospheric pressure. The opening of the lid of an autoclave, when temperatures and pressures inside the bottles are high, may lead to a dangerous explosion due to thermal shock.

METHODS AND MATERIALS

Several experiments were made to demonstrate the temperatures reached in laboratory autoclaves. Thermocouples were placed in different sites and the recordings were made using a 12 point multichannel strip chart recorder of high accuracy. In each experiment all buckets were filled with the load.

A downward displacement vertical autoclave was used in which the steam enters near the top and air is displaced through a chamber drain. In the normal process the steam is turned on and allowed to flush out the air for 3–5 min., after which the exhaust is manually closed, leaving the 'near to steam' trap to permit the escape of any residual air and water of condensation. The temperature within the chamber is determined by a probe on the inside near the bottom of the chamber, and is shown on a dial thermometer on the front of the apparatus. When the temperature reaches 121° C. the usual practice is to allow a specified time for sterilization, a minimum of 15 min., after which the steam is turned off, and the autoclave allowed to cool. When the dial thermometer indicates 90° C. the exhaust valve may be opened to reduce any vacuum developing in the chamber. When the temperature reaches 80° C. the lid is usually opened. The timing of the sterilizing period should be varied according to the load but it is frequently set at one specific time of 15 min., which as experiments described later show, is far too short as insufficient time is allowed for the whole of the load to attain 121° C.

In the first series of experiments a solid rubber bucket was used full of miscellaneous bottles and tubes discarded from a bacteriology laboratory. Two thermocouples were placed in the fluid inside the bottles, one at the top and the other at the bottom of the bucket. A third thermocouple was placed on the probe in the chamber and recorded the temperature as shown on the dial thermometer of the autoclave.

In the second series of experiments metal buckets were used with one inch holes in the sides one inch from the bottom.

In experiments 3–5 120 ml. bottles with 25 ml. water were used as test pieces. Thermocouples were put in two bottles, one at the top, the other at the bottom of the container, in the chamber drain, and in experiments 4 and 5 on the probe in the chamber. In experiment 3 a solid dry bucket was used, in the fourth 3 in. of water was added to the bucket, in the fifth a wire basket was used.

Experiments 6–8 were performed to show the difficulties of attempting to sterilize fluid media in bottles by a downward displacement autoclave. Two wire baskets were used, one above the other, each containing 20 'flat' bottles of 500 ml. capacity filled with water up to the shoulder. Thermocouples were inserted in 10 bottles, in the chamber drain and beside the chamber probe. In run 6 all bottles had loose caps except one: in run 7 all caps were sealed and in run 8 all the bottles had cotton wool plugs.

RESULTS

In the first experiment using a solid rubber bucket the chamber probe reached 121° C. in 12 min. (Fig. 1*a*), but the top bottle did not reach 121° C. until 20 min. had elapsed and the bottom bottle after 46 min. If one had counted the sterilizing time of only 14 min. from when the dial had reached 121° C. at which time the bottom of the load would just have reached 70° C. the load would have reached only 115° C. when the steam was turned off, and would not have been sterilized. When the load had reached 121° C. it was held at this temperature for 15 min. On cooling, the chamber probe reached 80° C. in 16 min. but at that time the temperature in the bottom bottle was 103° C. and in the top 99° C. If the lid had been opened at this stage the bottles would have been liable to explode from thermal shock. After another 5 min. the probe reached 70° C., the bottom bottle 100° C., and the top bottle 96° C. Five minutes later the probe reached 54° C., the bottom bottle 96° C. and the top 84° C., at which time the load was removed but the bottles were really too hot for safety, especially if any bottles were closed with tight screw caps, when they could have exploded if subjected to thermal shock.

The effect of extending the free steaming stage was then tested. Using a solid metal bucket with a load of bottles and tubes extending the free steaming for half an hour did not accelerate a rise in temperature of the load. If a light load of glass Petri dishes with metal lids was used, by free steaming until the temperature of the top and bottom dishes equated, the heating stage could be reduced from 24 min. to 18 min. However, when this was repeated with agar in the Petri dishes (Fig. 1*b*), the heating stage was extended to 30 min., but it took a further 14 min. before

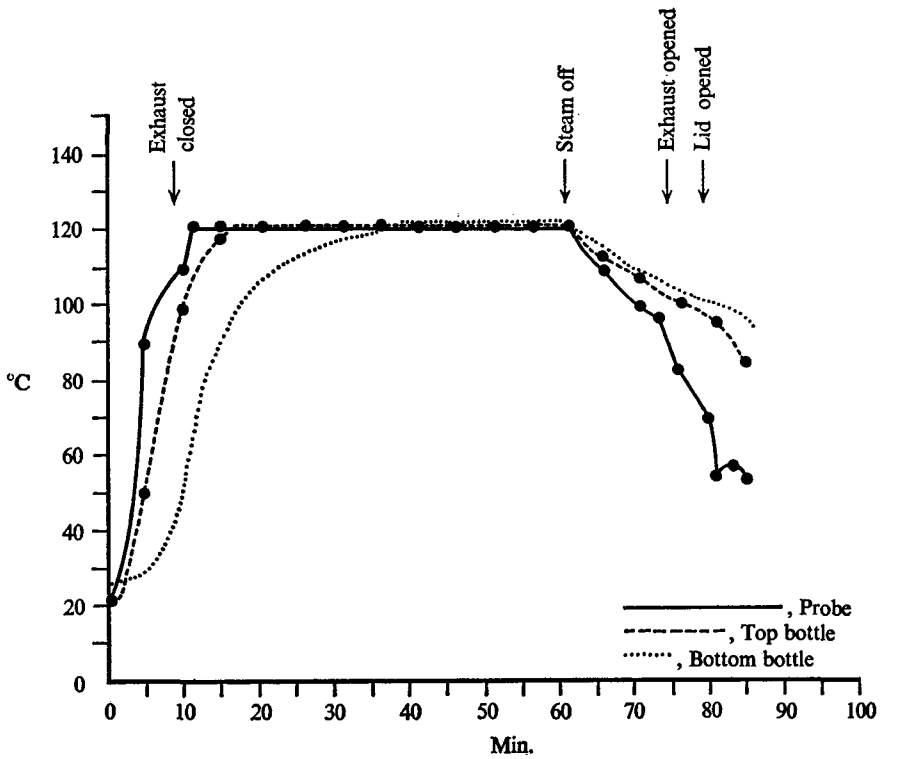

Fig. 1*a*. Temperature curves in autoclave loaded with miscellaneous discarded bottles and tubes contained in solid rubber buckets.

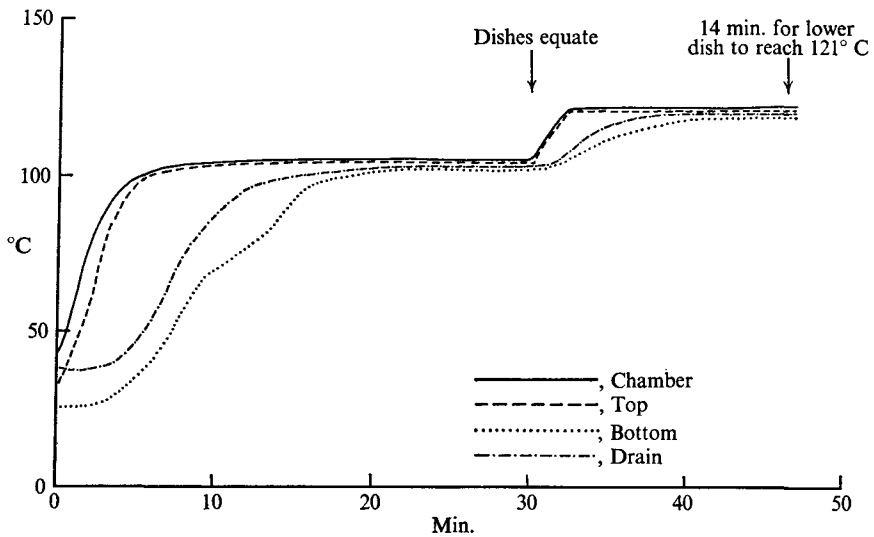

Fig. 1*b*. Autoclave loaded with Petri dishes containing agar, held in solid metal buckets. Free steaming was continued until temperatures in top and bottom dishes were equal.

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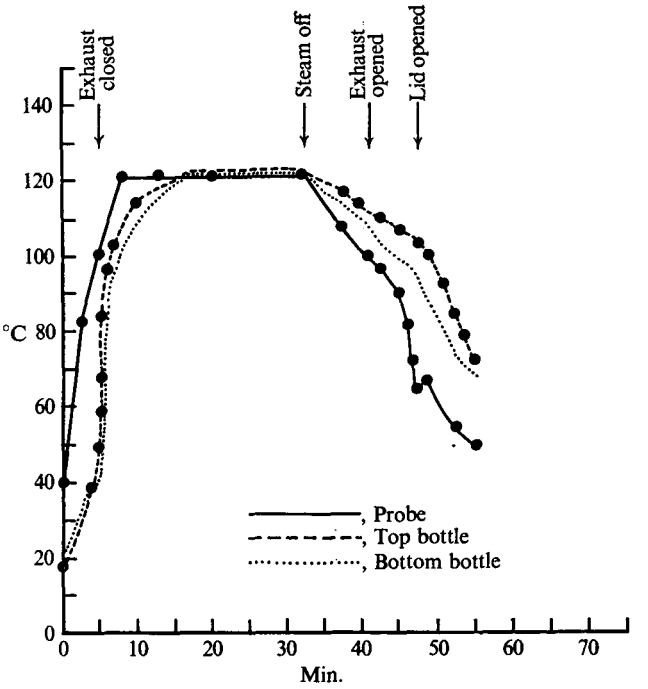

Fig. 2. Temperature curves in autoclave loaded with bottles in metal buckets perforated near base of wall.

sterilizing temperature of 121° C. was reached, so that only about 14 min. were saved.

In the second experiment (Fig. 2) in which metal buckets with holes in the sides were used the air escaped more easily yet the solid bottom collected any escaping fluid or agar if not excessive in amount. The temperature as determined by the probe reached 121° C. in 8 min., and in both bottles in 18 min. A further 15 min. were allowed for sterilizing after which the steam was turned off. On cooling, the probe reached 80° C. in 14 min. but the temperature in the bottom bottle was 97° C. and the top 105° C. The lid was opened at this time but the top bottles were still dangerously hot. After a further 7 min. the probe temperature was 52° C., the bottom bottle 72° C. and the top 80° C. The total cycle time was 54 min.

In a comparative experiment a rubber bucket was used with quarter inch holes two inches from the bottom but in which was placed a metal perforated tray two inches from the bottom above the holes. The temperature rise was rapid similar to that in the perforated metal bucket. It was felt that any spillage would be easily contained in the bucket and all air would be allowed to escape from the very bottom of the load.

In the third experiment (Fig. 3) using 120 ml. bottles containing 25 ml. fluid in a dry bucket the top bottle reached 121° C. in 27 min., but the bottom bottle reached 121° C. only after 60 min. at which point the steam was turned off. When the experiment was repeated (Fig. 4) with three inches of water in the bucket, the temperature reached 121° C. in the drain after 9 min., and in the top bottle after

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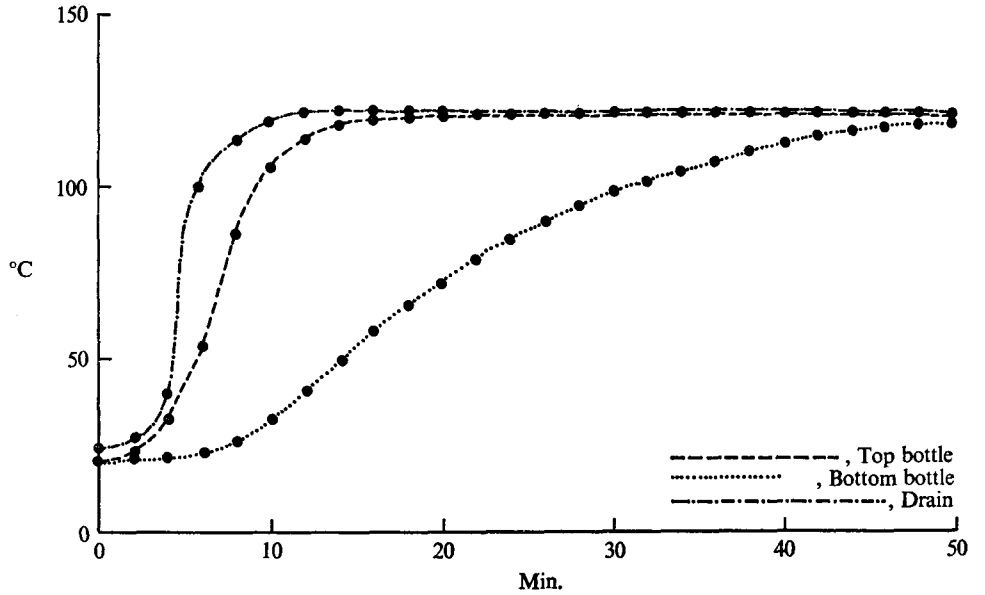

Fig. 3. Temperature curves in autoclave loaded with 120 ml. bottles each containing 25 ml. fluid, in solid metal buckets.

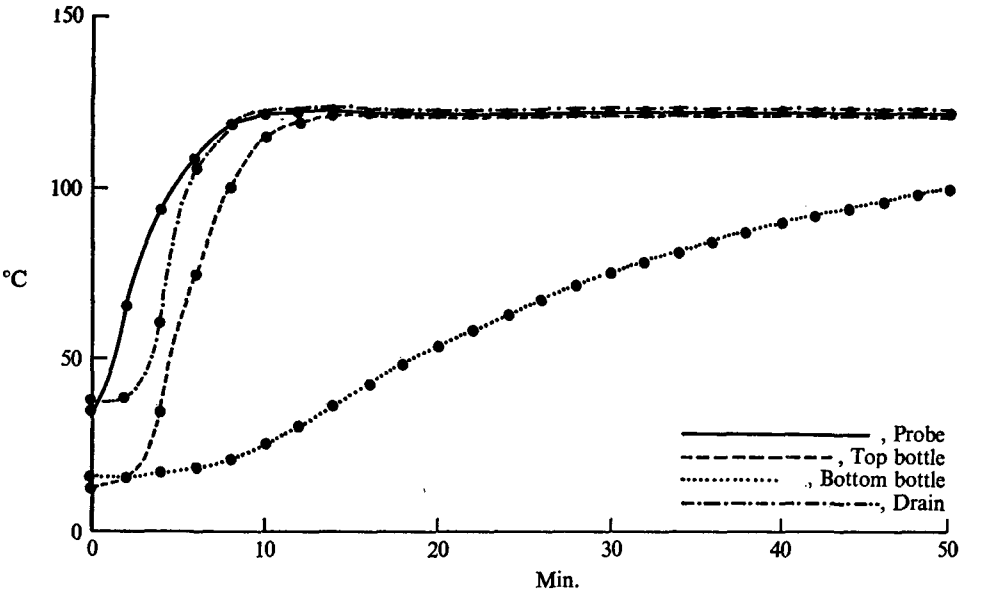

Fig. 4. Similar to Fig. 3, but the buckets contained water to a depth of three inches.

15 min., at which time the bottom bottle had reached only 40° C. After 50 min. the bottom bottle had attained only 100° C. and did not reach 121° C. until a total time of 2 hr. 20 min. had elapsed. The presence of the water had delayed the rise in temperature so much that the bottom bottles never reached sterilizing temperatures.

When the experiment was again repeated using wire baskets (Fig. 5) a tem-

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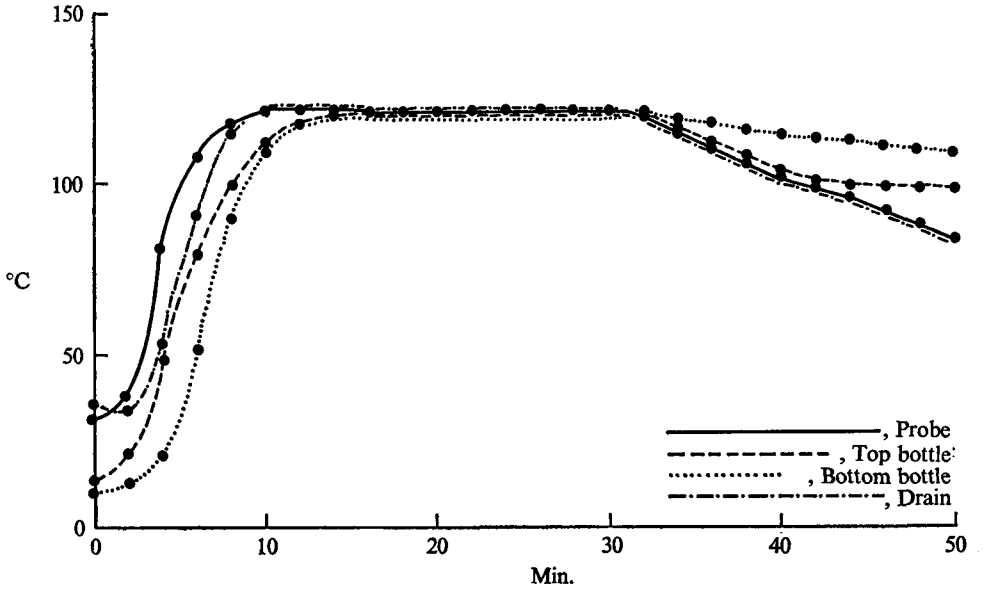

Fig. 5. Similar to Fig. 3, but the bottles were held in wire baskets.

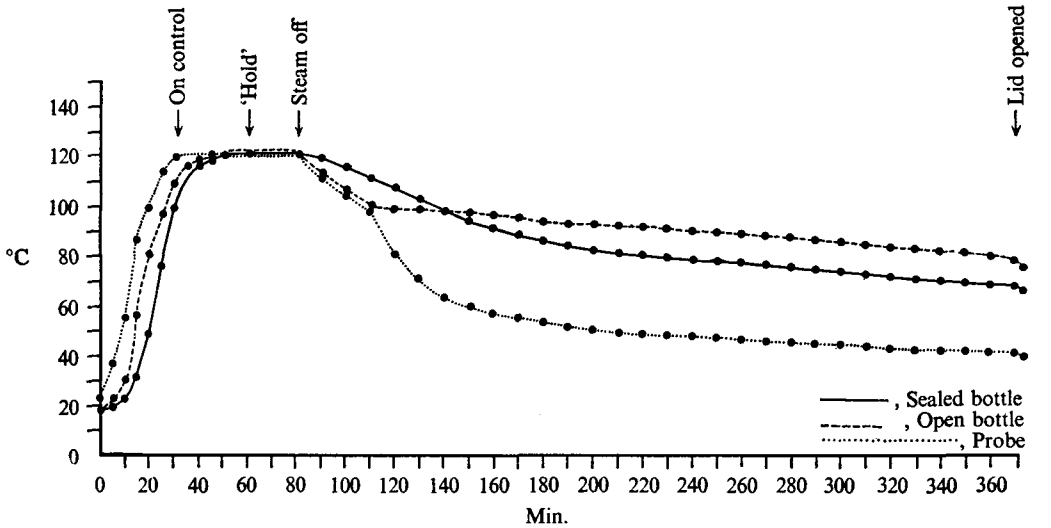

Fig. 6. Autoclave loaded with two layers of 500 ml. bottles in wire baskets. Each bottle contained fluid up to the 'shoulder'. All bottle caps were loose except one, which was tightly sealed.

perature of 121° C. was reached in the chamber probe and chamber drain in 10 min. and in the top and bottom bottles in a further 7 min. On cooling, the probes in the chamber and chamber drain reached 80° C. in 20 min., but the temperature in the bottom bottle was 110° C. and in the top bottle 98° C. showing that there was slow cooling in the bottom of the load. The above three experiments were repeated in a machine working at 134° C. with similar results.

The next three experiments (6–8) describe attempts to sterilize fluid media in 500 ml. bottles. In the first (Fig. 6) the screw caps were left loose except for one in

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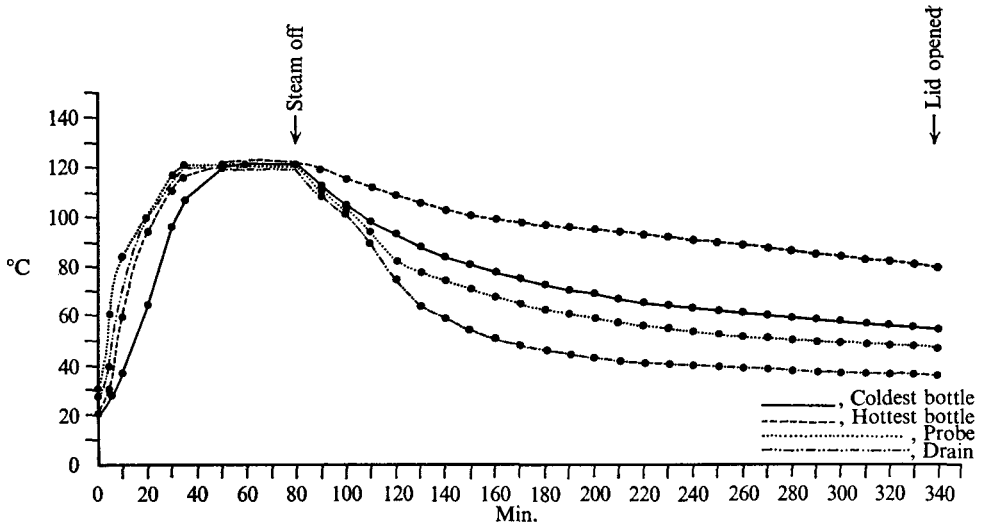

Fig. 7. Similar to Fig. 6, but all bottles were tightly sealed.

which a probe was inserted and the cap screwed very tightly and further sealed with insulating tape. It took exactly 30 min., for the chamber probe to reach 121° C. and a further 30 min. for all the bottles to reach 121° C., i.e. one hour from start. The bottles were held at this temperature for 20 min. and the steam shut off. On cooling, the temperatures in the chamber probe and in the open bottle fell to 100° C. in 30 min., at which time 112° C. was recorded in the sealed bottle. In just over a further 10 min. when the chamber probe had reached 80° C., the so-called 'safe' temperature, the open bottle remained at 100° C. and the sealed bottle had fallen slowly to 108° C. (equivalent to 25 lb./in.^{2} within the bottle) obviously too high a temperature and pressure to attempt opening the lid and removing the load. In a further 20 min. when the chamber probe reached 64° C. the temperature in both the open and the sealed bottles was 98° C. After this time the temperature in the sealed bottle fell more rapidly than in the open bottle indicating a vacuum effect as a result of fluid having escaped whilst the bottles were being heated in spite of the so-called 'seal'. From the start of cooling it took $2\frac{1}{2}$ hours for the temperature in the sealed bottle to reach 80° C., at which time the chamber probe indicated 48° C. and the open bottle 90° C. For all bottles to be below 80° C. it took 4 hr. 40 min. from the start of cooling. At that time the chamber probe recorded 42° C. demonstrating that the chamber probe temperature gave no indication of the temperature in the bottles. The complete cycle time was 6 hr.

In a repeat experiment (Fig. 7) all the caps were screwed as tightly as possible and 10 bottles, in which thermocouple probes were inserted, were further sealed with tape. Thermocouples were placed also on the chamber probe and in the chamber drain. On heating until 121°C. was reached as shown by the probe there was a wide difference in the rate of rise in temperature in the bottles – as much as 30° C. The chamber probe and chamber drain reached 121° C. in 35 min. and all the bottles reached 121° C. in a further 25 min. – i.e. 60 min. from the start. They were held at this temperature for 20 min. and the steam shut off. On cooling, the chamber

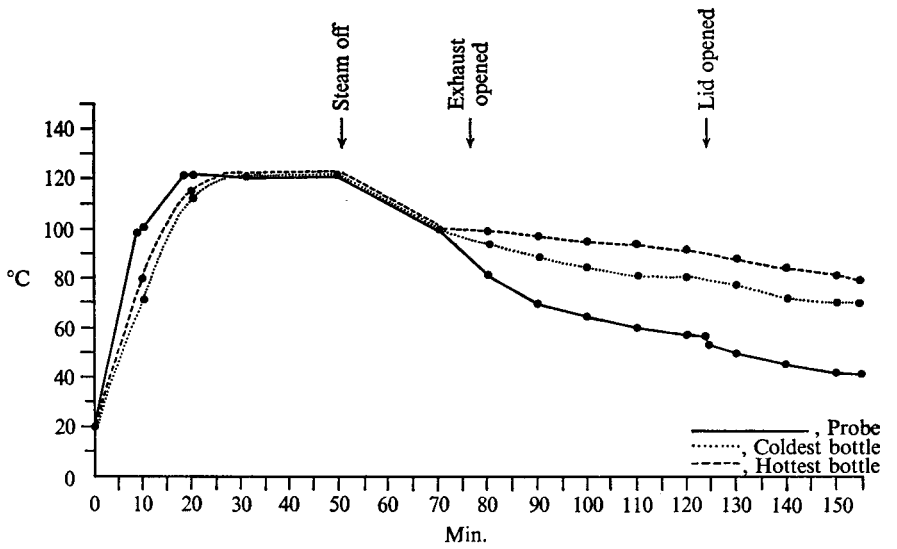

Fig. 8. Similar to Fig. 6, but the autoclave contained only one wire basket of bottles, which were closed only with cotton-wool plugs.

probe reached 100° C. in 23 min., but the temperature in the bottles again showed variation in cooling, some being as high as 114° C. at that time. From the start of cooling the chamber probe reached 80° C. in just over 40 min., at which time the hottest bottle recorded 108° C. After a further 40 min. cooling the chamber probe recorded 68° C. but the hottest bottle 100° C. After a further 80 min. the chamber probe had fallen to 54° C. but the hottest bottles only to 90° C. – still too high to handle for fear of explosion as a result of thermal shock. It took a further 40 min. cooling for all the bottles to reach 87° C. when the probe recorded 50° C. and a further 50 min. to reach 80° C. when the probe recorded 48° C. The whole cooling process had taken 4 hr. 10 min. Thus the total cycle of the whole process from start to finish was $5\frac{1}{2}$ hr.

In the final experiment (Fig. 8), using one basket of 20 bottles, all were plugged with cotton wool with no caps. A temperature of 121° C. was recorded by the chamber probe in 18 min., and in all the bottles in 30 min. from start. They were held at this temperature for 20 min. and the steam shut off. On cooling the bottle temperatures fell rapidly together to 100° C. in 20 min. In another 7 min. when the probe recorded 85° C. the exhaust valve was opened. When the chamber probe reached 80° C. in a further 5 min., the hottest bottle recorded 98° C. In a further 40 min. the chamber probe was 56° C. and the hottest bottles 90° C. after which the lid was opened when the load was still too hot to handle. After a further 30 min. the bottles had reached 80° C., that is a total cooling time of 100 min. The total cycle of the process was $2\frac{1}{2}$ hr.

DISCUSSION

On considering the results of the first two experiments, when using solid buckets for sterilizing discarded cultures one must not time the sterilizing period of 15 min. from when the chamber temperature reached 121° C. One must allow a further

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40–60 min. according to the loading to allow all the load to reach 121° C., and then be maintained at 121° C. for the sterilizing period (not less than 15 min.). For safety, on cooling, the lid should not be opened until the indicated temperature is 70° C. and a further 15–30 min. allowed before the load is removed. Thus the complete minimum sterilizing cycle could be as follows:

(i) 15 min. for the chamber temperature of 121° C. to be reached;
(ii) 45 min. for the whole of the load to be held at 121° C. for 15 min;
(iii) 60 min. for cooling to a safe condition for handling.

This would give a minimum total cycle time of 2 hr.

By using buckets with holes in the sides to allow the air to escape the heating up time was reduced by about half an hour. For safety a suggested cycle could be, for the probe temperature to reach 121° C., 10 min.; heating up and sterilizing load, 25–30 min.; cooling 25 min., a total cycle time of about one hour.

On considering the results of experiments 3–5 it is obvious that when using a downward displacement autoclave the fluid in bottles contained in solid buckets may never reach sterilizing temperature. The addition of 3 in. of water only delayed the rise in temperature so that even the top bottles never reached the desired sterilizing temperature of 121° C. in one hour. Many users believe that the presence of water drives off the air and speeds up the heating, but in a downward displacement autoclave this is untrue as the temperature in the load does not rise more quickly. When wire baskets were used there was a more rapid and uniform rise and fall of temperature in the bottles. Adequate time must be allowed for cooling.

The experiments on sterilizing fluid media in sealed bottles demonstrated an uneven and slow rise in temperature which could lead to inadequate temperatures being reached. By using non-sealed, plugged bottles there was a rapid rise in temperature on heating and also more rapid cooling when compared with sealed bottles. What was more frightening was the danger of explosion on rapid cooling. One should note that the pressure inside a sealed bottle is due to the expansion of the air and its water vapour, which can be compressed, together with expansion of the liquid. The more nearly filled the bottle is, by increasing the volume of liquid and thereby reducing the air space, the greater will be the internal pressure as the temperature rises.

These experiments show how the methods of sterilizing discarded cultures in tubes and bottles could be dangerous by reason of inadequate sterilization unless one adheres to certain elementary rules. The problem of removing air from a chamber and from the load is well recorded (Cruickshank, Duguid, Marmion & Swain, 1973; Perkins, 1969; Rubbo & Gardner, 1965), but is often forgotten in practice. Autoclaves may be manufactured properly but often lack adequate controls and instructions on how to ensure that they are performing the function of sterilizing. They often lack the necessary indicating thermometers which should register the temperature of the load at all stages from the start of the cycle to the end of cooling when it should be safe to open the lid or door.

Downward displacement autoclaves can be used safely as long as adequate time is allowed for the air to escape and for the temperature to rise, but this should be monitored by a probe simulating the load. Until such simulators can be fitted,

autoclaves must be tested individually according to the loads to be sterilized by using the results of test runs monitored by thermocouples. Once the proper time and temperature relationship has been demonstrated a schedule of times should be laid down for different types of load. Such times could be obtained from some of the runs in the experiments reported.

One should consider the best methods of removing the air as for example by using, instead of solid sided buckets, perforated buckets, preferably with a perforated tray three inches from the bottom, or open baskets if the type of load permits their use. On heating, time should be allowed for free steaming but prolonging the free steaming stage even up to half an hour in our machines did not remove the air more efficiently and thus did not accelerate the heating of the load.

When considering the sterilization of fluid media in bottles, sealed or unsealed, one is up against the same problems as the pharmacist. When the temperature of the fluid rises there is expansion of the fluid and also of the air in the space above the fluid in the neck of the bottle. If the bottle is completely sealed no air or fluid will escape so that a very high pressure can develop inside the bottle, much greater than the pressure in the chamber even although they may be at the same temperature. One must realize that because of the bulk of the fluid the temperature in the fluid will not rise as rapidly as in the chamber in which the temperature may be indicated by a thermometer probe in the chamber. In controlling the temperature of the fluid one may use a thermocouple in a sealed bottle but because sealing such a bottle is difficult and as the wire may be easily broken it is not always practicable. It is better to use a simulator which gives the same temperature conditions as the fluid within the bottles. One should be able therefore to control the time necessary to bring the fluid up to sterilizing temperature and the time necessary to ensure sterility. The cooling cycle must also be controlled in sealed and non-sealed bottles. If the temperature and pressure in the chamber were allowed to fall rapidly, sealed bottles could explode owing to the high pressure inside the bottles. At a temperature of 110° C. the pressure is about 30 lb./in.^{2} in a nearly full 500 ml. bottle. At 105° C. it is approximately 26 lb./in.^{2} and at 90° C. approximately 17 lb./in.^{2} Any sealed bottle over 80° C. when subjected to thermal shock is liable to explode with disastrous results. This risk of explosion can be prevented by allowing the chamber and the bottle temperatures and pressures to fall very slowly over a period of 6–12 hr. – i.e. overnight. However, in properly designed autoclaves one can add to the chamber air under pressure which can gradually be reduced as the temperature in the bottles falls. Accelerated cooling may also be achieved by using a finely atomized water spray or by filtered air cooled by refrigeration. However, when using simple laboratory downward displacement autoclaves one should be fully aware of the dangers of explosion associated with an unsatisfactory cooling cycle. Excess heating can also damage the ingredients in some specialized media. It is suggested that such simple machines should no longer be used for sterilizing media in sealed bottles as autoclaves are now available in which accelerated cooling together with bottle pressure compensation using filtered refrigerated air under pressure allow the cycle to be completed in under 2 hr.

With unsealed bottles the real problem is not of increased pressure inside the

Table 1. *Sterilizing times*

Time required for	Solid buckets (min.)	Perforated buckets (min.)
(1) Chamber to reach 121° C	15	10
(2) Whole of load to reach 121° C and maintained for at least 15 min.	45	25–30
(3) Cooling to a safe condition for handling	60	25
Total cycle time	120	65

bottles but of the slow rise and fall of the temperature of the fluid during the various cycles. Unless a proper load temperature simulator is used one cannot control the rise of temperature in the heating cycle. The experiments described showed that if one relies on the temperature indicated in the chamber or in the chamber drain, the heating cycle could be stopped well before the fluid had reached sterilizing temperature. Similarly on cooling, temperature gauges in these locations give no indication of the very high temperature remaining in the bottles for some time, long after the chamber temperature is low and the pressure at or below atmospheric. One can of course do test runs monitored by thermocouples for specific loads and draw up the necessary schedule for timing the cycles. However, one feels that for complete safety in sterilization and for preventing accidents to staff, a simulator should be used as recommended for sealed bottles.

RECOMMENDATIONS

In using laboratory downward displacement autoclaves for sterilizing discarded cultures and equipment it is essential that all the air is removed from the load to facilitate obtaining adequate sterilizing temperatures. Time must be allowed for this to occur. Autoclaves must be checked by test runs using thermocouples to provide a schedule of proper times for the cycles. The use of a load temperature simulator would ensure better control of temperatures reached in the load. Solid buckets hinder the removal of air. Buckets with perforations in the sides preferably with a perforated tray 3 in. from the bottom are better but wire baskets are the best. In this type of laboratory autoclave the time cycles as shown in Table 1 could be followed. When using solid buckets adding water to the bucket does not improve performance.

For sterilizing fluid media in sealed bottles a proper fully automatic fluid sterilizer should be used in which all temperatures and time cycles are controlled by a load temperature simulator. Cooling can be accelerated by the use of refrigerated filtered air under pressure, or by some other similar device. If a downward dispacement laboratory autoclave is used for sealed bottles it is essential that test runs monitored by thermocouples are performed so that a schedule of temperature and time can be determined for specific loads. It would be easier to control such loads if a simulator was used. It is difficult to assess truly the temperature within a load by the use of thermometers in the chamber or chamber

drain. Adequate time must be allowed for the temperature in the fluid to rise to sterilizing temperature when it can be maintained for the necessary sterilizing time. On cooling it is essential to allow the temperature of the fluid to fall to 80° C. or below before the lid or door is opened.

If non-sealed bottles are used by leaving the caps loose, or by inserting cotton wool plugs, there is not the same risk of explosion as when sealed bottles are used. However, test runs must be performed to ensure adequate heating of the fluid and also sufficient cooling to ensure safe handling. If the bottles are not sealed there is always the risk of post-sterilization contamination.

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