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Autoclaving practice in microbiology laboratories: report of a survey

THE PUBLIC HEALTH LABORATORY SERVICE SUBCOMMITTEE[1] ON LABORATORY AUTOCLAVES[2]

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SUMMARY The performance of autoclaves in 27 laboratories, operated in accordance with the normal routine of local practice, has been monitored using thermometric equipment. Sterilising performance was unsatisfactory on 10 of 62 occasions, and cooling was inadequate on 52 of 60 occasions.

As part of the general concern over safety standards, laboratories in the Public Health Laboratory Service (PHLS) were encouraged to obtain thermometric equipment to monitor the performance of their autoclaves. This provided an opportunity to discover whether, when the autoclaves were operated in accordance with the normal practices in use in those laboratories at the time of acquisition of the equipment, sterilising performance was satisfactory and cooling of the loads was adequate to allow the autoclaves to be opened safely. The results of a survey of these aspects of autoclaving practice in 27 laboratories are reported here.

Survey material

LABORATORIES AND AUTOCLAVES

In all, 46 downward-displacement autoclaves in 27 laboratories were tested. Twenty-four of the autoclaves were vertical and cylindrical, and 22 were horizontal. Some were the responsibility of the PHLS Board, some of Area Health Authorities and, in one case, of a university department.

Twenty-three of the laboratories were engaged, to a varying extent, in clinical microbiology and four were reference laboratories in special microbiology.

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Method

Tests were made when the autoclaves were loaded, operated, and unloaded in their normal manner, according to local custom and practice. Most temperature measurements were made with copper/constantan thermocouples (0·2 mm diameter) insulated with polytetrafluoroethylene (PTFE) but unsheathed. Usually these could be led into the autoclave chamber between the door and the seal. They were inserted at those points in the load where experience had shown that performance was likely to be unsatisfactory as regards either sterilising or subsequent cooling. For example, when testing the processing of discarded cultures, a thermocouple was placed inside one of the cultures making up the load and this was placed at the centre and about one-third of the way up the discard container. Similarly, when observing cooling times, thermocouples were placed in the largest units of volume making up the load, since it was in these that cooling was likely to be most prolonged.

Thermocouple signals were recorded on a suitable instrument; these were checked at intervals against a mercury-in-glass thermometer which had been certified by the National Physical Laboratory.

Not surprisingly, the choice of sterilising temperatures or times among the laboratories was not uniform, thus making comparison difficult. Furthermore, distinction has to be drawn between the treatment given to discarded cultures and other materials that have to be sterilised to make them safe, and that accorded to culture media which require to be heated to the minimum temperature and for the time which experience will have shown

to be just adequate to produce an acceptable yet sterile medium. Because of these considerations sterilising performance was expressed as the ratio of the minimum load temperature at the end of the sterilising period to the intended sterilising temperature or to that actually reached in the chamber during the sterilising period. Both possibilities had to be allowed for, because not infrequently either the chamber pressure-gauge or the chamber-drain temperature gauge, or both, were inaccurate. (On one instrument when the chamber pressure gauge read 18 lb psig (0·22 MN/m²)* the chamber-drain temperature gauge read 116°C. The true figures were 15 lb psig (0·2 MN/m²) and 121°C.)

Sterilising cycles were judged satisfactory if the ratio, expressed as a percentage, was above 95%, doubtful if between 90 and 95%, and unsatisfactory if below 90%.

Results

STERILISING PERFORMANCE

Applying the above criteria, 40 operating cycles were considered satisfactory, 12 were doubtful, and 10 were unsatisfactory. The distribution of these results between the two types of autoclaves is shown in Table 1.

Table 1 *Sterilising performance and autoclave type*

Result	*Vertical/Cylindrical*	*Horizontal*	*Total*
Satisfactory	19	21	40
Doubtful	8	4	12
Unsatisfactory	4	6	10

The results were further analysed according to the age of the autoclaves and are shown in Table 2.

Table 2 *Sterilising performance and age of autoclave*

Result	*Age (years)*		
	Up to 5	*5-10*	*Over 10*
Satisfactory	23	7	8
Doubtful	9	1	2
Unsatisfactory	7	2	1

Two instruments, age unknown, have been omitted.

PROBABLE CAUSES OF FAILURE

There was no apparent correlation between the type or age of autoclaves and their sterilising performance. This is further borne out by analysis of the 10 cycles (in 9 autoclaves) judged to be unsatisfactory. Two cycles were for the preparation of culture media and eight were 'sterilising' cycles to render discarded material safe. The causes of failure appear frequently to lie not in the autoclaves themselves but in other factors. These are listed in Table 3, in which the temperature reached is shown as a percentage of the temperature intended for the sterilisation of discarded materials.

Table 3 *Probable causes of failure in eight cycles for sterilising discard loads*

Probable cause of failure	*Temperature reached as a percentage of that intended*
Container	
1 Overlarge containers	81
2 Plastic bags impervious to steam	85
3 Lids on containers	73
4 Occlusion of container opening by other loads	85
Load	
5 Water added to container	89
6 Homogenous load of small tissue culture tubes	61
Other factors	
7 Overfast chamber heat-up	88
8 Inadequate air removal from autoclave chamber	78

Only in examples 7 and 8 in Table 3 could failure be ascribed to the autoclaves, and in one of these it was its installation rather than the instrument itself which was at fault. High pressure steam (110 lb psig; 0·8 MN/m²) was supplied to this autoclave, the outlet of which had been fitted with a pipe bypassing the trap in the chamber-drain. Consequently temperatures rose rapidly in the chamber but not in the load (Rubbo and Gardner, 1965). At the end of the sterilising period, when the chamber temperature had reached 132°C, the load temperature was only 117°C.

The attainment of this temperature in a load which then had to cool slowly might not be regarded as evidence of unsatisfactory performance in sterilising a load of discarded cultures, and consequently the method of judging sterilising performance might be considered to be unrealistic. However, selecting load temperaures at the finish of the sterilising period as the criterion for evaluating the process provided, on the whole, a generous assessment of it, and, furthermore, the operators and their supervisors were unaware—and without the essential thermometric equipment could not have made themselves aware—of the actual temperatures attained. Thus when a difficult load (example 6 in Table 3) was autoclaved in the same instrument, this, combined with the over-rapid heating of the chamber, resulted in a maximum load temperature of only 78°C.

COOLING

The results given in Table 4 indicate that cooling of

*10^5 N/m² (or pascals) = 1 Bar = 14·7 lb per square inch

Table 4 *Cooling of autoclave loads in 60 operating cycles*

Cooling load temperature on opening (°C)	*Vertical/Cylindrical*	*Horizontal*
Satisfactory (80 or less)	4*	4*
81-90	3	0
91-100	17	21
> 100	5	6
Total	29	31

*Includes one cycle in which the load temperature did not exceed 80°C during the sterilising period

most of the loads was inadequate—a not unexpected finding in view of previous observations (Everall and Morris, 1975). In only eight of 60 cycles were loads at the recommended temperatures of 80°C or below when the autoclaves were opened (Department of Health and Social Security and Welsh Office, 1972). In two of the eight the load temperature had not risen to 80°C during the whole 'sterilising' period.

As will be seen from Table 4, there was little difference in the adequacy of cooling between horizontal and vertical models.

Discussion

Most of the causes of failure in sterilising performance should be well known as they are reasonably well documented. However, the concept of the 'difficult' load is one which may not be widely appreciated. As the volume of laboratory work has increased and become more specialised, discarded materials requiring sterilisation have tended to become more homogenous. The loads for sterilisation from a virus laboratory may contain little but tissue-culture tubes and rubber bungs or loads may consist of large numbers of bijou (7-ml) bottles and little else. It is easy to show that air removal is more difficult from homogenous loads of small individual articles than from mixed loads containing larger items. Although this is not surprising, it is not well recognised and should be made known more widely.

In 11 of 62 cycles autoclaves were opened with load temperatures above 100°C (Table 4). Should some of the bottles making up the loads have had tight caps or have been tightly sealed in some other way, there would have been a real risk of explosion. One laboratory recorded a temperature of 113°C in 2½-litre bottles of water on opening its autoclave. At such temperatures, and depending on the head space above the fluid, the pressure inside a sealed bottle will certainly be in excess of two atmospheres (0·2 MN/m²).

Conclusions

Working standards for holding times and temperatures should be ascertained by means of thermocouples distributed throughout a typical load. Only in this way is it possible to determine the length of time taken to reach sterilising temperatures and on cooling to have reached a temperature of less than 80 C before opening the chamber.

Until laboratory autoclaves are equipped with a means for accelerating the cooling of loads it is difficult to offer any advice on the unloading hazard other than that already given in some interim advisory notes by this Subcommittee on the safe and efficient use of laboratory autoclaves, in which the factors influencing the performance of autoclaves in sterilising discarded materials are also considered. These notes are summarised in the Appendix.

The Subcommittee thanks those laboratory directors who kindly supplied details of the observations made on autoclaves in their laboratories and is particularly indebted to two of its members, Mr P. H. Everall, Public Health Laboratory, Shrewsbury, and Mr M. W. Scruton, Public Health Laboratory, Coventry, for many of the temperature measurements made during the survey and for the preparation of the original interim advisory notes (summarised in the Appendix).

APPENDIX

Safe and efficient use of laboratory autoclaves: summary of advisory notes

These notes are intended to supplement the advice given elsewhere (Department of Health and Social Security and Welsh Office, 1972; Collins *et al.*, 1974) and do not describe the detailed operation of an autoclave. The three main hazards considered here are: (*a*) failure to sterilise; (*b*) the pressure vessel hazard; and (*c*) the unloading hazard.

FAILURE TO STERILISE (the making safe of infected materials)

The commonly used holding times and temperatures are: 126°C for not less than 10 min; and 121°C for not less than 15 min. Other times and temperatures sometimes used are 134°C for not less than 3 min and 115°C for not less than 25 min. The times do not include the penetration or heating up time, which will vary with the physical characteristics of the load and which can be determined only by sensing the load temperatures.

Current practice is to measure the holding period from the moment the chamber drain thermometer

indicates phase boundary steam, that is, removal of air as shown by correspondence between temperature and pressure, but there is frequently no direct correlation between chamber drain thermometer readings and load temperature in the early stages of the cycle.

The rate at which air is displaced by steam is affected (among other things) by (*a*) the method by which steam is supplied to the chamber, and (*b*) the size, shape, and material of the containers.

STEAM SUPPLY

1 *Autoclaves (vertical and horizontal) with independent steam supply*

Maximum turbulence should be produced during the air removal stage, the duration of which should be determined by temperature measurements in typical loads.

2 *Autoclaves with steam generated by heating water within the chamber (the pressure cooker principle)*

Sufficient time must be allowed for the temperature of the load to reach the temperature indicated by the autoclave thermometer during the free-steaming process before the outlet is closed and the chamber is brought up to the desired pressure.

THE CONTAINER

The deeper the container, the longer will be the time needed for removal of air from it. Perforated and wire mesh containers are not suitable for discarded cultures since their potentially infectious contents may leak before reaching the autoclave. Molten agar may also escape within the autoclave and, on cooling, block the pipework.

Experimental results suggest that, rather than hastening air removal, the addition of water to a container prolongs the heat penetration time (Gillespie and Gibbons, 1975).

Working standards for holding times and temperatures should be ascertained by means of thermocouples distributed throughout a typical load. Only in this way is it possible to determine the time taken to reach sterilising temperatures.

STERILISATION INDICATORS

Chemical indicators should be used only with full knowledge of their limitations. Only when they indicate failure can the results be relied upon; a 'pass' does not necessarily indicate that sterilising conditions were maintained. If used, they should be placed about one-third of the way up a discard container.

Autoclave tape conspicuously positioned, for example, across the top of a discard bucket, is recommended solely as a check on whether the container has been subjected to the autoclaving process. A colour change in the tape does not indicate that the contents of the container have been satisfactorily disinfected.

Spore papers are not recommended for routine testing of laboratory autoclaves.

THE PRESSURE VESSEL HAZARD

'*When the door is opened at the end of the cycle there may be residual pressure in the autoclave which is not detectable by the pressure gauge*' (Department of Employment, 1974). Most modern autoclaves have interlocking safety devices on their doors (or lids). Even if such devices are fitted, it is important before using the autoclave to:

1 Inspect for any visible damage to the door seal.
2 Check the load to ensure that all caps are loose on screw-capped bottles and that there is a generous headspace (not less than 1/3) above the liquid in each bottle. Remove any cracked or otherwise defective bottles.
3 If the inclusion of sealed containers is unavoidable or if the load is unusually large or consists of large unit volumes, display a notice that special care is necessary on this account.
4 Close the door in the approved manner (if necessary referring to the written operating procedure, which should be displayed in the same room) and do not misuse the closing gear.
5 Check that the door is properly closed.
6 Make no attempt to override any safety mechanism.

At the end of the run and *before* attempting to open the autoclave door:

1 Ensure that the main steam inlet valve is closed (or its equivalent if steam has to be generated on the site).
2 Open the air break valve and the exhaust valve.

THE UNLOADING HAZARD

'*An autoclave must never be opened until the atmospheric pressure is reached in the chamber, the vent is open and sufficient time has elapsed for the contents to be at a safe temperature*' (Department of Health and Social Security and Welsh Office, 1972). Most laboratory autoclaves, however, are not equipped with means of measuring the load temperature. On no account should the reading of a chamber drain thermometer during the cooling period be taken to indicate load temperature. Past experience will have taught the user the necessary cooling times for various loads and, until load temperatures can be measured, such experience must remain the best guide.

To minimise the risk from bottle explosion, apart

from the precautions described under pressure vessel hazard (*b*) above, the following should be observed:

1 Wear a safety visor when opening the door (if possible, the door should be only 'cracked' open ($\frac{1}{2}$ to $1\frac{1}{2}$ in.; 12 to 36 mm) and the autoclave left for 15 min to accelerate the further cooling of the load before the actual unloading). During these operations the door should, as far as possible, be kept between the person of the operator and the chamber contents.
2 Wear insulated gauntlet gloves and a visor when unloading.
3 Avoid mechanical and thermal shocks (from cold, draught, etc) to the load.
4 When possible leave large loads, or loads made up of large unit volumes, overnight in a locked autoclave to cool.
5 Use containers for culture media as small as compatible with efficiency and convenience in use.

References

Collins, C. H., Hartley, E. G., and Pilsworth, R. (1974). *The Prevention of Laboratory Acquired Infection* (Public Health Laboratory Service Monograph Series, No. 6), p. 35. HMSO, London.

Department of Employment (1974). *Safety at Quick-Opening and Other Doors of Autoclaves* (Technical Data Note, 46), p. 2. HMSO, London.

Department of Health and Social Security and Welsh Office (1972). *Safety in Pathology Laboratories*, p. 11. Department of Health and Social Security, London, and Welsh Office, Cardiff.

Everall, P. H., and Morris, C. A. (1975). Some observations on cooling in laboratory autoclaves. *Journal of Clinical Pathology*, **28**, 664-669.

Gillespie, E. H., and Gibbons, S. A. (1975). Autoclaves and their dangers and safety in laboratories. *Journal of Hygiene*, **75**, 475-487.

Rubbo, S. D., and Gardner, J. F. (1965). *A Review of Sterilization and Disinfection as applied to Medical, Industrial and Laboratory Practice*, p.221. Lloyd Luke, London.