Biological monitoring of sterilizers and sterilization failures in Norwegian dental offices in 1985 and 1996

Nils Skaug, Egil Lingaas, Øyunn Nielsen and Charles John Palenik

Laboratory of Oral Microbiology, University of Bergen School of Dentistry, Bergen, Norway;
Sterilization Monitoring Service, Department for Hospital Hygiene, Rikshospitalet, Oslo, Norway;
Infection Control Research & Services, Indiana University School of Dentistry, Indianapolis, Indiana, USA

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It is essential that dental office sterilizers be regularly challenged with biological indicators (BIs) in order to prove that the test spores are being killed during sterilization. The aims of the study were to biologically monitor Norwegian dental office sterilizers and to identify factors contributing to sterilization failure. In 1985, participants received a packet containing: (i) 4 BI units; (ii) a set of instructions; (iii) a questionnaire concerning operation (including biological monitoring) of the office sterilizer(s), and (iv) a return-address envelope. In 1996, offices were sent (i) a survey which included demographic questions and inquiries concerning instrument sterilization processes; (ii) 2 sets of 3 BI units with instructions for their use on 2 different days; (iii) 1 control BI unit that was not to be processed, and (iv) a return-address envelope. Both private and public offices participated. Response rate to the 1996 study was 60%, which was 9.1% of all dental offices in Norway. Testing results indicated a 6.3% overall sterilization failure rate. Three out of 163 steam autoclaves (SAs) (1.8% of total) and 14 out of 109 dry heat (DH) ovens (12.8% of total) failed. DH ovens were over 7 times more likely to fail BI testing than were SAs (χ^2, $P < 0.01$). Demographic or hygiene procedural factors could not be correlated to sterilization performance (χ^2, $P > 0.05$). The failure rate for SAs ($n = 216$) in 1985 was almost 5 times greater than in 1996 (8.8% vs 1.8%). Improvement in sterilizer performance during the decade may be related to issuance in 1986 of Norway's 1st infection control guidelines for dentistry and greater awareness of infection control practices and/or to increases over the previous 10 years in the number of postgraduate courses offered in infection control. The current Norwegian guidelines on infection control practices in public health services, including dentistry, recommend regular biological monitoring of sterilizers without specifying how often. There is a lack of information among Norwegian dentists as to how frequently dental office sterilizers should be regularly monitored by BI. □ *Asepsis; biological indicators; dentistry; infection control; spore testing*

Nils Skaug, Laboratory of Oral Microbiology, Armauer Hansen Building, N-5021 Bergen, Norway. Tel. +47 55 97 46 53, fax. +47 55 97 46 89, e-mail. Nils.Skaug@odont.uib.no

Sterilization of orally soiled instruments between patients is one of the most important infection control procedures performed by a dental office/clinic. The recommended procedure involves the application of heat. The 2 most common types of office sterilizers worldwide are the gravity steam autoclave (SA) and the dry heat (DH) oven. In some areas, unsaturated chemical vapor and ethylene oxide gas sterilizers are more widely used. In Scandinavia, prevacuum autoclaves are increasing in number. Each method of sterilization has operational advantages as well as some disadvantages. Today, the SA is the most common type of sterilizer found in dental offices/clinics.

Routine sterilization of reusable instruments has become the expected behavior in many countries and is a fundamental aspect of infection control in dentistry. Numerous federal, state, local agencies and professional associations now require or strongly recommend uniform sterilization (1–13).

A major factor that can negatively affect sterilizer effectiveness is operator (procedural) errors such as incorrect loading, use of improper wrapping materials or suboptimal operating intervals and/or temperatures (14, 15). Some sterilizer failures are due solely to mechanical malfunction (16).

In order to increase the chances of sterilization success, treatment processes must be regularly monitored, which involves routine procedures verifying sterilizer effectiveness. Three forms of sterilization monitoring—physical, chemical and biological—should be used. Physical monitoring involves direct observation of a sterilizer in action. This is usually done by monitoring gauges and printouts ensuring the proper temperature and/or pressure. Physical monitoring also measures cycle length and the presence of any abnormal sounds that may indicate a problem. Chemical monitoring includes the use of color-change or other indicators (especially marked packaging materials, labels and autoclave tapes) on the inside and outside of packs, bags, trays or cassettes. Rapid-change (throughput) indicators change color quickly after exposure to certain temperatures, thus helping to prevent the use of non-

Table 1. Representative studies on dental office sterilizer performance worldwide showing biological indicator (BI) failure rates (%)

First author	Year	Country	Studied group	BI failure rates*		
				GSA**	DH**	UCV**
Engelhardt (17)	1976	Germany	All practitioners	11.6		
Simonsen (18)	1979	USA	All practitioners	33.0		
Christensen (19)	1980	USA	All practitioners	13.0		
Skaug (20)	1980	Norway	All practitioners	0.0		
Skaug (21)	1983	Norway	Oral surgeons	22.7	50.0	
Palenik (22)	1986	USA	Endodontists	6.1	26.8	12.5
Skaug (23)	1986	Norway	All practitioners	9.5		
Scheutz (24)	1988	Denmark	All practitioners	4.5		
Gleason (25)	1990	USA	All practitioners¶	9.8	12.0	9.9
Hastreiter (15)	1991	USA	All practitioners¶	3.0	12.0	8.0
Messieha (26)	1991	USA	All practitioners¶	43.0	67.0	47.0
McErlane (27)	1992	Canada	All practitioners	2.3	7.3	4.9
Scoville (28)	1994	USA	All practitioners	2.8	16.3	2.9
Molinari (29)	1994	USA	All practitioners¶	2.8	8.4	3.4
Bancescu (30)	1998	Romania	All practitioners	6.5	1.7	
Burke (31)	1998	UK	All practitioners	1.8		
Dahlén (34)	1998	Sweden	All practitioners	57.9		

* No. of offices and sterilizers evaluated varied greatly.
** GSA = gravity steam autoclave; DH = dry heat oven and UCV = unsaturated chemical vapor sterilizer.
¶ Offices and sterilizers evaluated came from new and current users of a biological monitoring service.
Blank space means sterilizer type not tested.

processed items, which can look similar to processed ones. Some wrapping materials, bags and tapes contain rapid-change indicators that can be used on either the inside or the outside of instrument packages or cassettes. It would be useful if every pack, tray or cassette contained a rapid-change monitor. Slow-change or integrated indicators are multiparameter indicators requiring more than just a specific temperature. Such monitors change at a slower rate, responding to a combination of time and temperature. It is recommended that in each load slow-change monitors be placed inside wrapped instrument packs or in the center of a group of unwrapped instruments for each load (5, 6, 11).

Biological monitoring involves demonstrating the effect of the sterilization process on live bacterial spores, which are more difficult to kill than all the common disease-producing microorganisms. An appropriate biological indicator (BI) is placed within a tray, pack, bag or cassette. The spore challenge may be in the form of a strip, ampoule or vial. The usual sterilization cycle is performed and the BI is retrieved and cultured to determine if the spores were killed. If growth is detected, corrective measures concerning operation or the use of the sterilizer must be taken.

Reports describing BI testing of sterilizers in dental offices began in 1976. A representative collection of such studies is presented in Table 1. All the studies reported SA results, while over half also tested DH ovens. In all cases save Bancescu (32), a much greater failure rate was reported for DH ovens. A non-adequate warm-up period has been commonly blamed for this higher failure rate. The Bancescu study was performed in Bucharest, Romania, where the majority of DH ovens were kept in special centralized sterilization facilities. Sterilization of instruments was their sole function. Conversely, most SAs were in private and public offices that processed instruments in-house.

The aim of this study was to biologically monitor Norwegian dental office sterilizers. Objectives included monitoring office sterilizer performance, comparing results with a study completed in 1985 in Norway, comparing results with similar studies conducted in other locations, and determining if demographic and/or infection control practices affect sterilizer performance.

Material and methods

Test population and study design

The 1985 study. A letter concerning monitoring of SAs using a BI appeared in a 1984 issue of the Norwegian Dental Association Journal. Offices/clinics (practices) were offered free biological monitoring of their SA by returning a completed reply slip. All respondents were sent a package containing (i) 4 BI units, (ii) information how to place one BI unit on top of the instruments at the back, in the middle and in the front of the autoclave, and among the instruments (inside instrument cassettes and instrument packages/on instrument trays), (iii) a form to be filled in for information about the SA (producer, brand name, type, age, frequency of use, sterilization time and temperature, previous BI monitoring) and the number of instruments sterilized at the monitoring, and (iv) a prepaid return

envelope. The 4 BI units and the completed form were returned to the Laboratory of Oral Microbiology in Bergen for incubation and reading of BI units and processing of data.

The 1996 study. Study participants were randomly selected from the national register of dental practices (public and private clinics/offices) in Norway until the desired sample size was reached. This population reflected the distribution of dental practices throughout Norway. Offices were pre-screened by calling dentists and asking about their willingness to participate in the study. Only practices with a working SA and/or a DH oven were sent study materials. Participants received a package containing an introductory letter describing the nature and significance of the study and an invitation to participate. Also included were 2 sets of 3 BI units (spore strips), a single BI unit that was not to be processed and which would serve as a control, and instructions on monitoring. Participants were asked to place 3 BI units within their sterilizer on the 1st day of the given work-week and again on the last day of the same week. BI units were to be placed in the back, middle and front of the sterilizer chamber (inside instrument cassettes and instrument packages/on instrument trays). Finally, the study packages contained a 13-question survey and a return envelope. The self-reporting questionnaire contained demographic inquiries (e.g., number, experience and gender of the dentists, practice location, training) and questions concerning the types of infection control equipment present and the specific infection control procedures used. All participants were assured that their monitoring results and survey responses would be kept confidential and that the results would be published.

Biological indicators

The 1985 study. Attest® No. 1262 BI (3M Company, St. Paul, MN, USA) was used for SA monitoring and Attest® Biological Incubator for Steam Sterilization (3M Company) for BI incubation. BI units that did not become positive (change of color from purple to yellow) after 24 h were incubated for another 24 h. BI units remaining purple counted as negative (spores killed). All BI units with yellow color scored positive (spores not killed). Attest® No. 1262 BI control units (not sterilized) were incubated along with the test (sterilized) units.

The 1996 study. BI units each containing 3.20×10^5 *Bacillus stearothermophilus* spores were used to monitor SAs, and BI units with ca. 1.0×10^6 *Bacillus subtilis* per unit served as the microbial challenge for DH ovens. The *B. stearothermophilus*-containing BI was produced by the Sterilization Monitoring Service, Department of Hospital Hygiene, Rikshospitalet, Oslo (Norway) and the BI with *B. subtilis* spores by the Laboratory of Biological Indicators, Department of Clinical Microbiology, Statens Seruminstitut, Copenhagen (Denmark). Offices were asked to use their normal sterilization processing, including cycle length. The 7 BI units were returned by post to Rikshospitalet in Oslo for culturing. They were aerobically incubated at 37°C (*B. subtilis*) or at 56°C (*B. stearothermophilus*) immediately upon their return. The culture medium was TGY (Bacto Plate Count Agar, Difco Laboratories, USA). Tubes were examined daily for 7 days of incubation. Results were recorded as 'growth' or as 'no growth'. Aliquots from tubes demonstrating microbial growth were subcultured and gram stained.

Information obtained from the survey responses was statistically compared using a χ^2 analysis to a variety of factors. Some included the type of sterilizing equipment present in the practice, demographic delineators, sterilizer operation (e.g. location of BI within the chamber and the test day of the week) and BI results.

Results

The 1985 study. The BI units from 222 monitored SAs were received. Six SAs were discarded because their BI units were crushed ($n = 3$) and/or melted ($n = 3$). Therefore, the study population consisted of 216 SAs, 212 of which were used daily for instrument sterilization. All were gravity autoclaves. Only 13 (6.1%) were routinely monitored. One-hundred-and-ninety-seven of the monitored SAs killed all BI units. The 19 (8.8%) failing SAs killed 0–3 BI test units. All BI control units turned out positive. All but one of the failing SAs were used daily for instrument sterilization. Offices with failing SAs received a letter of information and a new set of BI units for retesting. They were advised that retesting should be performed under conditions as similar as possible to those existing at the initial testing. There was no obvious difference in instrument load between failing and not failing SAs. Of the autoclaves, 30, 13, 21, 12, 32 and 99 were 1, 2, 3, 4, 5 and 6 years old, respectively, while 8 were of unknown age, giving a mean age of 8.4 years for autoclaves with known age.

The 1996 study. Packages were sent to 454 dental offices throughout Norway and 272 offices (59.9%) returned them complete (processed BI units and completed survey). This represents 9.1% of all dental offices in the country. Analyses of the participating offices showed that they were representative of all regions of Norway. There were 475 dentists working in the 272 participating practices (178 female and 297 male). The average period in practice was 20.1 years. Test sterilizers included 163 SAs and 109 DH ovens (Table 2) with mean ages of 6.9 years and 11.3 years, respectively (cf. Table 6). The brand names of the autoclaves shown in the completed questionnaires indicated that very few had prevacuum and none of them pulse evacuation. Of the 272 sterilizers tested, 17 (6.3%) failed to kill at least 1 of the 6 test spore strips. In all cases, the control strips grew upon incubation. Ten offices had a single failure and 2 failed to kill any of the 6 BI units. DH ovens produced significantly higher ($p < 0.0\ 1$) rates of failure than SAs. Only 3 SAs (1.8%) failed, while there were 14 DH oven failures (12.8%). Forty-eight percent of

Table 2. Biological indicator testing results (1996 study)

Sterilizer	Pass	Fail*	Total
Steam autoclaves	160	3	163
Dry heat ovens	95	14	109

* Failure to sterilize all 6 biological indicator units.

the SAs and 36% of the DH ovens had been monitored with BI during the previous 3 years. Location of BI units within the unit chamber and day of testing could not be related ($p > 0.05$) to sterilizer success or failure (Table 3). In all cases, the control BI units grew upon incubation.

All participating practices returned completed surveys. Save sterilizer type and age, no demographic or infection control practice could be correlated ($p > 0.05$) to sterilization success. Survey data that could not be correlated to sterilizer performance are presented in Table 4. Table 5 reports those factors that could be correlated to BI kill ($p < 0.0l$). DH ovens were more likely to fail and were older on average than SAs. Older SAs failed more often than younger ones, while the reverse was true for DH ovens. Offices with failing sterilizers were informed immediately and were provided with additional BI and instructions on improving sterilizer performance.

Discussion

These studies represent the only nationwide sterilization monitoring schemes for dentistry conducted in Norway. Results of biological monitoring of sterilizers in dental practices in a Norwegian county (20) and among oral surgeons in Norway (21) as well as preliminary results of the 1985 study (23) have been published. The 1985 and 1996 studies had comparable numbers of involved practices using SAs. The former study did not evaluate DH ovens. In a 10-year period, SA failure rates decreased almost 5-fold. Improvement may be due to a number of factors. Possibly, the most important factor was the issuance in 1986 of the first Norwegian infection control guidelines for dentistry, which recommended that SAs be spore tested every 3rd month and DH ovens every 6th month. Other factors include a greater general awareness of the importance of infection control, more practices subscribing to a sterilizer monitoring service, and an increasing number of continuing education courses dealing specifically with infection control. Purchase of new equipment and the implementation of different techniques (e.g., chemical monitors, process printouts and new wrapping materials) could also affect sterilizer performance. Another aspect is the relative distribution of SAs and DH ovens in Norwegian dental practices in 1985 and 1996. While DH ovens were predominant in 1985, the SA was the preferred sterilizer in 1996. This is reflected by the lower mean age of SAs in the 1996 study compared to the 1985 study, and the much higher mean age of DH ovens than of SAs (Table 6).

The response rate (60%) to our 1996 study was lower than the mean value of 64% obtained when response rates by dentists to questionnaires were recently assessed (33), but was higher than the 47.7% response rate to a recent sterilizer monitoring study among dentists in the United Kingdom (31). The response rate to our study was in fact low when it is taken into consideration that the study population was dentists who during pre-screening had indicated their interest in participating. We can only speculate on the reasons for this, but it could be that some dentists did not follow up simply because they were already monitoring their sterilizers regularly and therefore judged this as unnecessary, while others who did not monitor by BI may have considered that completing the form and doing the monitoring were too time-consuming and/or too complicated. It could also be that the materials sent had simply been misplaced or lost.

The 1996 study showed that failure rates for SAs and DH ovens were similar to those in studies performed in other countries after 1990 (cf. Table 1). As in most reports, the failure rate for DH ovens was significantly higher ($p < 0.01$) than that of SAs. Although DH oven operation

Table 3. Biological indicator testing failures* by test day and location (1996 study)

	Strip location			
Test day	Front	Middle	Back	Total
First workday of the test week	11	4	6	21
Last workday of the test week	7	3	6	16
Total	18	7	12	37

* Out of a total of 1632 biological indicator units used in the study.

Table 4. Factors not affecting the results of the biological indicator (BI) testing in the 1996 study (χ^2, $P > 0.05$)

Testing on Monday versus Friday
Placement of BI units (front, middle and back)
All demographic factors concerning dentists, staff and the offices
Level of continuing education experience concerning infection control
General infection control procedures
Specific infection control processes concerning instrument sterilization

Table 5. Factors affecting the results of the biological indicator testing in the 1996 study (χ^2, $P < 0.05$)

Dry heat ovens more likely to fail than steam autoclaves
Dry heat ovens were older on average than steam autoclaves
Older steam autoclaves were more likely to fail than younger ones
Younger dry heat ovens were more likely to fail than older ones

Table 6. Comparison of two studies investigating Norwegian dental steam autoclaves

	Sterilization failure rates / mean age of sterilizers	
Year of testing	Steam autoclaves*	Dry heat ovens**
1985	8.8% / 8.4 years	N.E
1996	1.9% / 6.9 years	14.7% / 11.3 years

* No. in 1985 = 177, no. in 1996 = 178; ** No. = 112; N.E. = Not evaluated.

is simple and inexpensive, problems with DH sterilization have been noted previously (22, 26, 28, 29). Because most sterilization failures can be related to human error (14, 15), the greater failure rate could be due to inadequate instrument exposure intervals. Of special concern are sufficient warm-up times, equipment overloading, poor internal air circulation and improper wrapping.

Very recently, Dahlén and Möller (34) published the results of a 1-year BI monitoring of SAs used in dental practice in Gothenburg, Sweden. Their most significant findings were that as many as 57.9% of the gravity SAs ($n = 133$), 11.9% of the prevacuum autoclaves ($n = 42$) and 9.6% of the pulse evacuation autoclaves ($n = 166$) tested did not kill the *B. stearothermophilus* spores. The authors relate these exceptionally high failure rates to insufficient handling or inadequate functioning of the autoclaves and conclude that gravity SAs should be used to sterilize unwrapped instruments but definitely not to sterilize air turbines, handpieces and contra angles.

Information collected from all participants of the 1996 study concerning dental practice demographic data and infection control processes used was compared with sterilizer failure or success. Only 2 factors influenced sterilization: the type of sterilizer used and their age. In general, it is difficult to correlate sterilizer effectiveness with a single personal or practice trait. Although the major causes of sterilization problems are well documented, the application of one such parameter to individual situations may be suspect. In the 1996 study, younger DH ovens were more likely to fail biological monitoring than older units. The opposite pattern was true for SAs. The reason for these results is not obvious because a properly operated and maintained older sterilizer should have a very low failure rate (30). However, in the present study, human operational factors and/or unit maintenance problems could have played important roles in sterilizer performance.

Some BI units of the 1985 study had to be discarded because the polypropylene ampoule had melted during the sterilization. This indicates a much too high sterilization temperature; the melting point of polypropylene being approximately 150°C. BI units with melted polypropylene ampoules after monitoring dental office SAs have been reported (21, 31).

In both studies, failing SAs killed no or only some BIs. This may have been due to varying sterilization conditions in different parts of a sterilization chamber and/or it reflects killtime variation among the BI units used. In our 1996 study, placement of the BI in the sterilizer chamber did not affect the result of the spore testing (cf. Table 4). This also shows that in given cases the number of BIs used will influence the outcome of the monitoring result (pass or fail). In the 1985 study, 4 BI units were used to monitor the SA, while in the 1996 study each office was given 6 BI units, 3 for the 1st workday of the week and the other 3 for the last workday of the week. There is concern about the number of BI units that should be run within a given test load. Some mail-in monitoring services provide 2 BI test units and a control. The most common recommendation is to monitor on 2 different occasions. However, some services ask that both BI units be placed in the same run. There is a movement to use a single BI unit, but with at least weekly monitoring. In fact, some services no longer send control BI units, preferring to perform in-house testing of spore viability. There is some advantage to using more than a single BI unit per sterilization cycle. Over 85% of failures in 1 monitoring service involved 1 positive BI unit and 1 negative unit (Miller 1998, pers. comm.). In a monitoring study of sterilizers used by endodontists, 55% of failures involved 1 failure and 1 successful kill (22). Finally, in a retrospective study of a commercial spore testing service it was found that 48.7% of evaluations using 2 BI units were single-unit positive (35). If both BI units are exposed in the same sterilization cycle but 1 is not killed, this could indicate variability (lack of uniformity) of sterilizer conditions within the unit's chamber (e.g. air pouches, leakage of the door rubber packing or sterilizer overloading). Many factors, including human error or mechanical malfunctioning, could affect killing. These data suggest that there are operational advantages to using more than 1 BI unit per monitoring run.

In the USA and Australia, at least weekly BI testing of dental office sterilizers is recommended (5, 11, 36, 37). For the European Community, such uniform recommendations exist currently only for big (e.g. hospital) sterilizers. The new European SA standard for small autoclaves (e.g. dental office) does not address monitoring of sterility (38). The recently issued Norwegian infection control guidelines for the public health services (2), which replaced the 1986 guidelines for infection control in dentistry, advise regular biological monitoring but, contrary to the 1986 guidelines, do not indicate how frequently. In their 1992 recommendations, the Norwegian Board of Health indicated that hospital sterilizers should be monitored regularly (e.g. every 3rd or 6th month for DH sterilizers and every 2nd month for SA) and always after repair (39). The many requests from Norwegian dentists about how often they should monitor their sterilizer(s) by BI indicate a need for more specific recommendations and information for dentistry in Norway.

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