Steam sterilization of air turbine dental handpieces

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The efficacy of autoclaving high-speed dental handpieces was tested in two types of downward displacement steam sterilizers (instrument autoclaves), commonly used in the dentist's office. Eight series of experiments were performed with various sterilization schedules. Lubrication oils with or without antimicrobial agent were used in four of the series. Each handpiece was inoculated with 1 ml of a suspension containing equal amounts of saliva and spores of *Bacillus stearothermophilus* (approx. 10^6 spores/ml). Neither sterilization at 120–124 °C for 20 min nor at 134–136 °C for 10 min resulted in complete inactivation of the spores in series in which the instruments were tested without oil or with oil containing no antimicrobial agent. However, when the handpieces were lubricated with oil containing isopropanol and formaldehyde and the schedule 134–136 °C/10 min was used, no growth was observed. The results indicate that instrument autoclaves with built-in programs of 120–124 °C/20 min and 134–136 °C/10 min could have insufficient capacity to sterilize lubricated or unlubricated dental handpieces. The use of oils containing an antimicrobial agent can overcome this problem. □ *Autoclaving; instrument care; microbiology; spores*

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Various pathogenic microorganisms may be present in and around the mouth (6). Sterilization of all possible instruments in dental office is therefore advocated. The problem of sterilizing dental handpieces has specifically been discussed (2, 4, 14). The possibilities for sterilizing these instruments are usually limited by the materials of the handpiece construction and the need for a water-repelling oil film (2, 9). Some air turbine handpieces are made to tolerate steam sterilization (autoclaving). Hegna et al. (8) found that handpieces that had been left to dry for various periods of time were not sterile after autoclaving. Other sterilization procedures such as the 'Steritube' method (9), ethylene oxide gas, chemicals, gamma radiation, and dry heat have also been discussed (2, 6, 14). However, the process time for some of these methods is long, the instrument may be damaged, and the chemicals are often tissue irritants (2, 4, 14). Thus, these methods have disadvanages that can be considered as greater than those existing with steam sterilization.

Only limited information is available about suitable temperatures and times for steam sterilization of air turbine dental handpieces (8, 17). It was therefore considered of interest to investigate further various sterilization programs for handpieces, using steam sterilizers (autoclaves) commonly used in the dentist's office.

Materials and methods

Handpieces and lubricating oils

Ten high-speed handpieces (HP) of the ball-bearing type were used (KaVo 'Super Torque'® Turbine Contra-Angle Handpiece 625, Kaltenbach & Voigt, Biberach/Riss, FRG). In a separate study, three low-speed HP of the gear type were also tested ('Micro-Mega' Contra-Angle E-handpieces®, Micro-Mega SA, Besançon, France).

For the high-speed HP, two types of lubricating oil were used, Quick Spray® and Stericlave Spray® (Kaltenbach & Voigt). The latter contains a 5% mixture of isopropanol and formaldehyde. For the low-speed HP, the lubricating oil i-Spray® (Micro-Mega SA) was used. The lubrication was

performed in accordance with the manufacturer's recommendations.

Autoclaves

The study was performed with two downward displacement sterilizers (5) manufactured by Helios, Skärhamn, Sweden. One had fixed sterilization programs (Citomat 16L®) and one an adjustable program (Citomat 200®). In these autoclaves, four standard dental trays can be sterilized at the same time.

Saliva–spore suspension

A spore suspension containing approximately 10^6 spores/ml of *Bacillus stearothermophilus* was used in all series of experiments. In Scandinavia this type of spore is recommended for control of steam sterilizers (1). The suspension was kindly provided by Dr. Ingmar Juhlin, Department of Medical Microbiology, Malmö General Hospital, Malmö, Sweden. Before every test, the suspension was concentrated to double strength by centrifugation. Five tenths milliliter of the concentrated suspension was mixed with 0.5 ml freshly collected human whole saliva. The number of colony-forming units (CFU) in the saliva–spore suspension was estimated on brain–heart infusion agar (BHI agar; E 418, Difco Laboratories, Detroit, Mich., USA) incubated at 57°C for 4 days. There were never less than 10^6 spores/ml in any test.

Experimental procedure

The ten high-speed HP were disassembled as described in Fig. 1. The components, placed in a nylon foil bag, were autoclaved in a high-vacuum steam sterilizer (127°C; total sterilization time, 20 min; System Corona, CTC Ltd, Gothenburg, Sweden). After sterilization, all procedures were performed in a horizontal laminar flow workbench. Nine HP were contaminated with 1 ml of the saliva–spore suspension at eight locations (Fig. 1). They were immediately assembled and stored in room temperature for 30 min. One of the HP was not treated any further

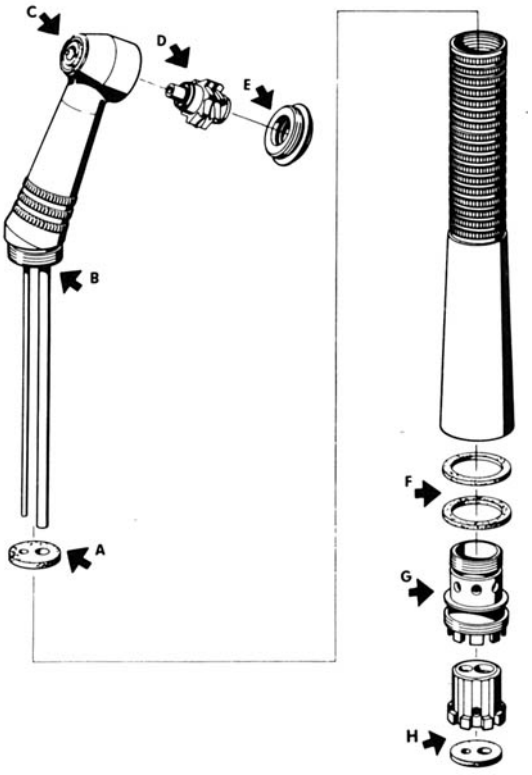

Fig. 1. A disassembled high-speed handpiece (KaVo 'Super Torque' Turbine Contra Angle No. 625), showing the eight locations (A–H) where contamination with the saliva–spore suspension was done.

and constituted the 'positive' control. Another HP was assembled without contamination with the saliva–spore suspension and constituted the 'negative' control.

The handpieces, except for the positive control, were placed on an open tray made of stainless steel, which was placed in the middle of the autoclave. Two trays, each filled with 2.5 kg of metal instruments, were also placed in the autoclave, one above and one below the tray containing the HP. The temperature was measured with two thermoelectric elements (Type TE7, Ellab Instruments, Copenhagen, Denmark). The first was placed inside one of the HP and the second in the cassette with metal instruments below the tray with the HP.

In two of the experiments, the safety margin for sterility was calculated using the so-called D-value. The death of microorganisms

under adverse influence can be given in terms of number of surviving organisms in relation to time. The time at which there are 10% surviving organisms or 90% reduction of the original number has been defined as the decimal reduction time of the D-value (15). The D-value was calculated by the method described by Stumbo et al. (16). The temperature registered inside the HP was used in these analyses.

After the autoclaving all ten HP were disassembled as described in Fig. 1. Each HP was placed in three separate test tubes containing Clausen Medium (dithionite-thioglycollate broth; HS-T broth; CM 353 Oxoid Ltd, England). Rubber and felt packings were placed in a test tube with 5 ml broth, smaller metal components in a tube with 10 ml broth, and larger metal components in a tube with 135 ml broth. The three test tubes were shaken for 15 min to release the spores. The components were then transferred to three new test tubes containing the same quantities of broth. All the test tubes were incubated at 57°C for 5 days and observed daily for growth. Growth in one of the six test tubes was considered a 'positive' result. To verify the presence of *Bacillus stearothermophilus* in the tubes with growth, material from the tubes was transferred to BHI agar and incubated at 57°C for 4 days, after which representative colonies were gram-stained. To detect any contaminating organisms, material from these tubes was also cultured on blood agar (10) and on *Staphylococcus* Medium 110 (B 297, Difco Lab.) incubated aerobically at 37°C for 4 and 2 days, respectively.

The experiments were divided into eight series on the basis of the sterilization process (Table 1). Each series consisted of five tests. In each series 40 HP (5 tests × 8 HP) plus 5 positive and 5 negative controls were tested, with the exception of series 8 and series 5, in which 80 HP (10 tests × 8 HP) and 55 HP (40, 5 × 8, of type KaVo, and 15, 5 × 3, of type Micro-Mega), respectively, were used.

Antimicrobial effect of the lubrication oils

Suitable dilutions of a *Bacillus stearothermophilus* spore suspension were inoculated on BHI agar. Filter paper discs were soaked with the three lubrication oils. Half of these paper discs were autoclaved before the test. They were placed on the inoculated BHI agar, which had been kept at room temperature for 3 h (prediffusion time) before incubation at 58°C for 4 days. The plates were examined daily. No zones of inhibition were found in any of the tests with the Quick Spray and the i-Spray. When the Stericlave Spray was studied, zones of inhibition appeared around the non-autoclaved oil-soaked discs but not around those discs that had been autoclaved before the test.

Table 1. Inactivation of *Bacillus stearothermophilus* spores in two types of dental handpieces with and without lubrication at different sterilization temperatures and times in two types of autoclaves

Handpiece type	Series	Autoclave	Lubrication oil	Sterilization schedule*		No. of handpieces	
				Temp. (°C)	Time (min)	Tested	With growth
KaVo	1	200		120–124	15	40	3
	2	16L		120–124	20	40	2
	3	16L		134–136	10	40	1
	4	200		134–136	15	40	0
	5†	200	Quick Spray	134–136	10	40	3
	6	200	Quick Spray	134–136	15	40	1
	7	200	Quick Spray	134–136	20	40	0
	8	16L	Stericlave Spray	134–136	10	80	0
Micro-Mega	5†	16L	i-Spray	134–136	10	15	2

* According to the manufacturer's description.
† Two types of handpieces were tested.

In separate tests with the Stericlave Spray, 0.01 M histidine was added to the BHI agar as an antidote (11). No zones of inhibition were observed. Therefore, in the series in which the HP were lubricated with the Stericlave Spray, 0.01 M histidine was added to the HS-T broth.

Results

The results of all series are given in Table 1. When the autoclave process was carried out at 120–124 °C during 15 min, growth was observed from 3 of the 40 HP (series 1). Neither sterilization at 120–124 °C for 20 min nor at 134–136 °C for 10 min resulted in a complete inactivation of the spores (series 2 and 3). A sterilization time of 15 min at 134–136 °C inactivated all the spores (series 4).

In the series in which the HP were lubricated, only the sterilization temperature 134–136 °C was investigated (series 5–8). When the Quick Spray was used, a sterilization time of 20 min (series 7) but not 15 min (series 6) gave complete inactivation. On the other hand, when the Stericlave Spray was used, 10 min was enough to inactivate all spores (series 8).

Growth appeared in 2 of the total of 15 Micro-Mega HP lubricated with the i-Spray and autoclaved at 134–136 °C for 10 min (series 5).

In the 'positive' controls, growth always appeared after 1–2 days' incubation, and the verifying tests showed the presence of gram-positive rods growing on BHI agar at 57 °C but failure to grow on blood agar and 110 agar at 37 °C. The same pattern of characteristics was also observed in all positive tests. In the 'negative' controls, growth never appeared.

Discussion

Besford (2) showed that the inside of handpieces becomes saliva-contaminated when used. Therefore, to imitate the clinical situation, the handpieces were contaminated at various locations on the instrument with the saliva–spore suspension. One million spores were used, since this number is recommended for control of autoclaves (1). On the other hand, 10^6 microorganisms are generally in excess of the number of bacteria existing on relatively clean hospital supplies, where usually less than 10^3 microorganisms per instrument are found (5). However, the external and internal configurations of handpieces hold many debris-retaining foci (13). It may therefore be justified to use a relatively high number of spores when testing this type of instrument.

The handpieces used in the present investigation contain various types of metals—for example, in the solderings. Metal ions might be released to the broth media during the incubation, which could have a bacteriostatic effect. It was therefore considered that the HS-T broth should be used. This medium is suggested in the *Pharmacopoea Nordica* (18) as a control medium for microbial-contamination testing (3). Since a tryptone-glucose-yeast extract broth is commonly used for the spore germination of *Bacillus stearothermophilus*, a separate study was performed, which showed that the spore germination was similar in the tryptone and the HS-T broth. To decrease further the possibility of a bacteriostatic effect of ions, all the components of the HP were shaken in HS-T broth and then transferred to another tube with fresh broth. Analysis of the broth with and without the components of the HP revealed a random distribution of growth. Thus, any metal ions in the broth did not seem to interfere with the germination of the spores.

Commonly used autoclaving schedules for downward displacement sterilizers are 121 °C/20 min and 134 °C/10 min. Such sterilization procedures (series 1–3) were not sufficient to inactivate all spores. However, when the sterilization time was increased (series 4), no growth was obtained in any of the test tubes. The results may indicate that the configuration of the HP, which includes components fitting to each other with precision, may impede penetration of the steam.

When series 3 was compared with series 5 and series 4 with series 6, in which the sterilization procedures were the same, it is

Table 2. Calculation of the safety margin for sterility by using the D-value (decimal reduction time)

Series	Sterilization time of the HP ≥134°C* (min)	Calculated D-value†	Calculated time for reaching the safety margin (min)
5	8	1.12 } mean, 1.28	13–14 } mean, 15–16
6	11	1.43 }	17–18 }

* Temperature measured within the HP using thermoelectric elements.
† According to Stumbo et al. (16).

obvious that the lubrication oil impedes the penetration of the steam. The findings are in agreement with previous observations (7, 12). Inactivation of all spores in the lubricated HP was observed first if a sterilization time of 20 min at 134–136°C was used. This is in contrast to the results reported by Hegna et al. (8) and Wirthlin et al. (17), who found that standard sterilization procedures were efficient. The difference in results may at least partly be explained by differences in methods and numbers of HP tested.

Within series 5, a few gear-type HP (Micro-Mega) were also tested. The HP were lubricated with the i-Spray, which did not contain any antimicrobial agent. The result was in line with that for the high-speed HP of the same series. Thus, for gear-type HP too, the usual sterilization and lubrication procedures seem not to be 'safe'. A suitable oil containing antimicrobial agent is not at present available for this type of HP.

In Scandinavia, the safety margin for sterility is expressed in the following manner: 'After sterilization there should be a very low probability that the articles in question contain more than one cultivable microorganism per million units' (1, 19). The safety margin was calculated with the so-called D-value, as described by Stumbo et al. (16). This was done by using the results in series 5 and 6. The analysis is presented in Table 2. The mean D-value for the two series was 1.28. The number of viable spores initially present was approximately 10^6. The calculated risk of finding only one non-sterile HP among 1 million HP should therefore be reached when the sterilization time approaches 15–16 min. Thus, when the 134–136°C/20 min schedule as performed in series 7 is used, the safety margin is passed.

No growth appeared on any of the 80 HP tested in series 8 when the instruments were lubricated with an oil containing isopropanol and formaldehyde. It was therefore considered of less interest to run further sterilization schedules. This excludes the possibility of calculating the D-value. However, on the basis of the number of HP tested, it might be concluded that efficient results should be obtained with ordinary sterilization schedules—in this case 134–136°C/10 min—if oils containing suitable antimicrobial agents are used.

In conclusion, the results confirm previous observations that lubrication oil impedes steam sterilization of dental handpieces. The usual autoclaving schedules for downward displacement sterilizers with fixed programs, commonly used in the dentist's office, were not sufficiently effective to give a probability of 10^{-6} or less of surviving microorganisms being present. The problem may be overcome if suitable antimicrobial agents such as alcohols and formaldehyde are included in the oil blown into the handpieces before autoclaving.

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