

**Keywords***Handpieces**Infection control**Cleaning procedures**EN 15883**DAC**Assistina***Martina Schmid-Schwap<sup>1\*</sup>, Margit Bristela<sup>1</sup>, Alexander Franz<sup>2</sup>, Michael Kundi<sup>3</sup>, Eva Piehlsinger<sup>1</sup>, Friedrich Stauffer<sup>4</sup>**<sup>1</sup> Department of Prosthodontics, Bernhard Gottlieb University Clinic of Dentistry, Medical University of Vienna, Austria<sup>2</sup> Bernhard Gottlieb University Clinic of Dentistry, Central Research Unit, Medical University of Vienna, Austria<sup>3</sup> Institute Environmental Health, Medical University of Vienna, Austria<sup>4</sup> Bernhard Gottlieb University Clinic of Dentistry, Hospital Hygiene, Medical University of Vienna, Austria

# Evaluation of the cleaning efficacy of instruments for processing of handpieces

## Summary

**Background:** The present study was designed to test the cleaning efficacy of a new dental autoclave (DAC) versus that of rinsing procedures performed by instrumental processing for lubrication (Assistina) of handpieces in experimental settings.

**Methods:** External and internal soiling with sheep blood or protein and cleaning in the DAC was done according to EN 15883. In addition, experimental soiling of air and water channels of handpieces/turbines with albumin and cleaning with DAC and Assistina was performed.

**Results:** After experimental soiling with sheep blood no visible contaminations could be seen. The protein content after experimental soiling of air and water channels of handpieces/turbines with albumin showed differences between Assistina und DAC.

**Conclusion:** The results show, that initial flushing of water/air canals with water before flushing with alcoholic solution is necessary.

Hyg Med 2008; 33 [12]: 518–521.

This is especially true for the internal part of the handpieces. Although this internal part – the gear channel which also requires lubrication - shows no direct contact to the oral cavity, contamination of this part has also been reported [1, 5]. Lubrication of the gear of handpieces is required for mechanical reasons following use in each individual patient. Most automatic instruments for lubrication also provide for simultaneous rinsing of water/air channels in the same process. External cleaning must be done manually in all of these cases. Recently, a new dental autoclave (DAC) device was introduced allowing cleaning, lubricating and sterilisation of dental handpieces in one single step. The present study was designed to test the DAC for the fulfilment of the requirements stipulated by EN 15883 in respect to the cleaning efficacy. The DAC was also tested by an experimental soiling procedure for handpieces, which was established at the Bernhard Gottlieb University Clinic of Dentistry in order to assess the cleaning efficacy of rinsing procedures performed by instrumental processing for lubrication like the Assistina.

## Introduction

Handpieces are in direct contact with the oral cavity of patients and are simultaneously connected to the dental unit by the different lines (e.g. air/water).

Thereby microorganisms of the oral cavity could contaminate not only the outer parts of the handpieces/turbines, but also the inner parts. Contamination of the inner parts could also be caused by the water of the dental unit waterlines. Handpieces have been controversially discussed in their role as vectors of microbial pathogens in dentistry [1,2,3,4].

## Materials and Methods

### Dental autoclave

The Dental autoclave (Nitram Dental Systems, Denmark) DAC Universal dental autoclave can be loaded with 1 to 6 handpieces per turbines and features 3 different reprocessing programs. For experimental soiling according to EN 15883 the program was stopped before sterilization.

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For all other experiments the full cycle with sterilization at 134 °C was used (internally cleaning the instruments with cold water, instrument lubrication, cleaning of outside surface with cold water, cleaning of outside surface with hot water, heating to 134 °C, back-flush – forcing saturated steam into the instruments, sterilization, back-flush, drying).

### Instrumental processing for lubrication

The Assistina 301 plus (W&H Dentalwerke Buermoos GmbH, Austria), an automatic instrument provides lubrication of the internal components of dental handpieces with service oil, and additional rinsing of excess spray water and spray air channels with a solution consisting of an alcohol mixture (1-propanol, ethanol) and flushing with compressed air. The full cycle takes 35 seconds. External cleaning must be performed manually. According to the manufacturer's instructions, this device does not fulfil the requirements of the EN 15883.

### Test soils and methods according to the EN 15883

#### Outside surface

External soiling was performed using heparinised (10 IU/ml) sheep blood (Fa. Acila AG, Germany) spiked with 0.15 ml protamine. On each handpiece (Sirona 1:5, W&H 1:5) and turbine (Sirona) 0.1 ml of the blood was applied and distributed using a hair brush.

The soiled handpieces/turbines were left to rest for 60 minutes at room temperature and then were cleaned in the DAC with a cleaning tablet containing citric acid and sodium carbonate (Nitram Dental Systems, Denmark) added.

One cleaning cycle with 6 handpieces and with 6 turbines and 6 cleaning cycles with one handpiece each were run. Because only the cleaning performance was tested, the cycle was stopped before the sterilization process was started as required by the EN 15883.

Thorough visual inspection was performed after removing the cover loaded with the handpieces. A surface protein test (Pro-Tect, Medisafe GesmbH, Germany) was performed, and results were obtained after 10 minutes.

#### Inner surface

In order to assess the cleaning performance for the internally soiled air and water channels a specific cover (Metal Work A/S, Denmark) was used to allow the attachment of Teflon tubes with a diameter of 2 mm by specific adapters modified at Nitram Dental A/S, Denmark composed of the Material CW614N (CuZn39Pb3 brass partly coated with SnNi (65/35%). These tubes were used instead of metal tubes [6] to allow the visual control of the cleaning effect.

Again, 10 ml of heparinised (10 IU/ml) sheep blood (Fa. Acila AG) were spiked with 0.15 ml protamine and 1 ml each was inserted into each Teflon tube using a sterile syringe.

The Teflon tubes then were dried internally with 4 ml of air. Thereafter, the tubes were left to rest at room temperature for 60 minutes in a horizontal position, followed by cleaning with the adapters in the DAC. For one cycle the DAC was fully loaded with 6 tubes, in a next step 6 runs were performed with a single tube each. Because only the cleaning performance was tested, the cycle was stopped prior to the sterilization process as required by the EN 15883.

After removal of the cover loaded with the Teflon tubes a visual inspection was performed and the tubes were flushed with 10 ml SDS followed by protein determination using the Micro BCATM Protein Assay Kit (Pierce, USA). This test cycle (loading 1 × 6 and 6 × 1 tubes) was repeated with soiling with 10 ml of a protein solution containing 10 g albumin fraction V (derived from bovine serum; Merck, Germany).

#### Experimental "soiling" of water and air channels of handpieces with protein

Prior to the experimental tests all handpieces (W&H 1:5) used for "soiling" were cleaned using disinfectant wipes (Green&Clean WD; Metasys: ethanol, isopropyl alcohol, quaternary ammonium salts, glyoxal) and autoclaved with the DAC Universal.

All rinsing cycles were performed using a sterile adapter fitting to the end which is connected with the dental unit specifically developed for this purpose (W&H) to provide easy access to both channels.

For each rinsing cycle an irrigation solution containing 10 ml sterile 0,9 %

sodium chloride was drawn up using sterile devices (10 ml syringe with mounted needle). The air channel was rinsed first by hand with a velocity of approximately 10 ml/20 s (corresponding to an average pressure 0.59 MPa). Then the water channel was rinsed in exactly the same manner.

The rinsing liquids were collected in test tubes and designated as zero samples. Subsequently, water and air channels of each handpiece were each filled with 10ml of a protein solution containing 10g albumin fraction V (derived from bovine serum; Merck, Germany) using the same devices described above. These protein solutions were allowed to interact for 60 minutes in the channels with the channels in horizontal position. The following approaches were compared:

A1 – Cleaning with sodium chloride (sterile 0.9% NaCl). Samples of the first and the last (fifth) rinsing process were collected for evaluation.

A2 – Cleaning with DAC Universal with full charge (holding 6 handpieces)

A3 – Cleaning with DAC Universal with single charge (holding 1 handpiece)

A4 – Cleaning with Assistina

A5 – Cleaning with sterile 0.9% NaCl and Assistina

A first rinse was performed prior to cleaning with Assistina.

The procedures A1 and A3–A5 were repeated six times each and the eluates were collected for evaluation.

#### Detection of protein content

For the colorimetric detection and quantitative determination of total protein the Micro BCATM (Pierce, USA) Protein Assay Kit (working range 5–250 µg/ml) was used together with an adaptation of the BCA Protein Assay Kit (working range 0.5–20 µg/ml). Based on preliminary tests the detection limit and cut-off for protein content of samples was recalculated using analysis of variance. The cut-off was chosen as the concentration that significantly exceeded that of the control sample by means of Dunnett's tests (data not shown).

For homogenization of eluates an ultrasonic homogenizer (Sonopuls HD 2070; Bandelin Electronic, Germany) was used. Samples were homogenized for 15 s on ice. 1ml of each standard and of each sonicated eluate was transferred into a test tube and mixed with 1ml of working reagent. Tubes were covered

and incubated at 60 °C in a water bath for 60 minutes.

In addition, all tubes were cooled to room temperature. The spectrophotometer was set to 562 nm and the instrument was zeroed on a cuvette filled only with water. Subsequently, the adsorbance of all samples of an experiment was measured within a time period of 10 minutes.

Testing of the outside surfaces for protein residues was performed using Pro-Tect M (Medisafe GesmbH, Germany). Like the BCA test this product is based on the biuret reaction and is well suited for the testing of surfaces on account of its simple handling. [7,8] In a previous evaluation it had been shown to deliver reliable results at residual concentrations of more than 5 µg. [8]

### Testing of cleaning and oil solutions

A dilution series of all cleaning solutions and oils of the DAC and the Assistina system was prepared to detect any possible cross-reaction with the working reagent of the protein assay kit.

### Statistical Methods

Comparisons between different methods and procedures were done using analysis of variance after log transformation of protein concentrations. A posteriori comparisons were done using the Turkey's HSD test. All samples that included cleaning solutions and oils were corrected based on the volume of the solution in the sample. For all statistical procedures p values below 0.05 were considered significant.

## Results

### Cleaning capacity of the DAC Universal after experimental soiling according to EN 15883

Following external soiling with sheep blood with subsequent cleaning in the DAC no visible contamination was detected. The protein test performed did not show any positive reaction. Experimental soiling of Teflon tubes with sheep blood also did not produce any visible contaminations. The tests for proteins after flushing of the Teflon tubes with 1 % SDS after processing with the DAC showed no protein levels above the cut-off of 1.13 µg/mL. Similarly, levels were invariably below cut-off when soiling the Teflon tubes with the protein solution.

### Experimental "soiling" of water and air channels of handpieces/turbines with protein solution

Experimental soiling showed that the protein content after single flushing of air and water channels with an NaCl solution was significantly higher than in the baseline samples (zero samples) ( $p < 0.001$ ). In obvious contrast, it also showed that the protein content determined after five flushings was below or at detection limit. Following direct processing with the Assistina system, the protein content was above baseline values ( $p < 0.001$ ). However, when water and air channels were flushed once with NaCl solution prior to cleaning in the Assistina system, the protein content was below or at detection limit (Figure 1 and 2).

For processing in the DAC no difference was seen between single and full occupancy of the device. The protein content of the channels after processing showed no significant difference to that of the zero samples with both single and full occupancy of the device (Figure 1 and 2).

## Discussion

The DAC was found to meet the general requirement according the EN 15883, in respect to the cleaning efficacy. Moreover, it showed an excellent cleaning performance for the soiling especially developed for handpieces. The importance of cleaning prior to sterilization has been emphasized earlier [9].

In water and air channels of handpieces being processed with an automatic lubrication device (Assistina) the protein concentration was higher with experimental soiling with albumin.

Alcoholic solutions may cause fixation of proteins [10]. This may be the reason for the higher residual protein concentration in the channels upon higher protein load. Therefore, initial flushing with water followed by subsequent disinfection or sterilization of water and air channels as practiced with the DAC would provide for particular safety. This also correlates with the results reported for other studies emphasizing the importance of precleaning prior to sterilization [11] or recommending flushing for reducing microbial load [12].

The reduction of the microbiological load was not tested in this study, but for a safe processing of handpieces between

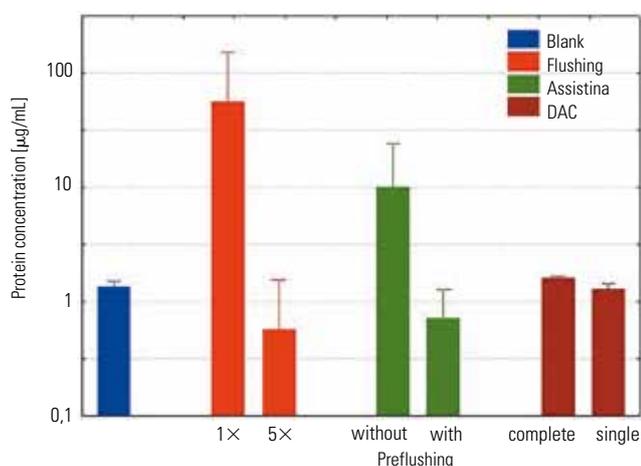


Figure 1: Protein concentration (geometric mean and standard error) of the water channels under the individual conditions (n=6 per measurement).

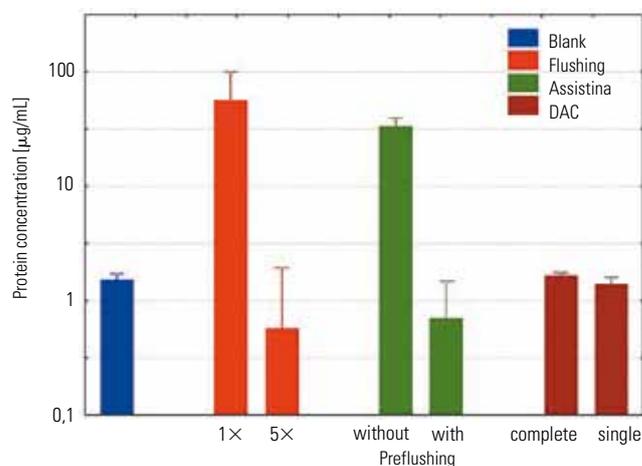


Figure 2: Protein concentration (geometric mean and standard error) of the air channels under the individual conditions (n=6 per measurement).

two patients this reduction is as important as the cleaning performance [13].

## Conclusions

The results show, that initial flushing of water/air canals with water before flushing with alcoholic solution is necessary. The DAC device showed good cleaning performance both with experimental soiling (experimental soiling with protein solution and soiling according to EN 15883).

## Acknowledgement

We acknowledge Eberhard Lorbeer (Department of organic chemistry, Vienna-University) for support with residual oil volume and Andreas Treidl and Daniel Schaffarzick (W&H-Austria) and Mads Hofmann and Gilbert Oliel (Nitram Dental) for technical assistance.

## Conflict of Interest

There are no financial or other relationships potentially causing a conflict of interest with regard to the enclosed paper. The Teflon tubes and the appropriate special covers were kindly provided by Messrs. Nitram for the tests. The rinsing adapter for the handpieces and turbines was provided by Messrs. W&H.

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