Investigating the steam sterilization of dental handpieces

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Abstract

Dental handpieces are used in a wide variety of dental treatment and oral surgery. During patient treatment handpieces becom contaminated with patient material. Due to the design and function of the dental handpieces, internal contamination of handpiece components frequently occurs during use, raising the risk of iatrogenic infection. Dental handpiece lumens represent a challenge for both cleaning and steam sterilization due to limited access. Manufacturers of handpieces and benchtop sterilizers as well as international standards and several guidelines recommend use of a vacuum steam sterilization process for lumen devices; however, non-vacuum is used in many UK dental practices.

Therefore the aim of this thesis was to investigate if benchtop steam sterilization processes commonly used in dental practice are appropriate for sterilizing dental handpieces. Critical variables affecting the outcome of steam sterilization, such as pre-cleaning and lubrication were assessed.

In order to investigate the above stated aim, four research questions were formulated:

1- Investigating steam penetration into dental handpieces and lumens in general (chapter 4), which was approached using thermometric measurements, chemical and biological indicators were used in different handpiece types (high-speed turbines, slow-speed motors, surgical handpieces) and process challenge devices using non-vacuum and vacuum sterilization cycles in a laboratory setting (chapter 4) and in general dental practices (chapter 6).

2- Investigating the effect of pre-cleaning dental handpieces, contaminated with different test soils from the standards or clinical contamination after patient treatment using a washer-disinfector or a handpiece cleaner-lubricator, which was assessed using the o-phtalaldehyde and G-box method (chapter 7).

3- Investigating the effect of handpiece lubricating oil on microbial inactivation by altering different parameters during a steam sterilization process using a BIER/CIER vessel in St. Paul (MN, US) (chapter 5).

4- Investigating the effect of different humidity levels on chemical and biological indicators using a BIER/CIER vessel in Neuss (Germany) (chapters 3).

Thermometric measurements as well as assessment of chemical and biological indicators suggest that not all handpiece types can successfully be sterilized in all non-vacuum benchtop sterilizers. Especially the surgical handpiece appears to be difficult to sterilize. All non-vacuum sterilizers in general dental practice failed to sterilize handpieces. The comparison of the cleaning efficacy of a washer-disinfector and a handpiece cleaner-lubricator showed that a washer-disinfector is more efficient in cleaning the outside of a handpiece. Handpiece lubrication oil appears to impair steam penetration into handpiece lumens. Pre-conditioning in high humidity (90% RH) causes chemical indicators to perform a colour change and indicate successful sterilization quicker than ones pre-conditioned in low humidity (14% RH), which suggests that it is moisture rather than saturated steam that causes chemical indicators to indicate pass conditions.

Non-vacuum sterilization benchtop sterilizers are not adequate for sterilizing dental handpieces. A vacuum process is highly recommended in the interest of patient and staff safety. Chemical and biological indicators are not necessarily reliable and results should be interpreted with care.

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Author's declaration

I declare that, except where explicit reference is made, that this thesis is the result of my own work and has not been submitted partly or in whole for any other degree at the University of Glasgow or any other institution.

Signature:.....

Printed name:.....

Abbreviations

The following abbreviations are used throughout this thesis:

μg	microgram
μM	micromolar
μL	microliter
ACTC	American type culture collection
AWD	automated washer disinfector
BA	blood agar
BBV	blood borne virus
BDA	British Dental Association
BDT	Bowie & Dick test
BfArM	Bundesamt für Arzneimittel und Medizinprodukte
BIER	Biological Indicator Evaluator Resistometer
BS	British Standard
BSA	bovine serum albumin
bsi	British standards institution
$CaCl_2$	Centers for Disease Control
CDU	central decontamination unit
CEN	European Committee for Standardization

CFU	colony forming units
CIER	Chemical Indicator Evaluator Resistometer
cm	centimeter
DCDU	dental central decontamination unit
Dhp	dental handpiece
EN	European Norm
FU	fluorescent units
g	gram
GDH	Glasgow Dental Hospital & School
GDP	general dental practice
h	hours
H_2O	water
HBV	hepatitis B
HIV	human immunodeficiency virus
HTM	Health Technical Memorandum
ISO	International Organization for Standardization
kg	kilogram
LDU	local decontamination unit
M	molar

MDD	Medical Devices Directive
MFU	mean fluorescent units
mg	milligram
min	minutes
ml	millilitre
mm	millimeter
NCG	non-condensable gasses
NCTC	National Collection of Type Cultures
nm	nanometer
OD	optical density
OPA	o-phthaldialedhyde
P	pressure
PCD	process challenge device
PBS	phosphate buffered saline
RKI	Robert Koch-Institut
RFU	relative fluorescent units
rpm	revolutions per minures
SD	stainless steel disc
SDS	sodium dodecyl sulfate

sec	seconds
SHTM	Scottish Health Technical Memorandum
SSD	sterile service department
t	time
temp	temperature
type B	vacuum sterilization
type N	non-vacuum sterilization
type S	special sterilization
UK	United Kingdom
W	Watt

Terms and definitions

Exposure time	time (min) of the load exposed to sterilization temperature
Heat-up time	time (min) in a sterilization process required to reach sterilization temperature
Holding time	time (min) of the load exposed to sterilization temperature
Plateau time	time (min) of the load exposed to sterilization temperature
Sterilization conditions	successful outcome of a sterilization process
Sterilization cycle	total time (min) of the sterilizer required to complete sterilization
Sterilization process	series of actions during a sterilization cycle
Sterilization temperature	134 – 137°C

Chapter 1 – Introduction

1.1 Aim

Dental handpieces are used for a variety of tasks, such as polishing teeth, drilling teeth, bone, and dental implants. Dental handpieces can become contaminated internally and externally with saliva and blood that contain a wide variety of microorganisms. In a study by Lewis et al., where a dye solution was used to simulate patient material, which following use of the handpiece was seen to spread throughout the instrument (Lewis and Boe, 1992). If microorganisms are spread in a similar way this presents a risk for transmission of infection in the dental work place. Therefore the cleaning, disinfection and sterilization of contaminated dental handpiece after every use are essential steps in reducing the risk of cross infection from blood borne viruses (Redd et al., 2007, Lewis et al., 1992, Radcliffe et al., 2013). Dental handpiece have multiple internal components, such as turbines, drive shafts and lumens, which constitute the air channels for driving the turbine and spray channels, which utilize water as a coolant. On the surface of dental handpiece there are grooves for gripping the device while handling, where the cleaning is more difficult than on plain surfaces (Cole et al., 1988, Weightman and Lines, 2004). Cleaning of such devices is difficult to validate because a wide range of factors must be considered that affect the efficacy of chemical disinfectants. These factors include the range and degree of bactericidal activity, as well as variations in pH and concentration of the reactive agent (15883-1:2009+A1:2014, BS EN ISO). The sterilization step is usually performed using steam sterilization (Smith et al., 2009b, 13060:2014, BS EN). However, steam sterilization can adversely affect material that is not thermo-stable, such as plastics or electrical components (13060:2014, BS EN). Many workers have described microbial contamination of dental turbines after use, both prior to sterilization and after sterilization (Andersen et al., 1999, Larsen et al., 1997).

The aim of this study is to investigate the critical factors influencing sterilization of dental handpieces, the effect of pre-sterilization cleaning and different air removal processes to allow steam penetration and factors influencing bacterial survival within the handpiece by means of reviewing the mechanism and kinetics of microbiocidal action during steam sterilization processes, using thermometric,

chemical and biological monitoring methods to identify, evaluate and verify the influencing factors.

1.2 Literature search and evaluation

For the introduction (chapter 1) a selection of books was used (Bacteriology, 1994, Block, 1991, Joan F. Gardner, 1991, Perkins, 1983, Russell, 1982, Russell, 2004, Sandle, 2013) and for section 1.5 and chapters 2-7 a keyword based literature search was conducted on NCBI Pubmed, Ovid MEDLINE, Web of Science and mhp Central Services, which is a journal focusing on decontamination of medical devices (Table 1-1). Central Services journal was additionally subscribed for and each edition was reviewed for relevant articles. Abstracts of search results after keyword search were reviewed and selected or rejected according to relevance for the study and discussed in meetings held twice each month to avoid bias die to selective inclusion (Page et al., 2014). Additionally, references found in books and selected publications were reviewed and relevant publications were obtained on either online or the Glasgow University Library. Decontamination specific standards, European Pharmacopoeia, Medical Devices Directive and guidelines, as well as relevant theses were obtained from the Glasgow University Library or provided by W&H, Austria. Literature written in languages other than English and German was not included.

The books used for the introduction provided a profound and extensive knowledge on the subject and background. References stated in the books, which were considered relevant and obtained dated back to the early 1900. Scientific work found using online keyword search and reference search on relevant publications, books and theses showed consistency across studies in terms of results, presentation and interpretation of data. Although, no published study showed high numbers, replicates and mixed methods compared to the present work, little variation in outcome of presented work was found.

Table 1-1 Keywords used for online literature search online and mhp Central Services

Chapter number	Chapter name	Keywords used in various combinations for online literature search
1 (section 1.5)	Introduction	Dental, decontamination, contamination, sterilization, bacterial contamination, viral contamination, dental unit water lines, handpieces, contra-angles, medical devices, bioburden, infection risk, cross infection
2	Materials & method Validation	Phtaldialdehyde, OPA, Proreveal, culturing bacteria, protein recovery, protein fluorescence methods, artificial saliva, composition saliva
3	Influence of different humidity levels on the sensitivity of chemical and biological indicators for steam sterilization	Chemical indicators, biological indicators, steam sterilization, humidity, dental, handpieces, process challenge device, helix, spores, carrier
4	Investigating steam penetration into dental handpieces using Benchtop sterilization processes in-vitro	Steam penetration, steam sterilization, dental handpieces, contra angles, lumen sterilization, hollow medical devices, sterility assurance level, d value, z value
5	Investigating steam penetration into lumens under controlled conditions	Steam penetration, steam sterilization, dental handpieces, lumen sterilization, hollow medical devices, material, diameter, stainless steel, PTFE, handpiece lubrication, oil, spores, *Geobacillus,* sterility assurance level, d value, z value, F0 value

6	In-vivo study investigating steam sterilization in general dental practice	Dental practice, decontamination, sterilization, steam, handpieces, benchtop sterilizer, survey, maintenance, bioburden, infection risk, cross infection, sterility assurance level, d value, z value, F0 value, integrated lethality
7	Investigating the effect of cleaning on handpiece sterilization	Cleaning, dental, handpiece, disinfection, sterilization, lubrication devices, bioburden, automated washer disinfector

1.3 Principles of thermal inactivation of microbes

Death of a microbial population, like growth, is exponential, or linear when plotted on a logarithmic scale. Inactivation of resistant strains takes longer due to a higher decimal reduction time (D value). The D value is a quantitative expression of the death rate and describes the time (min) required for a bacterial population to be reduced by a factor 10 or 1 log. The z value describes the change in temperature required to increase or decrease the D value by a factor of 10. The F value is used to calculate the integrated lethality of a process needed to kill a population of microorganisms, e.g. 121.1°C, in which case the F value is called F_0 and is the standard method to calculate integrated lethality in steam sterilization. The main areas of application of this theorem are the food industry, the drug industry and the sterilization of medical devices (Block, 1991, Joan F. Gardner, 1991, 17665-1:2006, BS EN ISO).

The equation used to calculate integrated lethality (F_0) is

$$F_0 = t\ (10^{((T-121.1)/z)}),$$

where t is the time interval of points of measurement; T is the temperature at the point of measurement; z is the z-value, assumed to be 10.

Further calculation of log reduction of the microbial population is performed by F_0/D_{121} (Pharmacopoeia, 2014).

The efficacy of an antimicrobial agent is influenced by several factors, such as population size, population composition (endospores, resistant strains, etc.), concentration of an antimicrobial agent, duration of exposure, temperature and local environment (pH, biofilms, etc.)(Block, 1991, Joanne Willey, 2007).

Gram-positive bacteria are more susceptible to antimicrobial treatment than Gram-negatives and spores have a higher heat resistance. There is a difference between viruses, fungi and mycobacteria, due to the variation in the complex cell physiology of these different microorganisms, which is unequal in terms of sensitivity to biocides. However, it is not only the variety of microorganisms that

has an impact on efficacy of biocides, environmental conditions also influence the kinetic process in inactivating microorganisms. One of the most important factors is the concentration of biocide. It is described as the concentration exponent η and is a measure of the effect of changes in concentration of biocide. Although it is easier for a biocide to be lethal when there are few microorganisms present.

Temperature is an influencing factor as well. Boiling and fire have been used for sterilization since the time of the Greeks (Russell, 1982). Environmental pH can have impact on the activity of the biocide in a variety of ways. For example, some substances, such as phenol, benzoic acid, sorbic acid and dehydroacetic acid are effective in an un-ionized stage. Glutaraldehyde is more stable at acid pH, but more effective at alkaline pH. Organic matter, such as serum, blood, food residues etc. can affect the efficacy of the biocide, because the biocide can interact with the organic matter such that less biocide will be left to inactivate microorganisms (Block, 1991, Russell, 2004). According to the Arrhenius equation, inactivation rates increase with increasing temperature. However, the dry heat destruction rate of microbial spores is a function of water content in the cell and the heating time, as it also is in steam. In a range between 100° and 135°C spores of intermediate moisture content are more resistant to the effects of heat (larger D values) than spores with greater or lesser moisture content. Relative humidity is a parameter that has a profound influence on the activity of gaseous disinfectants and steam. Moist heat kills viruses, bacteria and fungi by degrading nucleic acids, disrupting the cell membranes and denaturing enzymes and other essential proteins. Therefore the water content of microbial cells and their surrounding influences the development of heat resistance. Spores of *G. stearothermophilus* are extremely heat resistant. Even under moist heat conditions, their heat resistance is 50,000 times higher than the resistance of certain other spores (e.g. *C. botulinum*). Therefore moist heat sterilization must be carried out above 100°C. Moist heat is a specific condition where saturated steam/dry steam is used (dryness value of 1.0) (Russell, 2004).

1.3.1 Theories and models of microbial death by heat

Heat stress leads to microbial death by affecting the ability of the organisms to reproduce, either by the direct effect on the reproductive mechanisms or by disrupting cellular metabolic systems. An understanding of the mechanisms of microbial death has been derived from two areas of research: (1) research focusing on the molecular level of thermal destruction and (2) research looking at the death of microbial populations and different species under heat stress and different conditions. To develop an assay for microbial destruction a suspension of microorganisms is needed. The suspension can either be heterogeneous or homogenous. Heterogeneous samples contain a variety of different species that vary in their resistance to heat. A survival curve of such mixtures is generally semi logarithmic and shows the quantity of organisms with a lower resistance to heat as well as those with higher resistance. A homogeneous suspension contains a population of a single microbial species. To achieve a reliable result the lethal stress must be uniform, the recovery medium must be optimal for growth and additional environmental factors must be excluded. The data can be treated analytically to give information about the resistance to thermal stress and the rate-of-kill. For determining the effect of heat stress on a homogenous microbial population, aliquots of the suspension are exposed to heat for different periods of time. The number of surviving organisms for each heating time can be plotted as a function of the heating period. There are different ways to plot such data. When both, number of surviving organisms and heating period are plotted on an arithmetic scale, the result is an exponential decay curve, where the number of survivors approaches zero with increasing exposure to heat. However, the number of survivors never reaches zero. This is vital because it states sterility cannot be attained if this theoretical model is assumed. Therefore, a sterility assurance level (SAL) has been introduced. If the number of survivors is plotted on a logarithmic scale and the heating period on an arithmetic scale, the result is a linear decay curve (Bacteriology, 1994).

However, it is difficult to determine the assay parameters mentioned above due to the sensitivity of microorganisms, especially bacterial spores, which are used as a reference microorganism in biological indicators (BI) according to the standards (11138-1:2006, BS EN ISO) to heat as well as other factors, e.g., growth media, pH, incubation temperature, water content of spores, strain

differences, presence of lipids (Bacteriology, 1994, Brown and Gaze, 1988, Senhaji, 1977) (Smith et al., 1982, Guizelini et al., 2012, Kim and Naylor, 1966). Determination of spore resistance to steam is performed in absence of air, due to the fact that this is the most potent form of steam for killing spores. In practice, this is not always true, since steam-air mixtures decrease the germ-killing power severely. Large amounts of residual air in steam prolonged the heat-up time of the sterilization process without necessarily reducing the temperature (Scruton, 1989). Rubner found that steam at 100°C with 0% air killed spores within 3 min, however for killing spores effectively in steam –air mixtures more time was required under the following conditions:

91.6% steam + 8.4% air …….3 minutes

80.0% steam + 20.0% air …10 minutes

63.0% steam + 37% air ……30 minutes

Muntsch performed a study adding air to an autoclave. The test material utilized was soil with native spores. Overall it is shown that an increased volume of air results in not only more spores surviving, but quicker recovery after exposure (Konrich, 1938).

1.4 Sterilization by heat

1.4.1 History of sterilization

Many well-known scientists discovered the basics of infection, contamination and decontamination. Even centuries ago people heated, dried, smoked or salted food to preserve it without knowing the basis of infection, contamination and decontamination. Scientific studies from the 15th and 16th century describe ideas about disease transmission from one person to another (Girilamo Francastro). He thought that contagion was caused by either direct contact, by fomites, or through the air. The use of chemicals for disinfection was first reported in 1676 and the Dutch merchant Anton van Leeuwenhoek, who also manufactured microscopes, was the first person to describe bacteria. He called them “little animals”, which could be killed by vinegar, salt, sugar, wine and other chemical compositions. In 1750, John Pringle found that eggs spoil faster when inoculated with a small amount of already spoiled egg. Harrington and Walker found ethanol solution to be effective against bacteria, but was not able to kill bacterial spores in 1903 (Block, 1991).

Sterilization by heat has its roots in ancient times (Russell, 1982). Fire was used to destroy clothes and corpses of diseased people. The Italian physiologist, Lazzaro Spallanzani, discovered in 1776 that some microorganisms were more heat resistant than others. So he suggested boiling liquids for 1 hour to kill them. This procedure was modified to boiling for 15 minutes and sealing the bottles afterwards, which was applied for preserving food in 1810 by Appert. In 1878, Joseph Lister recommended heating glassware to a temperature of 150°C for two hours to prevent contamination and three years later, Robert Koch reported the use of hot air and steam as sterilizing agents. At the same time, Pasteur discovered the sterilizing effectiveness of superheated steam. According to the findings of Pasteur and Papin, autoclaves for laboratory use were developed. They were named Chamberland’s autoclaves (Block, 1991).

1.4.2 Defining instrument sterility

For a device to be labelled sterile it must go through a validated process. A medical device is sterile, if the theoretical probability of there being a viable microorganism present on or in the device is equal or less than 10^{-6}, i.e., less than < 1 in 10^{6} (556-1:2001, BS EN).

In order to achieve sterilization of a medical device it is important that several key parameters are fulfilled and validated. These parameters are temperature, cycle time and presence of moisture. Pressure and air removal are required to deliver steam to all parts of the load. If one of these parameters fails to work according to these requirements, the process cannot be claimed to be successful. Other factors influencing the choice of sterilization include the physical state of the medical device and the thermal and chemical stability of device materials (13060:2014, BS EN).

What the term “sterile” really means is the absence of all microorganisms. However, as described in section 1.2, the number of surviving microorganisms cannot be zero, in terms of sterilization of medical devices, the term “sterile” describes the probability that a microorganism has survived on a sterilized product and is expressed as the sterility assurance level (SAL) and is required to be 10^{-6}, i.e. a 12 log reduction of the initial population of microorganisms (Allison, 1999, 11138-1:2006, BS EN ISO). For terminal sterilization, the European Pharmacopoeia states that sterilization by saturated steam is the preferred method and has to achieve a SAL of 10^{-6} or better. The SAL can be calculated using the F_0 concept, as described in section 1.2 (Pharmacopoeia, 2014).

1.4.3 Steam sterilization

As described by Perkins (1983), a sterilization process consists of three main phases, the heat-up time, where all surfaces of the load are brought up to sterilization temperature. The heat up time after which the chamber has reached the required temperature is followed by the holding time, which is the time selected for the chamber to hold the sterilization temperature, followed by condensation and drying. In the case of vacuum sterilization, these phases follow active air removal, i.e. vacuum pulses (Perkins, 1983).

The three main steam sterilization processes are: non-vacuum processes for unwrapped solid instruments, vacuum processes for porous and hollow loads and processes especially designed for one particular type of medical device, such as the DAC for dental handpiece sterilization (Sirona) (17665-1:2006, BS EN ISO).

1.4.3.1 Background and historical aspects

This project focused on steam sterilization processes to demonstrate that air removal is important for sterilization to be achieved. Saturated steam can reach outer surfaces and accessible cavities, condensation causes release of energy (latent heat) on the surfaces and provides the biocidal action, therefore, the removal of air from the sterilizer chamber and sterilizer load is important to facilitate steam penetration (Perkins, 1983, Bowie et al., 1963).

The most common sterilization method in hospitals and dental practices is steam sterilization, saturated water vapour, at 134°C is added to or produced in the chamber of the sterilizer. A sterilizer must not be overloaded, in order to provide uniform steam distribution throughout the load (Bartels, 1931). Different methods of air removal are in use, depending on the type of sterilizer. The non-vacuum cycle is the simplest type; air is removed passively by gravity displacement or by the steam pushing the air out through a valve, and is used for un-wrapped solid items (556-1:2001, BS EN). A vacuum cycle removes air actively by using a vacuum pump. It is used for wrapped and non-wrapped solid items, hollow items and porous loads and has therefore the widest application range. A drying stage is included after the sterilization phase, which extends the total cycle time (556-1:2001, BS EN). A vacuum sterilizer is highly recommended

for sterilizing dental handpiece by manufacturers of dental handpiece (such as W&H) as well as manufacturers of sterilizers (W&H, Eschmann, Newmed, Prestige). Type-S-sterilizers, in which sterilization is carried out by steam at increased pressure, remove air in an active way also, but are only suitable for certain types of loads as specified by the sterilizer manufacturer, e.g., DAC (Sirona). This method can only be performed, if the load is thermo stable. This type of sterilization may damage materials such as plastics or electronics. Detailed technical descriptions of the design, construction and controls of modern steam sterilizers are contained in official standard regulations. Moist heat sterilization is defined by time, temperature and presence of moisture required to inactivate *Geobacillus stearothermophilus* (556-1:2001, BS EN). Saturated steam is delivered by pressure and air removal (17665-1:2006, BS EN ISO). If *Geobacillus stearothermophilus* can be successfully inactivated and a SAL of 10^{-6} is achieved, it is assumed, that all other microorganisms will be inactivated as well. For efficient sterilization the recommended parameters are 3 minutes at 134-137°C and 206 kPa pressure (Perkins, 1983, Walbum, 1931, MRC, 1959, 13060:2014, BS EN).

1.4.3.2 Producing steam

Sterilization by moist heat depends on the use of saturated steam above 100°C (121 – 134°C), which is only possible at an increased pressure level, so that the boiling point rises. While the time required for sterilization at 121°C is 15 min, at 134°C 3 min is the time required, where F_0 values are 15 and 40, respectively (Perkins, 1983). Saturated steam is water vapour, free of other gases (e.g., air), that is in equilibrium with water in liquid phase and the dryness of steam has a max value of 1.0. The microbiological efficiency of saturated steam depends on moisture content, heat content and penetration. Superheated steam is considered less potent in terms of microbial inactivation, due to the fact that swelling of protein of the microbes followed by coagulation, is what kills microbes exposed to steam. Superheated steam has greater energy than dry saturated steam. Therefore, it delivers a large amount of latent heat energy to the surface of the load before it can condense, which has to conduct its superheat energy to the surface first. Once this is done, the steam condenses, releasing latent heat of evaporation, rather than condensation, which happens in

dry, saturated steam. Therefore, the load takes longer to heat up when exposed to superheated steam (Konrich, 1938).

Impurities in the steam, like non-condensable gasses (NCG) or air can cause air pockets in the load and can therefore result in a non-sterile load. It has been suggested that the percentage of NCG should not exceed 3.5% in order to ensure sterilization (Scruton, 1989, Spicher et al., 1999, 13060:2014, BS EN). Steam is either produced in an external steam generator, followed by steam injection into the sterilizer chamber or is generated in the sterilizer chamber (285:2006+A2:2009, BS EN, 13060:2014, BS EN). The chamber of a sterilizer is made of metal, mostly stainless steel, constructed to withstand the maximum pressure that is required to raise the temperature of steam to the level required for sterilization (Konrich, 1938).

1.4.3.3 Large steam sterilizers

Large sterilizers are commonly used in hospitals. The standards give requirements in terms of sterilization temperature and time, which are 121°C, 126°C, 134°C +3°C for 15, 10 and 3 min, respectively. The NCG percentage must not exceed 3.5% and water for steam generation should be of “potable” quality. Large sterilizers are required to pass a Bowie Dick test and an air detector function test (if an air detector is fitted) weekly (285:2006+A2:2009, BS EN). Large sterilizers have to undergo daily, weekly, and annual testing (SHTM, 2010).

1.4.3.4 Small steam sterilizers

A small steam sterilizer (benchtop) is built according to the standard BS EN13060, 2014 and is mainly used in dental practices. They have a chamber not exceeding 60 liters. The maximum load depends on the manufacturers’ instructions. Parameters to consider are temperature, time and NCG and are the same as for large sterilizers (see section 1.3.3.3) (13060:2014, BS EN). In case of a vacuum model, a daily porous load (Bowie Dick) or hollow load (helix) test has to be passed and daily, weekly, quarterly and annual tests are required to be performed with success (SHTM, 2010).

1.4.3.5 Steam sterilization of lumens

Especially air driven high-speed turbines are prone to become contaminated internally, due to suction of saliva and patient material in the head of the handpiece when the turbine stops. Researchers found internal contamination throughout the air channels. In case of unsuccessful sterilization, this might pose a risk of cross infection (Checchi et al., 1998, Ojajarvi, 1996). Perkins (1956) established safe minimum standards for steam sterilization of 134°C for 2 minutes for non-vacuum and 1 min for vacuum processes, based on studies using garden soil containing heat resistant spores and bacteria, performed by Walbum (1931) and by Ecker (1937). Perkins (1956) referring to the earlier studies clearly stated that the exposure time for "needles, hollow, individually packed in glass tubes and moist lumens" should be 15 min in non-vacuum and 4 min in vacuum processes (Walbum, 1931, Ecker, 1937). The first Medical Research council (MRC) report (1959) added a one-minute safety margin to the 2 minutes established by Perkins (1956), in case the steam quality was not perfect for non-vacuum processes. Moreover, it is published that the exposure time for lumens in steam sterilizers consists of the time steam reached all surfaces and heats up to 134°C, the holding time (3 min) and the safety period (half the holding time for non-vacuum) (MRC, 1959). It is well known from the literature that steam penetration into lumens gets more and more difficult with decreasing diameter. A critical diameter to assure air displacement by gravity was found to be 0.4 cm. Using active air removal however, Kaiser et al., and de Borchers et al. suggest that wider lumens are harder to penetrate (Kaiser U., 1998, Borchers, 2004). Also the length of the lumen impacts on the sterilization outcome. As van Doornmalen et al. has shown, using infrared density measurements of steam in a dead-ended stainless steel tube (l=54 cm, d=0.5mm), even in a vacuum process the density of steam at the blind end of the tube was insufficient for steam sterilization. However, increasing the number of vacuum pulses increased steam penetration into lumens (J. P. C. M. van Doornmalen, 2013). It was also shown that a lumen in steam sterilization processes, which is open at both ends, behaves exactly like a lumen half the length, which is open at one and closed on the other end (Kaiser U., 1998).

1.4.3.6 Process challenge devices

A PCD is an ”item designed to constitute a defined resistance to a sterilization process and used to assess performance of the process” (11139:2006, ISO/TS). The most common process challenge devices (PCD) for sterilization are the Bowie Dick test for testing steam penetration into porous loads and the helix test, to test steam penetration into hollow loads. Both accommodate a chemical indicator, which changes colour to indicate when sterilization parameters have been achieved (867-5:2001, BS EN). Superheated steam has also been shown to have an effect on chemical indicators. Therefore, an inappropriate colour change can indicate the presence of superheated steam during the sterilization process (Everall et al., 1978).

1.5 Dental Handpieces

There are three main types of handpieces used in dentistry and oral surgery. The high-speed air turbine, which is an air driven handpiece, connected to the dental unit and is mainly used for drilling teeth, the slow-speed handpiece, used for prophylaxis, restorative, orthodontic and endodontic procedures, is driven by a motor, which is connected to the dental unit. Finally the surgical handpiece is a straight, motor-driven handpiece used for oral surgery (www.wh.com). Details and figures below are examples for the handpieces used in this project.

Dental high-speed turbine: Air driven, weight=42 g, three internal lumens (x2 spray channels D=0.9 mm, x1 drive air channel D=2.3 mm), total length 115 mm, speed=360,000 revolutions per minute (rpm) (Figure 1-1).

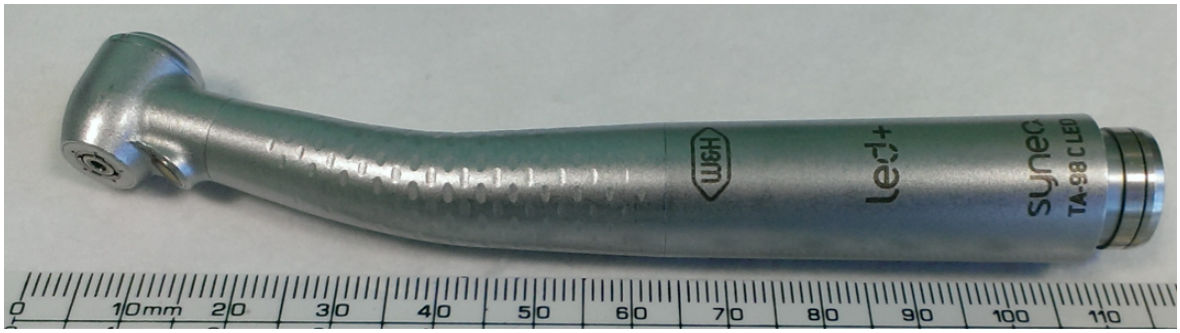

Figure 1-1 Example for dental high-speed turbine (TA-98 C LED, W&H, Austria)

Dental slow-speed motor: Motor driven, weight=66.5 g, two internal lumens (x2 spray channels D=0.9 mm), total length 95 mm, speed=40,000 rpm (Figure 1-2).

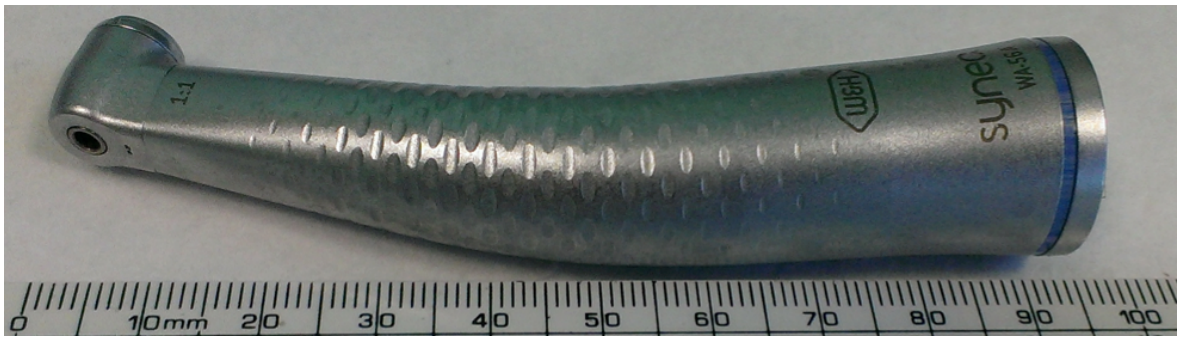

Figure 1-2 Example for slow-speed motor (WA-56, W&H, Austria)

Surgical handpiece: Motor driven, weight=101 g, one external lumen (spray channel D=0.9 mm), total length 115 mm, speed=50,000 rpm, dismantlable (Figure 1-2).

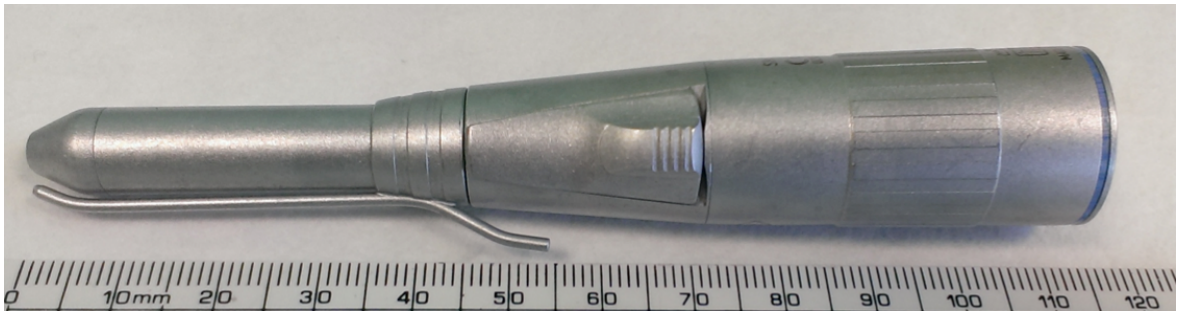

Figure 1-3 Example for surgical handpiece (S11, W&H, Austria)

1.5.1 History of dental handpieces

The history of dental handpieces has been mentioned in several publications describing the history of their development. The first reported rotating engines for dental use were Harrington's clockwork drill and Soper's 'spring motor' in 1864 and 1866, respectively. However, the first water powered rotating engine for one-handed use in dentistry was produced by the S.S. White Company in 1881. Its maximum speed reached 700 rpm, as published in 1944. The problem of heat generation during rotary cutting has long been recognized according to Henschel inn (1946). A handpiece fitted with a system for cooling the cutting instrument by water was in commercial production by 1874. In 1941 John W. Iseman designed a turbine handpiece, supplied with compressed air that was claimed to rotate at 25,000 rpm. Only 8 years later, in 1949 E.J. Steward and his team realized the concept of Sir John Walsh's (American Dental Association) to produce a contra-angled turbine rotor handpiece, driven by compressed air, which achieved a free running speed of 60.000 rpm. The turbine rotor was placed in the turbine head. The successful use of this handpiece was reported in Walsh's Doctoral thesis, submitted in 1950 (University of Melbourne). The device was patented as 'the original dental contra-angle turbine handpiece' in January 1964. Three years later in 1953 Dr Robert Nelson, Pelander and Mr John Kumpula (National Bureau of Standards, Washington) reported and described the construction of a hydraulic turbine contra-angled handpiece with a small turbine rotor in the turbine head, which was driven by water. The water was delivered to the rotor via a flexible tube, which was itself driven by a pump. This experimental device achieved a speed of 60,000 to 70,000 rpm, when free running. The first commercial dental instrument based on the turbine principle was the 'Turbojet', which was fluid driven and reached approximately 75,000 rpm, while free running. Walsh surpassed this speed with his air turbine and it was used for cavity preparation on humans. In the year 1956, the Chayes handpiece was introduced and one year later the Borden handpiece. In 1960 a cord driven handpiece by Borden was introduced; the Borden Airotor. The development of the 'Alston Mini-Head' was reported in 1959. Its head had a diameter of 8.7 mm, a length of 9.7 mm and was cord driven, but it was not in commercial use until 1972. It was named 'Super-Torque'.

Over the decades two main types of air turbine handpieces were introduced; air bearing turbine handpieces and ball bearing turbine handpieces. Air bearing handpieces reach a higher speed, lower torque (tends to produce rotation), to reduce vibration, and need a higher air pressure to be driven. The principle and design were described first by Morrant, Powell and Hargreaves in 1962. These are up until now the basis for all modern air turbine handpieces. However, the ball bearing air turbine handpieces experienced significant development in 1991. Star Dental introduced the LubeFree handpiece, which makes use of ceramic bearings. In the 1980s, the first turbine handpieces with push-button chucks built according to the dimensions of the ISO International Standard were introduced, although this mechanism was first conceived in 1933 (W&H). Dental fibre optics has been in use since the early 1970s. The first illumination units were independent, attachable to mouth mirrors, oral evacuator and other dental instruments. The most significant developments in the history of dental handpieces are summarized in a timetable (Table 1) (Dyson and Darvell, 1993a, Dyson and Darvell, 1993c, Dyson and Darvell, 1993b, Stephens, 1987)

Table 1-2 Summary of milestones in the development of dental handpieces

Year	Invention	Inventor/manufacturer
1864	Clockwork drill	Harrington
1866	Spring motor	Soper
1881	First water driven rotating engine for one-handed used in dentistry	S.S. White Company
1874	Handpiece fitted with a system for cooling the cutting instrument in commercial production	Not specified
1941	Turbine handpiece, supplied with compressed air, 25,000 rpm rotation speed	John W. Iseman
1949	contraangled turbine rotor handpiece, driven by compressed air, free running speed of 60.000 rpm → patent in 1964	E.J. Steward
1953	Hydraulic turbine contra-angled handpiece with a small turbine rotor in the turbine head, driven by water, 60,000 to 70.000 rpm running speed	Dr Robert Nelson, Pelander and Mr John Kumpula
1956	Cord driven handpiece	Chayes
1957	Cord driven handpiece	Borden
1959	'Alston Mini-Head'. Head diameter of 8.7 mm, length of 9.7 mm and cord driven, in commercial use in 1972, named 'Super-Torque'.	Alston
1960	Cord driven handpiece 'Airotor'	Borden
1933	First turbine handpieces with push-button chucks → commercially produced since the 1980s.	W&H
1991	Lubrication free handpiece, use of ceramic bearings	Star Dental

1.5.1.1 History of dental handpiece sterilization

Until the beginning of the 20th century, where the first sterilization methods were used, dental equipment was only cleaned, presumably. One of the earliest reports in sterilizing dental equipment appeared in 1902. Hot air at 200°C was used to sterilize dental equipment. Six years later, dental equipment was sterilized by heating in mineral machine oil to 120-150°C. In 1913 sterilization was performed by removal of the sleeve and by boiling in water. Two years later 0.25% NaOH was added to the hot water (80°C). The water was then removed by alcohol. Another two years later, the application of absolute alcohol with a cotton swab was the favored method, while in 1918 an additional autoclaving step was added. Wiping with a cloth (wet with alcohol) before the use of a dilute phenol solution or boiling in soap solution indicated a differentiation in cleaning and sterilization for the first time. Also immersing in 10% Lysol (disinfectant household cleaner) for 15 min was a common procedure. Boiling in 1% sodium bicarbonate before placing in a test tube with 95% alcohol was a procedure that occurred in 1919. In 1924, dental equipment was completely immersed in mineral oil at 185°C for 5 min and the oil was wiped off with sterile towel (Appleton, 1924). Since the 1950s, vacuum and non-vacuum steam sterilization are considered the most common procedure to sterilize medical devices, including handpieces (Perkins, 1983).

1.6 Background evidence for contamination of handpieces

The problem of dental handpieces becoming contaminated and that they are challenging to decontaminate has been well known since the 1970s, where Pelzner used a weight-load machine to determine contamination on six different high-speed turbines following cutting through tooth structure and after different disinfection or sterilization methods. The results show that dry cutting results in a lower amount of debris compared to wet cutting and that wiping with alcohol does not remove all of the debris and can therefore not be used as a single procedure to decontaminate handpieces (Pelzner et al., 1977). Especially high-speed turbines become contaminated internally, due to retraction of oral fluids when the turbine stops. Therefore, anti-retraction components were included in turbine design (Ozawa et al., 2010).

1.6.1 Bacterial contamination

There are several publications on bacterial contamination of dental handpieces. Dreyer and Hauman (2001) investigated internal contamination of high-speed turbines and found that water channels become more contaminated than air channels and found bacteria even after cleaning, disinfection and lubrication (Dreyer and Hauman, 2001). An in-vitro contamination involving *G. stearothermophilus* spores on slow-speed prophylaxis angles showed that the spores travelled all the way through the handpieces (Chin et al., 2006). Work performed by Herd et al. (2007) demonstrated that 75% of handpiece/prophylaxis angle systems used on patients were contaminated with bacteria (Herd et al., 2007).

1.6.2 Viral contamination

Herpes simplex in-vitro contamination of dental handpiece lumens was carried out with the result that even anti-retraction handpieces were contaminated internally and the necessity of thorough internal cleaning and disinfection was addressed (Epstein et al., 1993, Epstein et al., 1995). After invasive dental treatment on patients, Hepatitis B as well as HIV-DNA was identified on surgical devices, which addresses the risk of blood borne virus (BBV) cross infection

(Zhou et al., 2006). Hu et al. investigated the infection risk of Hepatitis B using anti-retraction turbines and discovered that such devices may reduce the risk but internal contamination is not eliminated (Hu et al., 2007).

1.6.3 Other contaminants

Dental unit waterlines pose a contamination problem as well. Viral contamination (Samaranayake, 1993) as well as a variety of bacteria were found in the water lines of the dental unit (Szymanska and Sitkowska, 2013). It was also mentioned in the literature that the use of an internally contaminated high-speed turbine can result in bacterial mixing with aerosols and contaminating surrounding equipment (Shpuntoff and Shpuntoff, 1993). A more recent study looked at protein contamination of high-speed turbines, slow-speed and surgical handpieces and found the surgical gear from inside the handpiece to be the most contaminated (Smith et al., 2014).

1.7 Legislations, Standards, Guidance

There are several legislations, standards and guidelines, which are relevant for this project.

1.7.1 The European Medical Devices Directive (MDD)

“Any medical device placed on the European market must comply with relevant legislation. Manufacturers' products meeting 'harmonized standards' have a presumption of conformity to the Directive. Products conforming to the Directive must have a CE mark applied. The core legal framework consists of three directives:

- Directive 90/385/EEC regarding active implantable medical devices (AIMD)
- Directive 93/42/EEC regarding medical devices (MDD)
- Directive 98/79/EC regarding in vitro diagnostic medical devices (IVDD)

All medical devices must fulfil the essential requirements set out in the above-mentioned Directives. Where available, relevant standards may be used to demonstrate compliance with the essential requirements defined in the Directives list of harmonized standards.”

(Source: www.medicaldevices.bsigroup.com)

1.7.2 Standards

A standard is a document providing requirements, specifications and procedures, such as test/validation methods for a process (e.g. sterilization), equipment (e.g. sterilizer) or accessories (e.g. packaging, PCD, etc.). Most standards are developed under a joint CEN/ISO banner with ISO usually taking the lead. This leads to parallel voting within ISO and CEN, which results in EN ISO standards. EN ISO’s must be published by EU members as local standards e.g. BS EN ISO 17665. Many standards are still published by CEN which have no ISO equivalent e.g. EN 13060. If an EN is harmonized, it means it has an annex ZA, which clearly

identifies which essential requirements are met and therefore offer a presumption of conformity to the MDD (personal conversation with Brian Kirk, PhD, 3M, Loughborough, UK). National recommendations can be accepted by the International Organization for Standardization (ISO) and may then be adapted as European standard (EN ISO) and following by a country (BS EN ISO) (Richard Bancroft, abhi conference for sterilization and microbiology standards, 2014).

1.7.3 Guidance

There are several guidelines for infection control in health care settings. The Robert Koch-Institute (RKI) in cooperation with the Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) gives recommendations on the level of protein residues acceptable after cleaning in dentistry (RKI, 2005). A document published by the Centers for disease control and prevention (CDC) "Guidelines for Infection Control in Dental Health-Care Settings" provides guidance on infection prevention in health care settings, such as hospitals or dental practice (CDC, 2003). Dental specific guidelines are given by the British Dental Association (BDA) and the Health Technical Memorandum (HTM) 01-05: "Decontamination in primary care dental practices" (BDA, 2013). In the Scottish Health Technical Memorandum (SHTM) 2010 part 3 recommendations for verification and validation of sterilization by means of daily, weekly, and annual testing are provided (SHTM, 2010).

These guidelines describe dental handpieces as "semi-critical" or "critical" medical devices, which have to undergo cleaning, disinfection and sterilization and go into detail regarding the level of residual protein tolerable after cleaning and periodic testing for benchtop sterilizers to be carried out in dental practices.

Chapter 2 - Materials & Method Validation

Laboratory methods and equipment used in this study comply with the relevant legislations, standards and guidelines, unless stated otherwise.

2.1 Protein

2.1.1 Standard curves

The o-phthaldialdehyde (OPA) assay is one of the recommended methods to assess efficacy of cleaning processes for medical devices (15883-1:2009+A1:2014, BS EN ISO). It is based on a chemical reaction of the OPA reagent with amino acids in proteins, in the presence of an alkaline solution and thiol compound such as mercaptoethanol (Roth, 1971). Excitation at 338 nm causes the product of the amino acid OPA reaction to fluoresce (Zhu, 2009), which is detected at an emission wavelength of 455 nm, measured in mean fluorescent units (mfu) and concentrations (μg/ml) can be calculated from a standard curve. The OPA assay has successfully been used in previous studies to investigate residual protein on surgical instruments (Smith et al., 2005).

In order to validate the method, stocks of 0.25 g/ml of mucin (from porcine stomach, Sigma) and bovine serum albumin (BSA, Sigma) were prepared in sterilized RO water or 1% sodium dodecyl sulfonate (SDS). Standard concentrations of 25, 50, 100, 200, 300 and 4000 μg/ml were prepared from these stocks.

For the preparation of the OPA reagent 40 mg Phtaldialdehyde (Sigma) were dissolved in 1 ml of methanol (Sigma) and 100 mg sodium 2-mercaptoethanesulfonate were dissolved in 50 ml of 0.1 M sodium tetraborate (1.9 g sodium tetraborate (Sigma) in 50 ml sterilized RO water). The pH was adjusted to 9.2 and was checked using a pH meter (Hanna instruments).

20 µl of all standard concentrations were pipetted into wells of a 96 well solid black microtitre plates (Costar) in triplicate and 300 µl of OPA reagent were added to each occupied well. Following incubation for 3 minutes at ambient room temperature, the assays were read on a plate reader (Omega Fluostar plate reader, BMG Labtech) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm (the closest filters available to the optimum

excitation and emission wavelengths). Raw data was exported to excel and standard curves were plotted in GraphPad Prism 5.01.

2.1.1.1 Bovine serum albumin (BSA)

The BSA standard curve (Fig 1) showed that the optimal range of measurement was between 1 and 100 μg.

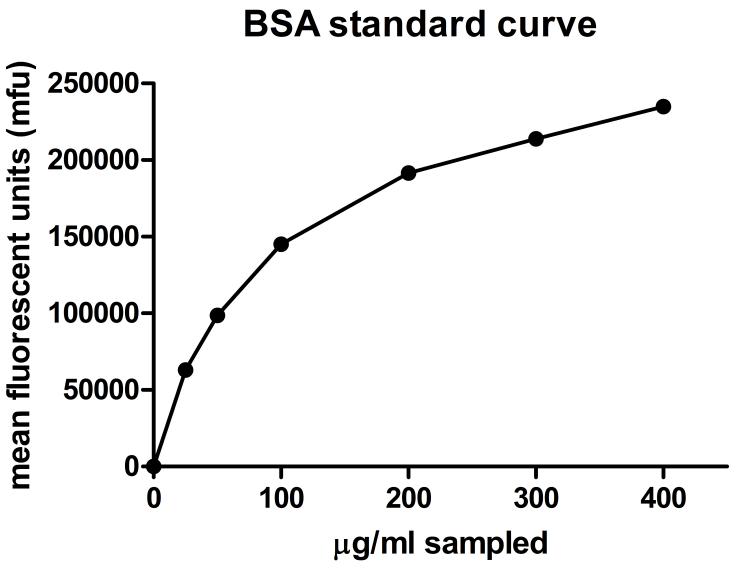

Figure 2-1 OPA standard curve BSA

2.1.1.2 Mucin

The mucin standard curve (Fig 2) reached a plateau at concentrations greater than 100 μg, which suggests that measurements of the higher concentrations are likely to be inaccurate.

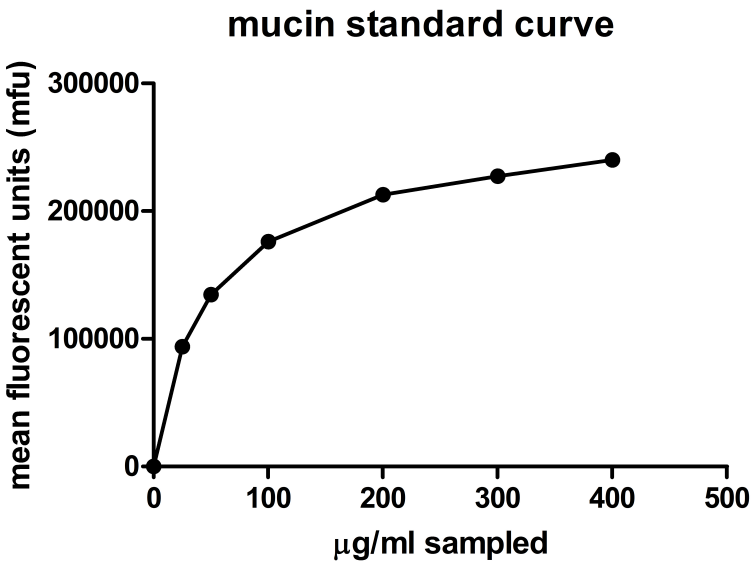

Figure 2-2 OPA standard curves BSA

2.1.2 O-phtaldialdehyde (OPA) method - Recovery of protein from stainless steel

In order to proceed to protein recovery from medical devices made of metal after their use on patients or artificial contamination of instruments in the laboratory, validation work was performed to determine protein recovery from stainless steel discs used as surrogates for the surgical instruments. This involved contaminating the discs with known amounts of protein and then drying at room temperature for 30 min or 60 min, see below. For this experiment, stainless steel discs were first cleaned in five steps:

1- Tap water rinse
2- Soaking in 1% Decon 90 solution for 1 h
3- Rinse with RO water three times
4- Wipe with methanol
5- Dry on a hot plate

After the discs had cooled down, amounts of 50, 100, 200, 300, 400, 500 μg of either BSA or mucin were both applied to six replicate sets of stainless steel discs (12 sets in total) and this was performed on three separate occasions. One half of each set was left for 30 min to dry at room temperature, while the other half was left to dry for 60 min. After the drying time, three different strategies to recover the protein were applied. Stainless steel discs from a single set with either BSA or mucin were submerged in 500 μl of 1% SDS. The BSA or mucin on stainless steel discs of the second set was wiped off using a moist swab, by dipping a sterile swab into phosphate-buffered saline (PBS) and perform 5 strokes across the stainless steel surface and submerging the swab in 500 μl of 1% SDS. For the third set, the same strategy was used, except the PBS was left out and the swab remained dry. All samples were left in 1% SDS for 60 min, as suggested by the standards (15883-1:2009+A1:2014, BS EN ISO). Samples were taken and measured in triplicate along with a standard curve prepared in 1% SDS using the OPA assay as described in section 2.1.1. The equation calculated from the standard curve was used to calculate values for the recovered protein from each of the discs. As stated above this experiment was repeated three times. Raw data was analyzed in Microsoft Excel and GraphPad Prism 5.01 was used to plot mean values and standard error.

2.1.2.1 Mucin

Figure 2-3 shows a comparison of the three recovery methods described in 2.1.2 after application of mucin and a 30 min drying time. The results showed that submerging discs in 1% SDS was the most effective recovery strategy, this was followed by the moist swab and dry swab.

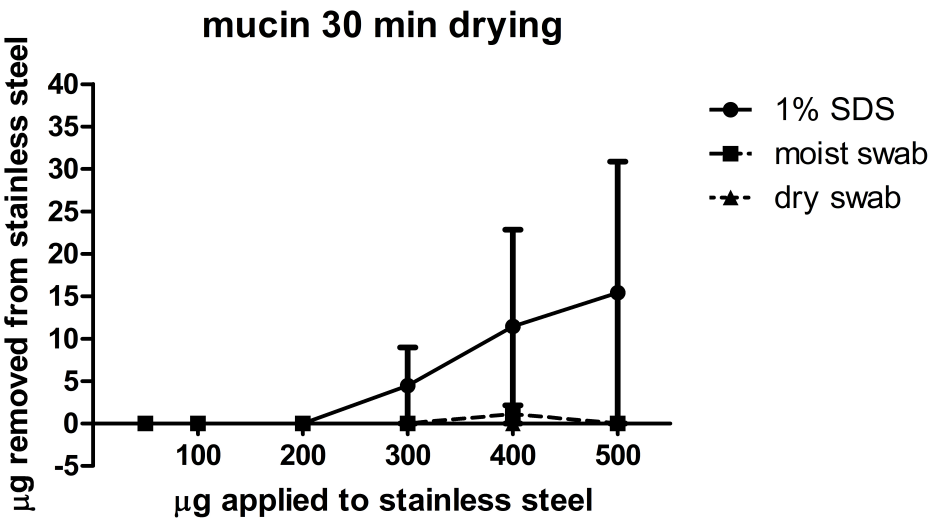

Figure 2-3 Recovery of mucin after 30 min drying at room temperature using 1% SDS, moist and dry swab

Figure 2-4 shows the same parameters, but after a 60 min drying time. Protein was only recovered from disc contaminated with the highest amount of protein and only submerging the discs or using a moist swab was successful at recovering the protein. The standard errors are high, which shows that the results from all three experiments showed a high degree of variance in the amount of protein recovered, but were consistent in determining the best method to use for this purpose.

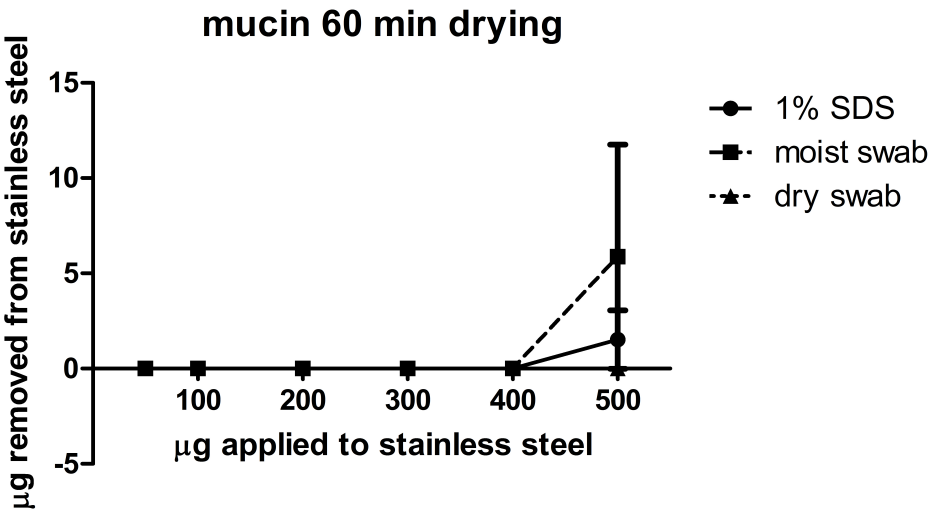

Figure 2-4 Rcovery of mucin after 60 min drying at room temperature using 1% SDS, moist and dry swab

2.1.2.2 BSA

In figure 2-5, the three recovery methods are shown after application of BSA on stainless steel discs and 30 min drying. As shown with mucin in section 2.1.2.1, submerging the contaminated discs in 1% SDS was the most effective recovery method. Again followed by the moist and the dry swab.

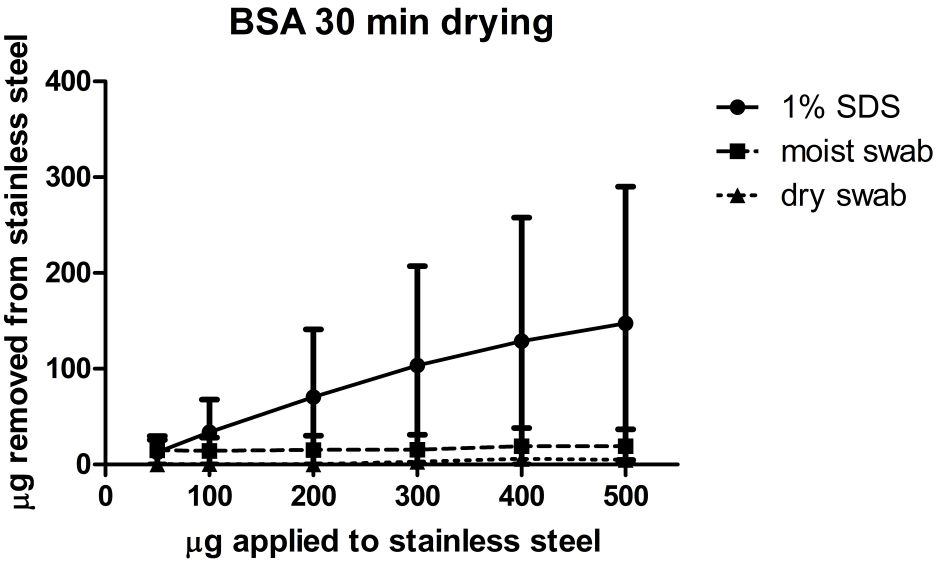

Figure 2-5 Recovery of BSA after 30 min drying at room temperature using 1% SDS, moist and dry swab

As shown in figure 2-6, an increase to 60 min drying had an effect on consistency of the method used and was highly variable in terms of the amount of protein recovered by each method used.

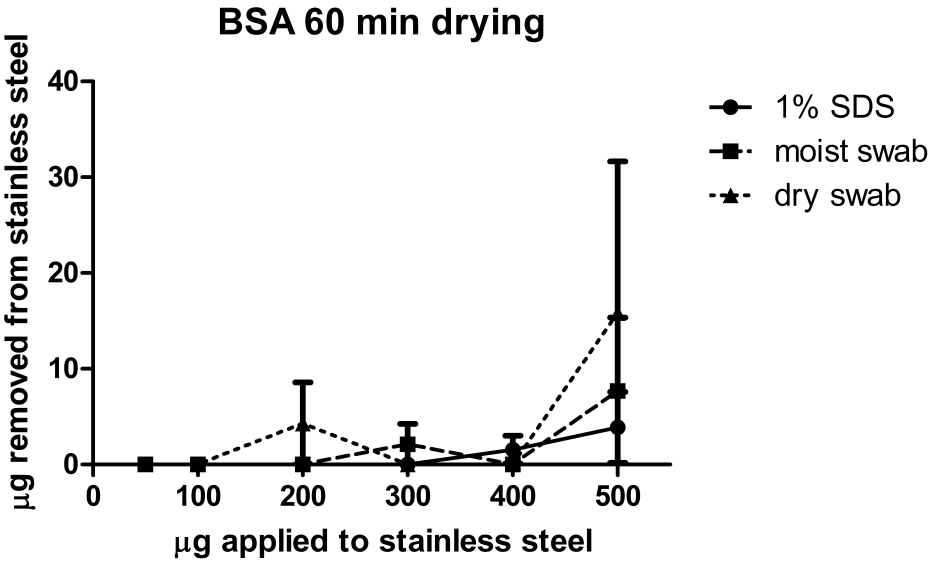

Figure 2-6 Recovery of BSA after 60 min drying at room temperature using 1% SDS, moist and dry swab

Overall, it can be said that BSA is easier to recover than mucin from steel discs and submerging discs in 1% SDS appears to be more successful than swabbing methods. However, it has to be considered that submerging in 1% SDS has the potential to add a high dilution factor that may reduce protein in the solution to a concentration below the limit of detection.

2.1.3 G-Box

2.1.3.1 Standard preparation

The G-Box (Syngene, Cambridge) was invented by Prof. Perrett and uses fluorescence and the OPA method to detect protein on metal surfaces. It is a semi-quantitative method and provides a sensitivity of 50 ng BSA (www.syngene.com/g-box).

It is very difficult to prepare protein standards on stainless steel tags, because the chemistry of surfaces and liquids are different. For preparing standards, the tags must be clean and the difference of protein adherence on the surface has to be considered.

The stainless steel tags were cleaned in five steps:

6- Tap water rinse
7- Soaking in 1% Decon 90 solution for 1 h
8- Rinse with RO water three times
9- Wipe with methanol
10- Dry on a hot plate

For preparing the standards it is important to know the correct density and temperature at which the protein (BSA) should be applied onto the tags. If the density is too high, then protein layers may form, which would result in an inaccurate determination of the amount of protein. The temperature has to be high enough to fix protein on to the surface without burning it. The optimum temperature is 120°C for 30 minutes. Known amounts of 0.125, 0.25, 0.5, 1, 2, 4 and 8 μg of BSA were used to set the standards (Fig 2-7).

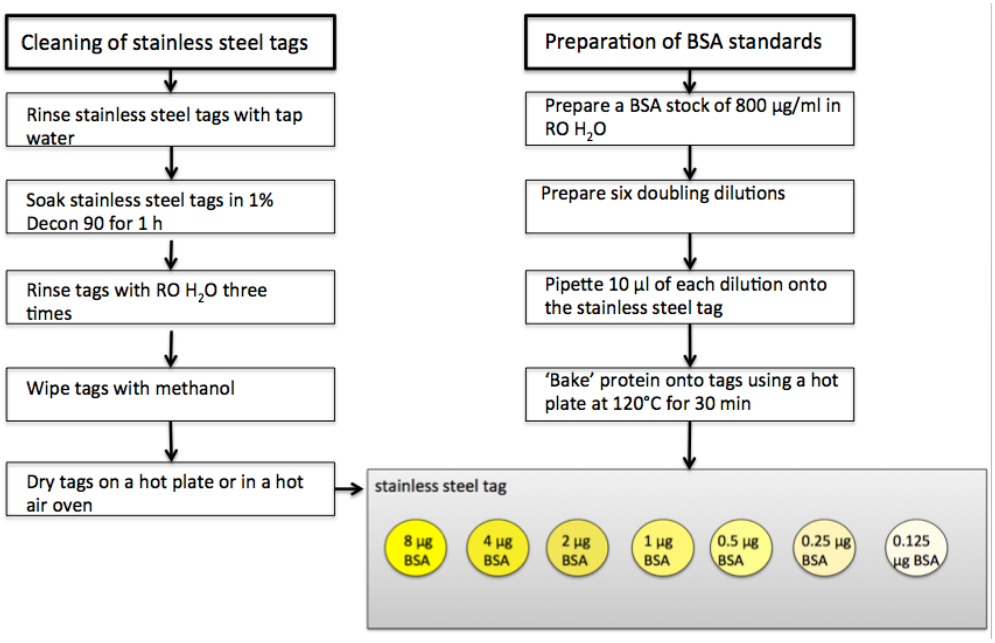

Figure 2-7 Diagram for cleaning stainless steel and preparation of G-box standards

2.1.3.2 Use of G-Box

Stainless steel standard tags were submerged in OPA reagent for 5 min and then placed into the G-Box onto black paper. The software imaged the tag using visible light and the protein using the suitable wavelength for OPA (425 nm). The image shows 'blobs' of protein. Relative fluorescence units (RFU) are shown in the 'results window'. A standard curve with an equation based on the best fit of the line obtained by plotting fluorescence intensity against known protein concentrations was then produced using Microsoft Excel (Fig 2-8).

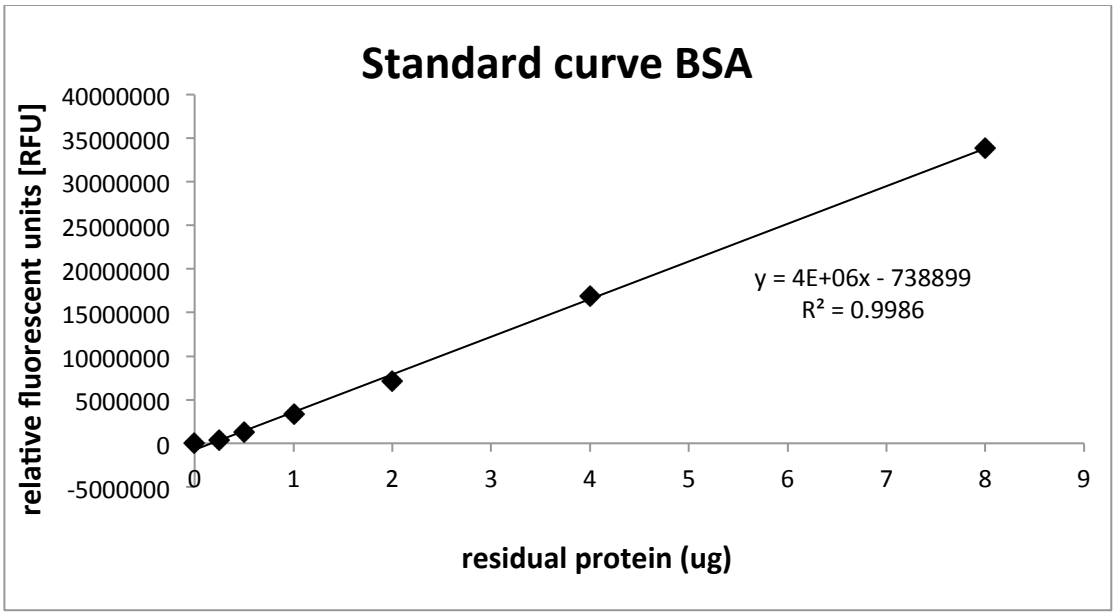

Figure 2-8 Example of G-box standard curve with BSA, produced in Microsoft Excel

According to this standard curve protein amounts from samples were converted from RFU to μg. Protein amounts were also calculated into μg/mm^2 or μg/specimen. To calculate the residual protein on the whole specimen, the amount detected was multiplied by two to obtain the total amount of protein on the instrument, as the G-Box only looks at one side of the specimen.

2.1.4 Bacteria

2.1.4.1 Preparation of microbiological growth media

All microbiological growth media was prepared according to manufacturers' instructions.

In brief, tryptone soy agar plates (TSA, OXOID) 12 g powder was added to 400 ml of RO water (Purelab Prima, ELGA), autoclaved (Prestige Medical, Omega Media), 20 ml distributed into Petri dishes (10 cm, Sterilin) and left for drying at room temperature. Blood agar plates were prepared by putting 400 ml adding 5% vol/vol (20 ml) defibrinated horse blood (E & O Laboratories Limited) to autoclaved Columbia blood agar base (Sigma) once it had cooled to approximately 40^{o}C. Tryptic soy broth (TSB, Sigma) was prepared by adding 16.8 g powder to 400 ml of RO water and autoclaving. Phosphate buffered saline (PBS) was prepared by dissolving 2 PBS tablets (Sigma) in 400 ml of RO water, followed by autoclaving.

2.1.4.2 Culture of microorganisms

Preparation of a bacterial suspension *Staphylococcus aureus* (ATCC 25923) was performed by picking up a single colony forming unit (cfu) from a Mannitol Salt Agar plate and inoculating into 20 ml TSB (Sigma), followed by 24 hour incubation at 37°C and 140 revolutions per minute (rpm) (KS 4000 i control, IKA).

A bacterial suspension of *Streptococcus mutans* was prepared (NCTC 10449) by picking up one colony forming unit (cfu) from blood agar plate and inoculating into 20 ml TSB (Sigma), followed by anaerobic incubation for 24 hours in an incubator (Hera cell, Heraeus) with (5% CO_2) at 37°C.

2.1.5 Growth curves

After transferring 1000 µl of the culture into a cuvette (Fisherbrand, semi-micro, PS) and measuring turbidity at 550 nm in a spectrometer (Colorimeter model 24, Fisher Scientific) using TSB only as the reference, the suspension was diluted to an optical density (OD) of 0.5. Serial dilutions ranging from 1 in 10 up to 1 in a million (10^{-6}) were produced and 20 µl of every dilution were pipetted into the

wells of a 96 well microplate (Costar) in triplicate. TSB only was used as a blank control. The kinetics of the growth of the organisms was determined over a 24 h period by measuring the absorbance (OD) at 550 nm on a plate reader (Omega Fluostar plate reader, BMG, Labtech), where measurements were taken hourly. Averages were calculated in Microsoft Excel and a graph was produced using GraphPad Prism 5.01.

2.1.5.1 Staphylococcus aureus

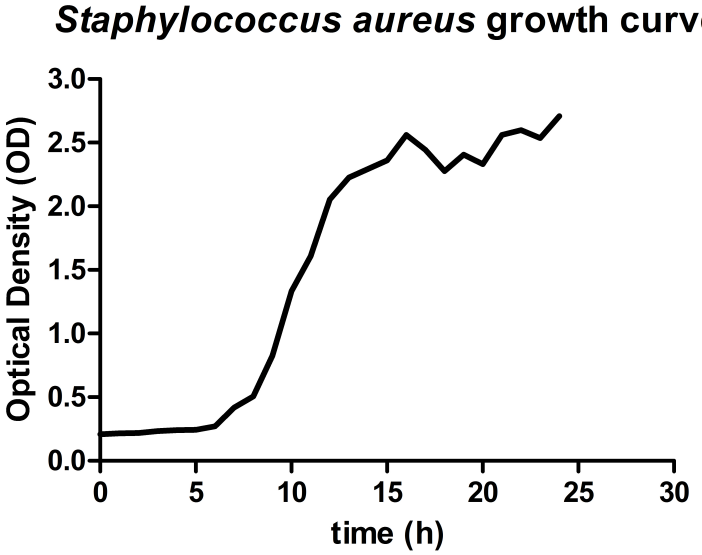

Figure 2-9 24 h growth curve of *Staphylococcus aureus*

2.1.5.2 Streptococcus mutans

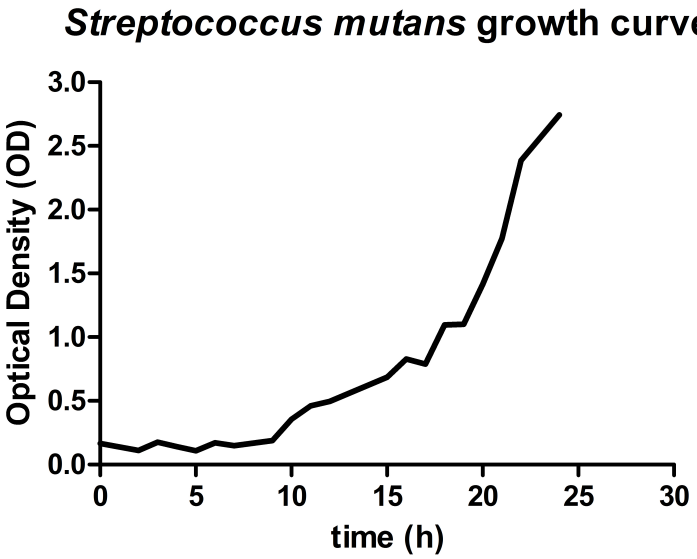

Figure 2-10 24 h growth curve *Streptococcus mutans*

2.1.6 Artificial saliva

For the preparation of 1 L artificial saliva the following ingredients were used. 2.5 g porcine stomach mucin (Sigma), 3.5 g Sodium Chloride (VWR), 0.2 g Potassium Chloride (Sigma), Calcium Chloride Dihydrate, 0.2 g $CaCl_2.2H_2O$ (VWR), 2 g Yeast Extract (Oxoid), 1 g Lab Lemco Powder (Oxoid) and 5 g Proteose Peptone (Oxoid). 1 L sterilized RO H_2O (ELGA) was added and sterilized using a lab sterilizer (MP 24 Control, Rodwell Scientific Instruments). 40% Urea (Oxoid) were added (1.25 mL) to artificial saliva after sterilizing. Artificial saliva was stored at 4°C (Leung and Darvell, 1997).

2.1.7 Standard inoculum

An overnight suspension of bacteria was centrifuged for 10 min at 3000 rpm (C20, Awel centrifuges). The pellet was resuspended in PBS and optical density (OD) was measured using a plate reader (Omega Fluostar plate reader, BMG Labtech) at 550 nm. Bacterial suspension was diluted in PBS to an OD of 0.5. A 1 in 10 dilution series was performed to 10^{-7} and 100 µl were plated onto TSA plates for *S. aureus* and blood agar plates for *S. mutans* in triplicate. TSA plates were incubated at 37°C for 24 h and blood agar plates were incubates at 37°C with 5% CO_2 for 24 h. The following day a colony forming units (cfu) count was determined by summing the number of colonies on the higher dilution plates. Each experiment was performed in triplicate and averages were taken to calculate a standard inoculum for OD=0.5 (Koch, 1970, Sutton, 2011).

The average colony count for *S. aureus* at OD=0.5 was 2.7×10^8 cfu/ml, while the average colony count for *S. mutans* at OD=0.5 was 8.4×10^8 cfu/ml

2.1.8 Standard test load for handpiece sterilization experiments with data loggers

For each cycle, a standard test tray consisting of 3 different types of handpieces were used: a dental air turbine TA-98 C LED (W&H, Austria), a straight surgical handpiece S11 (W&H, Austria), a slow speed motor WA-56 (W&H, Austria)) and a helix process challenge device (PCD) was used as a control (Fig 2-11). Three handpieces of each type were inoculated with chemical indicators (CI), which indicate a successful sterilization process by a colour change from yellow to blue and biological indicators (BI), which require incubation and indicate a successful sterilization process by the absence of microbial growth. CI (class 2, Browne) were placed in three positions in the turbine (turbine head, drive air channel, spray channels), in two positions in the surgical handpiece (chuck lever, handpiece back) and in one position in the slow speed (inside sleeve). BI (mini spore strips, Excelsior, D_{121}= 1.8 – 2.5 min) were placed in three positions in the turbine (turbine head, drive air channel center, drive air channel back), in two positions in the surgical handpiece (chuck lever, handpiece back) and in one position in the slow speed (inside sleeve). Handpieces for vacuum sterilization were places in sealable sterilization pouches (Steris) before sterilization.

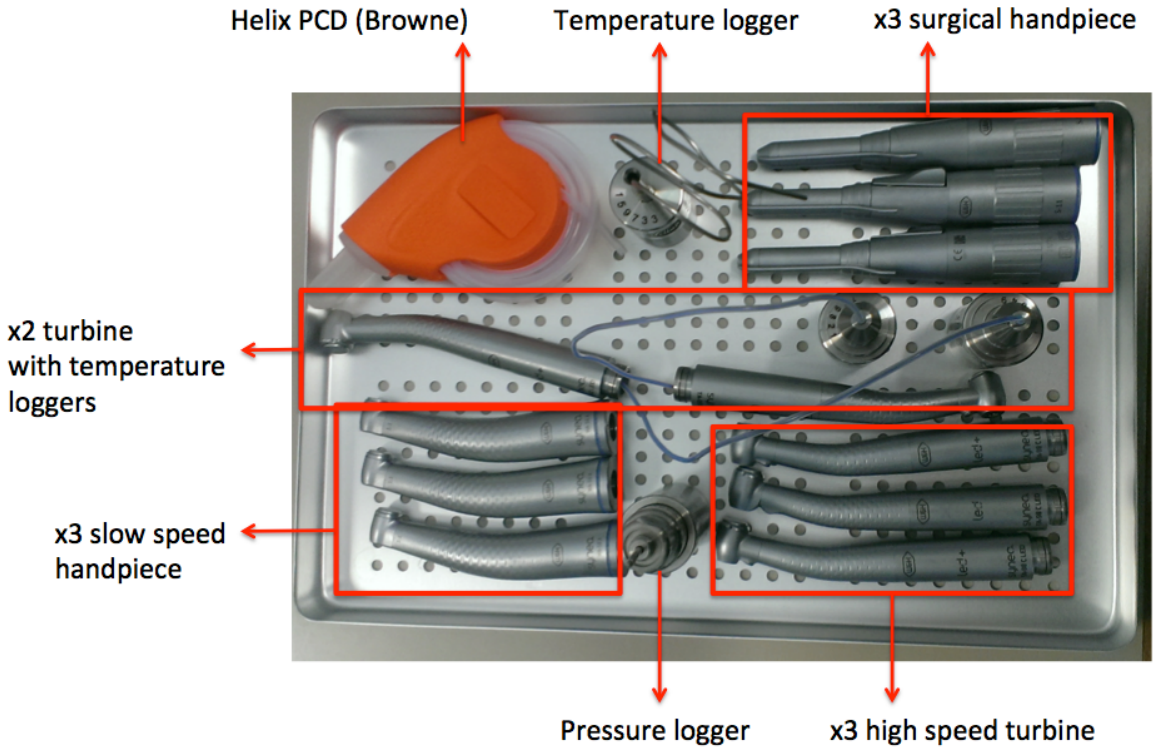

Figure 2-11 standard test load with 3x dental air turbine TA-98 C LED (W&H, Austria), 3x straight surgical handpiece S11 (W&H, Austria), 3x slow speed motor WA-56 (W&H, Austria), 1x helix PCD (Browne), 2x temperature data logger inside dental air turbine (TA-98 C LED (W&H, Austria), 1x temperature data logger "free space" and 1x pressure data logger (all data loggers ellab), total weight 0.5 kg

Chapter 3 - Influence of different humidity levels on the sensitivity of chemical and biological indicators for steam sterilization

3.1 Introduction

Exposure times to achieve a SAL of 10^{-6} using steam sterilization were first reported in 1956 by Perkins to be 1 minute plus an additional minute for safety at 134°C (Perkins, 1983). In the 1st Medical Research Council (MRC) report, published in 1959, another minute was added to ensure further safety, which resulted in a recommendation of 3 min at 134°C at a chamber pressure of 2.2 bar to achieve the sterilization conditions for the production of a sterile and safe to use medical device (556-1:2001, BS EN, MRC, 1959). In order to monitor steam sterilization processes, biological indicators (BI) (11138-1:2006, BS EN ISO) and chemical indicators (CI) (11140-3:2007, BS EN ISO) are commonly used in large as well as small steam sterilizers. Chemical indicators are also used in process challenge devices (PCD), such as the Bowie Dick Test (BDT) or in the Helix test device (867-5:2001, BS EN) to simulate porous and hollow loads, respectively. Previous workers such as van Doornmalen et al. (2012) showed that only one out of six different CI (class 6 – emulating indicators) tested, achieved their claimed properties (according to the manufacturer) in terms of sensitivity to time, temperature and presence of saturated steam in a vacuum sterilization cycle with 3 min, 3.5 min or 4 min at 134°C (J.P.C.M. van Doornmalen, 2012). Two different helix devices tested did not reliably represent a hollow medical device in a small or a large vacuum sterilizer at pre vacuum depths ranging between 300 and 400 mbar (S. Esen, 2012b).

Indicators for steam sterilization are manufactured to comply with International standards (867-5:2001, BS EN, 11138-1:2006, BS EN ISO, 11140-3:2007, BS EN ISO) and are designed to react to the presence of saturated steam after a specified time and temperature exposure. However, little is published about the potential influence that different humidity levels have in the indicators' environment and or storage conditions on the chemistry of chemical indicators and/or the spores of *Geobacillus stearothermophilus* in biological indicators. According to one of the manufacturers, the critical humidity for chemical indicators is 40% relative humidity (RH); below 40% RH chemical indicators rehydrate when in contact with steam and cause an exothermic reaction during the sterilization cycle. The requirement for a class 6 CI is that a pass is shown

when exposed to it's stated values and a fail when exposed to 1°C lower and 6% less time (11140-1:2009, BS EN ISO).

The aim of this study was to investigate the effect of humidity on the reaction of CI and BI to the presence of heat delivered by steam in order to be able to interpret CI results from further experiments in this study correctly.

3.2 Materials and methods

3.2.1 Preliminary experiment

Small screw top glass bottles (Schott) were used for this experiment. The volume of the glass bottle was 130 ml. Chemical indicator strips (class 2, Browne) were placed into four glass bottles. Water deionized by reverse osmosis (RO H_2O) was added into two of them; 20 ml (15.4%) in the first experiment, 1 ml (0.8%) in the second, 0.5 ml (0.4%) in the third and 0.25 ml (0.2%) in the fourth experiment. After sealing the bottles they were sterilized in a non-vacuum sterilizer (Little Sister 3, Eschmann). The colour change of the chemical indicators was examined after sterilization.

3.2.2 Main Study

Technical equipment comprised of thermocouples (type T), a BIER/CIER vessel (Resistometer Typ 219, Lautenschläger) and a hygrometer (rotronic). Three different chemical indicators were used in each experiment. A class 4 CI (3M 1250), a class 5 CI (3M 1243 Comply SteriGage) and a class 2 CI (Browne chemical indicator (helix PCD)). Also included in each experiment were biological indicators (3M 1262 Attest, D_{121}=1.5 – 3.0 min) and Excelsior mini spore strips (2 mm x 10 mm, population 2.5 x 10^5 spores per strip, D_{121}=2.3 min) were included in one set of experiments (5 min and 6 min).

The BIER vessel with a pre-heated chamber (80-100°C) was programed to run replicate cycles at a pressure of 45 mbar (pre-vacuum) with exposure times of 2, 3, 4, 5 or 6 min and a temperature set to at 134°C.

Solutions of Glycerol (Sigma) in combination with and silica gel (Sigma), Potassium carbonate (Sigma), Magnesium nitrate hexahydrate (Sigma) and Potassium nitrate (Sigma) in water were used to achieve different levels of humidity (17%, 43%, 60% and 90% RH, respectively) in sealed plastic boxes (ASDA) (Elshatshat, 2009). Chemical and biological indicators were placed inside the boxes and left to equilibrate overnight at room temperature.

Initial experiments focused on two extreme humidity conditions: Low (14 – 17% RH) and high (85 – 90% RH). Two sets of five glass vials, with 17% RH in set 1,

90% RH in set 2, were inoculated with 0, 1, 10, 50, 100 and 500 μL of water and a rubber bung placed in each tube to act as a platform (Fig 3-1). Within each vial were placed 3 different pre-conditioned CI and one pre-conditioned BI. In the BIER vessel the sealed vials were exposed to 134°C. A thermocouple (T type) was inserted inside a sealed vial through a hole in the cap (sealed with silicone (3M)). The temperature traces from inside the glass vials when compared to the chamber showed that the temperature took on average 32.8 sec longer to reach 134°C in the sealed glass containers than in the chamber (Fig 3-2), which had to be taken into consideration when analysing the results. The first two sets of experiments consisted of 2, 3, 4, 5 or 6 min exposure at 134°C, which equated to 1.5, 2.5, 3.5, 4.5 or 5.5 min actual exposure of the items in the glass vials to 134°C including the indicators. The second set of experiments consisted of 4 min exposure (Table 3-1). Experiments were performed in triplicate.

CIs were visually assessed while Attest BIs were incubated in the 3M Attest incubator for steam at 56°C for 24 to 48 h, followed by visual assessment. A colour change from purple to yellow indicates growth and therefore a fail. Excelsior mini spore strips were transferred into 2 ml TSB (tryptic soy broth, Sigma) and incubated for up to 8 days at 56°C and checked for growth every 24 h. Presence of growth indicated a fail.

For further analysis, colour changes of Sterigage (3M) and Helix CI (Browne) were measured using a ruler. Length values for Sterigage were divided by half of the total length of the indicator (15 mm), which indicates the pass line on the indicator, while values from Helix CI were divided by the full length of the indicator (18 mm), in order to achieve values where 1 is the pass line, as previously described by van Doornmalen (J.P.C.M. van Doornmalen, 2012). For acquiring values for class 4 (3M) indicators, a densitometer (X Rite 400 reflection densitometer) was used to achieve OD values. Values from 0.75 were considered a pass. Mean values and standard error were plotted using GraphPad Prism 5.01.

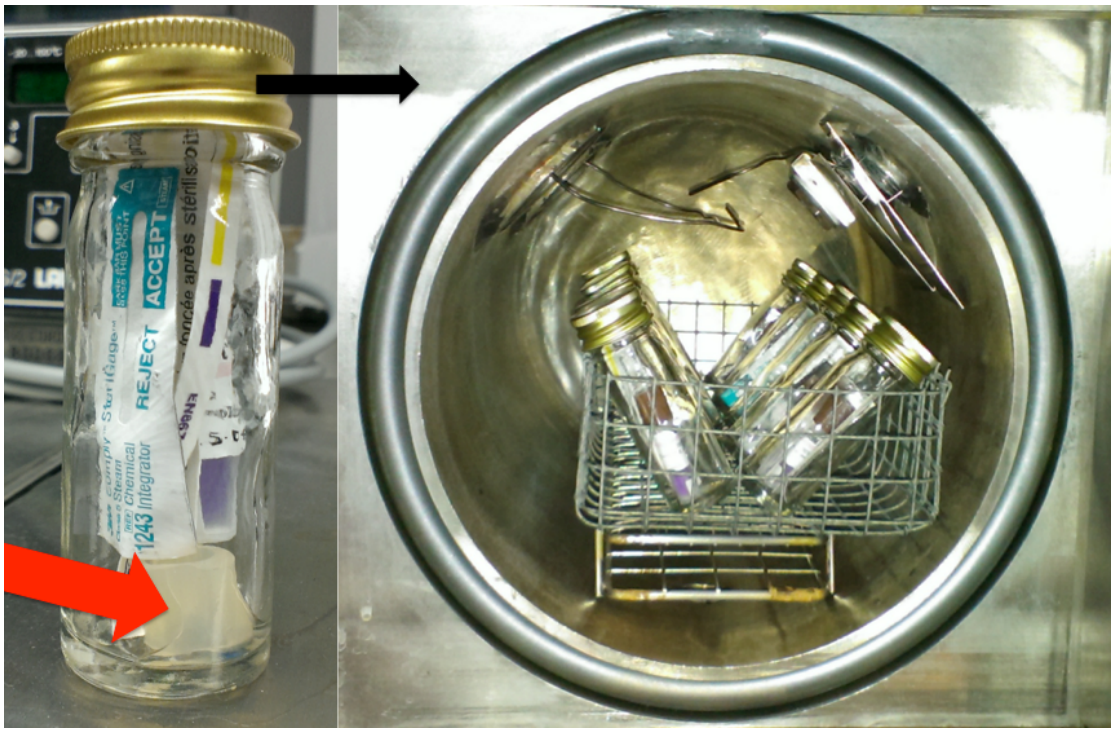

Figure 3-1 Left: glass vial containing CIs and BIs; red arrow indicates rubber platform; Right: glass vials in BIER vessel chamber

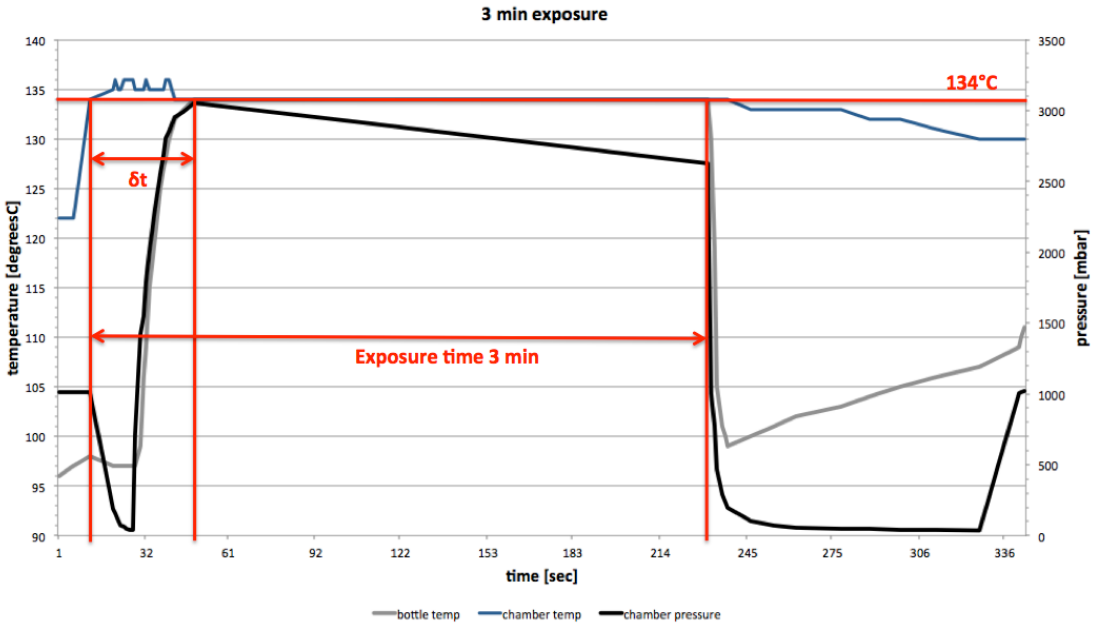

Figure 3-2 Temperature traces at 3 min exposure (δt = time difference between chamber and glass vial reaching sterilization temperature)

Table 3-1 Summary of experiments performed/CIs and BIs used

Water volume in vial (µL)	Set up 1 performed in triplicates for exposure times of 2, 3, 4, 5 and 6 min						Set up 2 performed in technical triplicates for exposure time of 4 min	
	Chamber control		Precondition Low (14 – 17% RH)		Precondition High (85 – 90% RH)		Low (14 – 17% RH)	High (85 – 90% RH)
	CI	BI	CI	BI	CI	BI	BI	BI
0	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	3x Excelsior mini spore strips	3x Excelsior mini spore strips
10	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	3x Excelsior mini spore strips	3x Excelsior mini spore strips
50	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	3x Excelsior mini spore strips	3x Excelsior mini spore strips
100	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	3x Excelsior mini spore strips	3x Excelsior mini spore strips
500	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	1x 3M SteriGage 1x 3M class 4 1x Browne helix CI	3M Attest	3x Excelsior mini spore strips	3x Excelsior mini spore strips

3.3 Results

3.3.1 Preliminary experiment

Water contents as low as 0.25 ml (0.2%) in a sealed vessel (total volume 130 ml) caused the chemical indicators to give a pass result. The chemical indicators in the bottles that did not contain water did not show a colour change and thus failed.

Not only temperature but also humidity (water content in vessel, wet steam saturated steam) is required to change the chemical indicator. Even the smallest amount of moisture results in a colour change (Figure 3-3).

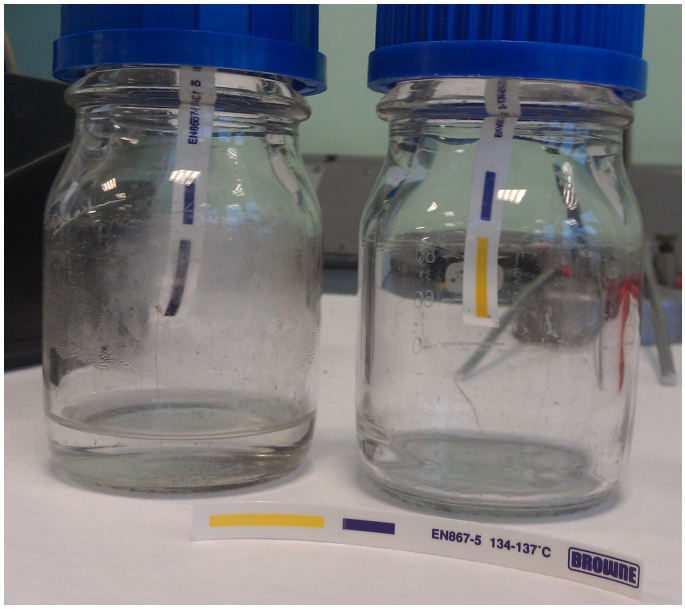

Figure 3-3 Left bottle with water shows colour change; right bottle without water does not show colour change; unused colour indicator strip in front of the bottles for comparison

3.3.2 Main study

Results of the low RH pre-conditioned CI showed that 3M SteriGage indicators gave passes at a water content of 10 μL with an exposure time of 6 min, while 3M class 4 and Browne helix CI started indicated pass results with a water content of 50 μL and 5 minutes exposure time (Table 3-2a). In all samples, it was observed that the higher the water content in the vial, the lower the exposure time required to achieve a CI and BI pass. Results of the high RH pre-conditioned CI showed that 3M SteriGage indicators passed when the water content was 0 μL with an exposure time of 5 min, while 3M class 4 and Browne helix CI indicating pass results with water content of 50 μL or greater after 4 min and 6 min exposure time. No passes were detected in all samples exposure for 2 min (Table 3-2b). There was a trend for CIs to perform passes as the amounts of water in vials and exposure time were increased. However, no clear difference was found between high and low humidity preconditioning. The chamber controls indicated passes at exposure times of 2 min (Table 3-2c).

Additional analysis measuring colour change confirms that Sterigage (3M) graphs show little difference between high and low humidity pre-conditioning (Figure 3-4). Class 4 CI (3M) showed sensitivity towards water amounts in vials as well as pre-conditioning. More samples that were pre-conditioned in high humidity showed a stronger colour change at earlier exposure times than samples pre-conditioned in low humidity (Figure 3-5). Looking at the graphs of helix CI (Browne) in figure 3-6, it appears that CI responded to time and the amount of water present in the glass vials, but not to the pre-conditioning. However, standard errors show a wide variation in colour change.

Biological indicators (Attest, 3M): only the chamber controls given an exposure of 2, 3, 4, 5 and 6 min were killed. All other tested indicators showed growth, as shown in table 3-3. Biological indicator strips (Excelsior) were used in one experiment (exposure 4.0 min) due to time limitations. Growth was detected in samples with preconditioning in the dry environment, while Spore strips preconditioned in the humid environment were inactivated in vials with water amounts of 0, 10, 100 and 500 μL. Paradoxically, growth was detected in vials with 50 μL of water (Table 3-4).

Table 3-2a CI results for low humidity preconditioning (✗=fail, ✓=pass)

Water volume in vial (µL)	Low preconditioning (14 – 17% RH)														
	3M SteriGage					3M class 4					Browne helix CI				
	Exposure time (min at 134°C)					Exposure time (min at 134°C)					Exposure time (min at 134°C)				
	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6
0	✗✗	✗✗	✗✗	✗✗✗	✗✗✗	✗✗	✗✗	✗✗	✗✗✗	✗✗✗	✗✗	✗✗	✗✗	✗✗✗	✗✗✗
10	✗✗	✗✗	✗✗	✗✗✗	✗✗✓	✗✗	✗✗	✗✗	✗✗✗	✗✗✗	✗✗	✗✗	✗✗	✗✗✗	✗✗✗
50	✗✗	✗✗	✗✗	✗✗✓	✗✗✗	✗✗	✗✗	✗✗	✗✓✓	✗✓✓	✗✗	✗✗	✗✗	✗✗✓	✗✗✓
100	✗✗	✗✗	✗✓	✗✗✓	✗✓✓	✗✗	✗✓	✗✓	✗✓✓	✗✓✓	✗✗	✗✗	✗✓	✗✓✓	✗✓✓
500	✗✗	✗✗	✗✓	✗✓✓	✓✓✓	✗✓	✓✓	✓✓	✓✓✓	✓✓✓	✗✗	✗✓	✓✓	✓✓✓	✓✓✓

Table 3-2b CI results for high humidity preconditioning (✗=fail, ✓=pass)

Water volume in vial (µL)	High humidity preconditioning (85 – 90% RH)														
	3M SteriGage					3M class 4					Browne helix CI				
	Exposure time (min at 134°C)					Exposure time (min at 134°C)					Exposure time (min at 134°C)				
	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6
0	✗✗	✗✗	✗✗	✗✗✓	✗✓✓	✗✗	✗✗	✗✗	✗✗✗	✗✗✗	✗✗	✗✗	✗✗	✗✗✗	✗✗✗
10	✗✗	✗✗	✗✓	✗✗✗	✗✓✓	✗✗	✗✗	✗✗	✗✗✗	✗✗✗	✗✗	✗✗	✗✗	✗✗✗	✗✗✗
50	✗✗	✗✗	✗✗	✗✗✗	✗✓✓	✗✗	✗✗	✗✓	✗✓✓	✗✓✓	✗✗	✗✗	✗✗	✗✗✗	✗✗✓
100	✗✗	✗✗	✗✓	✗✗✗	✗✗✓	✗✗	✗✓	✓✓	✓✓✓	✗✓✓	✗✗	✗✗	✗✓	✗✓✓	✗✓✓
500	✗✗	✗✗	✗✓	✗✓✓	✓✓✓	✗✗	✓✓	✓✓	✓✓✓	✓✓✓	✗✗	✗✓	✓✓	✓✓✓	✓✓✓

Table 3-2c CI results for unsealed chamber controls (✗=fail, ✓=pass)

Chamber control (unsealed)														
3M SteriGage					3M class 4					Browne helix CI				
Exposure time (min at 134°C)					Exposure time (min at 134°C)					Exposure time (min at 134°C)				
2	3	4	5	6	2	3	4	5	6	2	3	4	5	6
✗✓	✓	✓✓	✓✓	✓✓✓	✓✓	✓	✓✓	✓✓	✓✓✓	✓✓	✓	✓✓	✓✓	✓✓✓

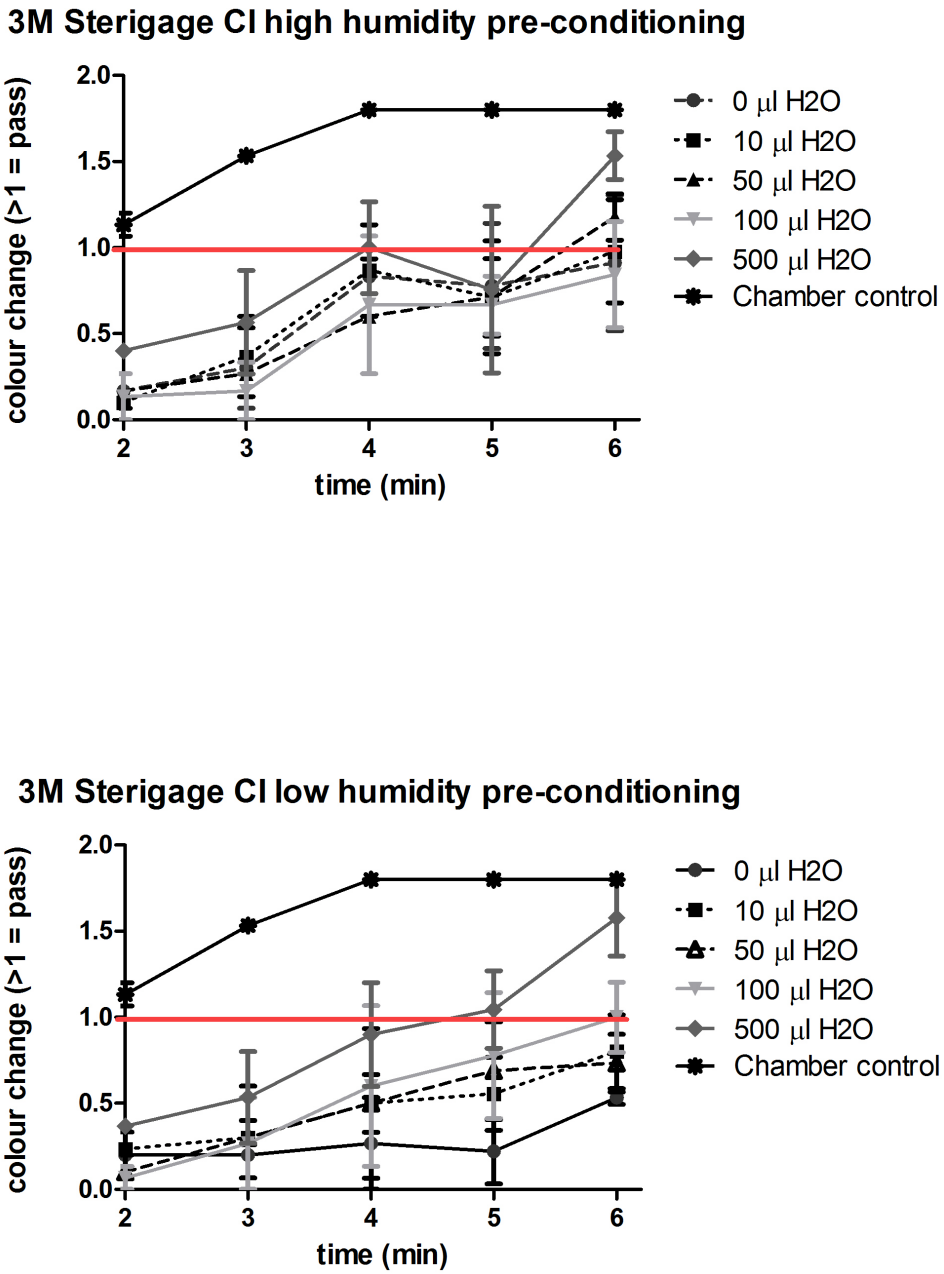

Figure 3-4 Colour change of Sterigage (3M) CI against time, in different amounts of water, pre-conditioned in high humidity (top graph) and low humidity (bottom graph); results on or above the horizontal red line are passes, results below are fails

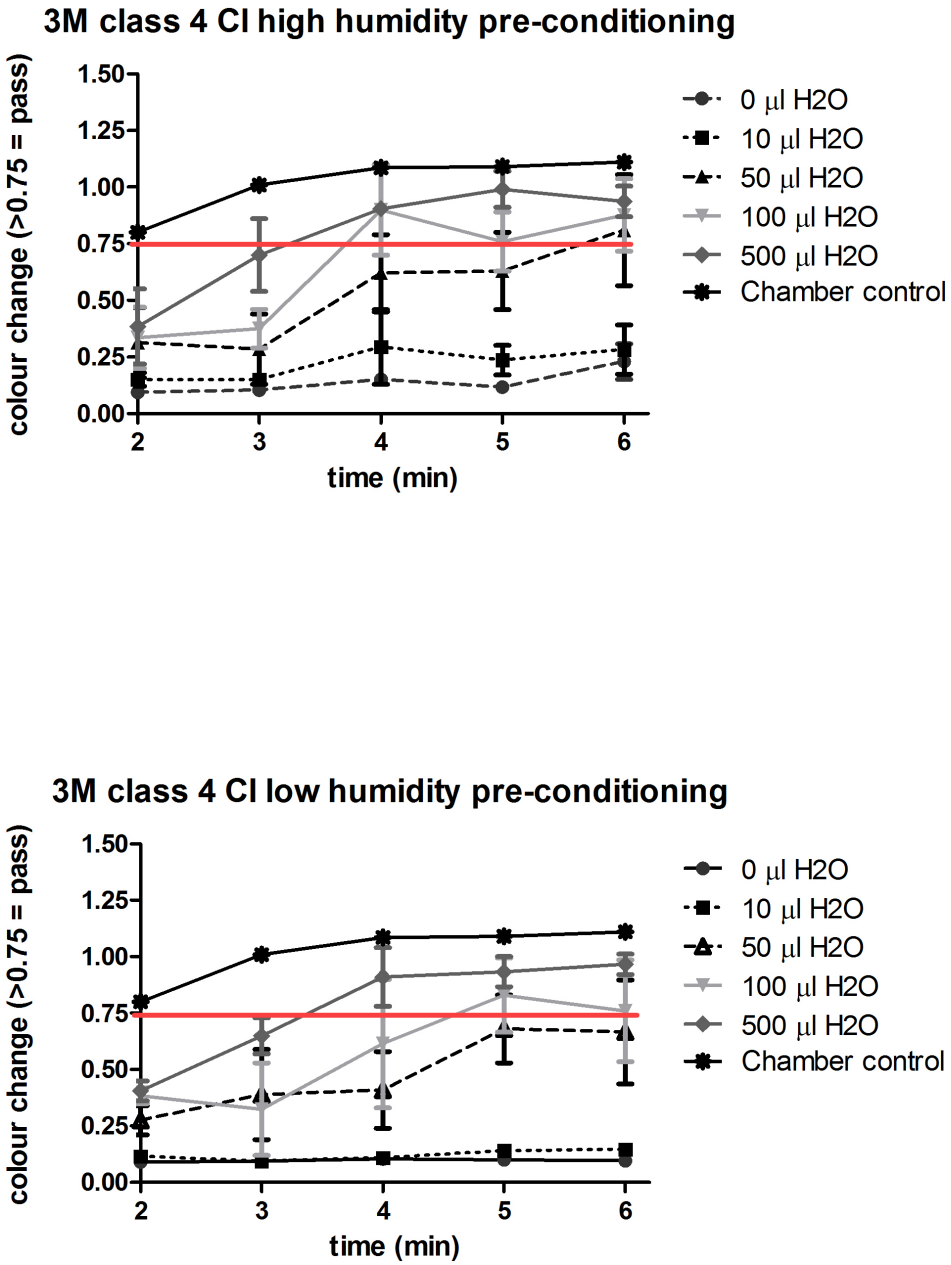

Figure 3-5 Colour change of class 4 (3M) CI against time, in different amounts of water, pre-conditioned in high humidity (top graph) and low humidity (bottom graph); results on or above the horizontal red line are passes, results below are fails

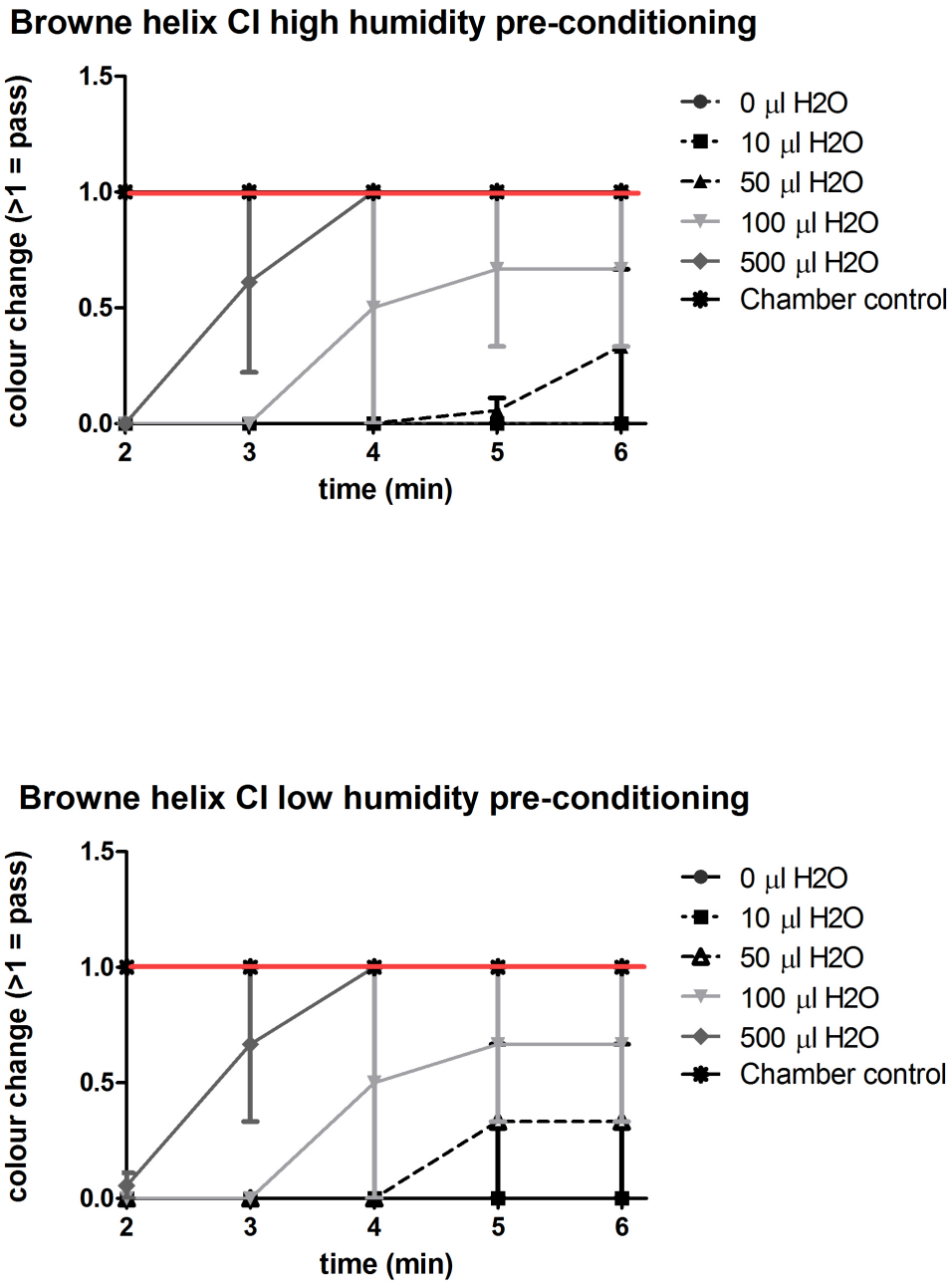

Figure 3-6 Colour change of helix (Browne) CI against time, in different amounts of water, pre-conditioned in high humidity (top graph) and low humidity (bottom graph); results on or above the horizontal red line are passes, results below are fails

Table 3-3 Results Attest

Water volume in vial (µL)	Set up 1 BI results for exposure times of 2, 3, 4, 5 and 6 min														
	Low (14 - 17% RH)					High (85 - 90% RH)					Chamber control				
	Exposure time (min) N=3					Exposure time (min) N=3					Exposure time (min) N=3				
	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6
0	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3
10	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3
50	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3
100	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3
500	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3

Table 3-4 Results Excelsior mini spore strips

Water volume in vial (µL)	Set up 2 performed in technical triplicates for exposure time of 4 min	
	Low (14 - 17% RH)	High (85 - 90% RH)
	BI (growth)	BI (growth)
0	1/3	0/3
10	3/3	0/3
50	3/3	3/3
100	3/3	0/3
500	-	0/3

3.4 Discussion

The present study suggests that different humidity levels affect how chemical indicators react to saturated steam and that pre-conditioning, sampled in different humidity levels, allows the matrix of the indicator to absorb certain amounts of moisture, so that during sterilization auto generation of steam can take place. In both, high and low RH pre-conditioned CI it was determined that 3M class 4 CI were more susceptible to humidity than 3M Sterigage and Browne helix CI, which behaved similarly. 3M Sterigage were less susceptible to the amounts of water in the glass vials, while Browne helix CI were more sensitive to the amounts of water added than to pre-conditioning. However, all CI tested indicated passes under sub-optimal conditions during exposure to 134°C indicating the achievement of false positive sterilization conditions. Glass vials were sealed, which suggested that the pressure inside the glass vials would be different. The BI results for the 3M Attest show no growth in any of the glass vials, but were inactivated in the chamber control at 2 min, which suggests that the BI used, which are self-contained, represent PCDs. It takes time for the internal space, in which the BI is housed, to equilibrate with the environment. However, the Excelsior mini spore strip results suggest that moisture in terms of wet steam seems to be sufficient to inactivate spores. This inconsistency may be due to differences in spore manufacturing conditions or spore recovery methods.

Previous workers such as Rutala et al. (1996) tested four *G. stearothermophilus* BI Attest, Assert, and Biosign and Proof Plus as well as five chemical indicators (Comply, Propper, Chemdi, Sterigage and Thermalog S) and found that some chemical indicators failed to indicate adequate sterilization (Rutala et al., 1996). Van Doornlamen et al. (2012) also found that CI are unreliable and can provide false results when six commercially available CI (class 6) were tested (J.P.C.M. van Doornmalen, 2012). Both studies (Rutala et al., 1996 and J.P.C.M. van Doornmalen, 2012) agree well with the results of the current study, as we also demonstrate that BIs and CIs can indicate pass conditions at exposure times of less than 3 min at 134°C. This may be due to the presence of wet steam in sealed glass vials, or superheated steam, where a sufficient quantity of water molecules was present. However, care should be taken in the interpretation of

these results from the context of sterility assurance. While the use of glass vials provided a suitable environment in which to assess the impact of different humidity levels on the reaction of CI's and BI's to the environmental, time and temperature challenges the aim was not to determine the suitability of CI's and BI's for monitoring sterilization conditions in glass vials.

Little work has been published on this topic, but our findings are consistent with others that suggest that not all CI's and BI's of the same class behave similarly to the same time and temperature exposures. Under some conditions the CI's and BI's can indicate false positives and care should be taken in their use, exposure and interpretation.

Chapter 4 - Investigating steam penetration into dental handpieces using benchtop steam sterilization processes in-vitro

4.1 Introduction

Dental handpieces become contaminated externally and internally during patient treatment (Herd et al., 2007, Chin et al., 2006, Dreyer and Hauman, 2001, Epstein et al., 1993, Epstein et al., 1995, Kellett and Holbrook, 1980, Shpuntoff and Shpuntoff, 1993, Zhou et al., 2006). Clinical evidence for cross infection risk is difficult to assign to a particular incident due to the fact that infections are difficult to trace back to a dental treatment (Hu et al., 2007), but there are reports of incidents involving HIV risk (Nottingham 2014) and Hepatitis B (Radcliffe et al., 2013). Technical evidence for the necessity for air removal from lumens has been provided by many studies investigating (J P C M van Doornmalen, 2013, Kaiser U., 1998, S. Esen, 2012a). Manufacturers of both sterilizers and dental handpieces recommend that this equipment be sterilized using a vacuum process, due to their complex construction and internal lumens (instructions for use by W&H and Eschmann).

There are 3 different benchtop steam sterilization processes described in standard BS EN ISO 13060. Type N, which is a non-vacuum and passive air displacement process, type B and S, which achieve air removal using a vacuum pump and special cycles, respectively. While the special cycle is specifically designed for a particular instrument the type S, type B sterilization uses a vacuum pump for active air removal and is recommended to be used for porous or hollow load (13060:2014, BS EN). However, there is no legislation that makes type B sterilization mandatory for dentists, even though dental handpieces are hollow devices. In UK dental practices non-vacuum sterilizers are still commonly used (Smith et al., 2009c).

Steam penetration into lumens can be measured using thermocouples (TC) to measure temperature (13060:2014, BS EN), which is the main method used to commission and validate a steam sterilizer (SHTM, 2010). Besides acquiring details from sterilizer print outs, biological and chemical indicators are used for monitoring the efficacy of the steam sterilization process by the operator, whereby chemical indicators are placed in process challenge devices (PCD), such as the helix or the Bowie Dick test pack, which simulate hollow or porous loads, respectively (867-4:2001, BS EN, 867-5:2001, BS EN, 11138-1:2006, BS EN ISO).

The following chapter describes the use of all three methods in order to investigate steam penetration into PCD and dental handpieces during non-vacuum and vacuum steam sterilization processes by using chemical and biological indicators to indicate sterilization conditions.

4.2 Material and Methods

In this investigation the non-vacuum and vacuum processes (Figures 4-1 and 4-2) were monitored by recording temperature and pressure measurements and determination of the time difference between the chamber and the inside of the handpieces reaching sterilization temperature, as shown in Figure 4-3.

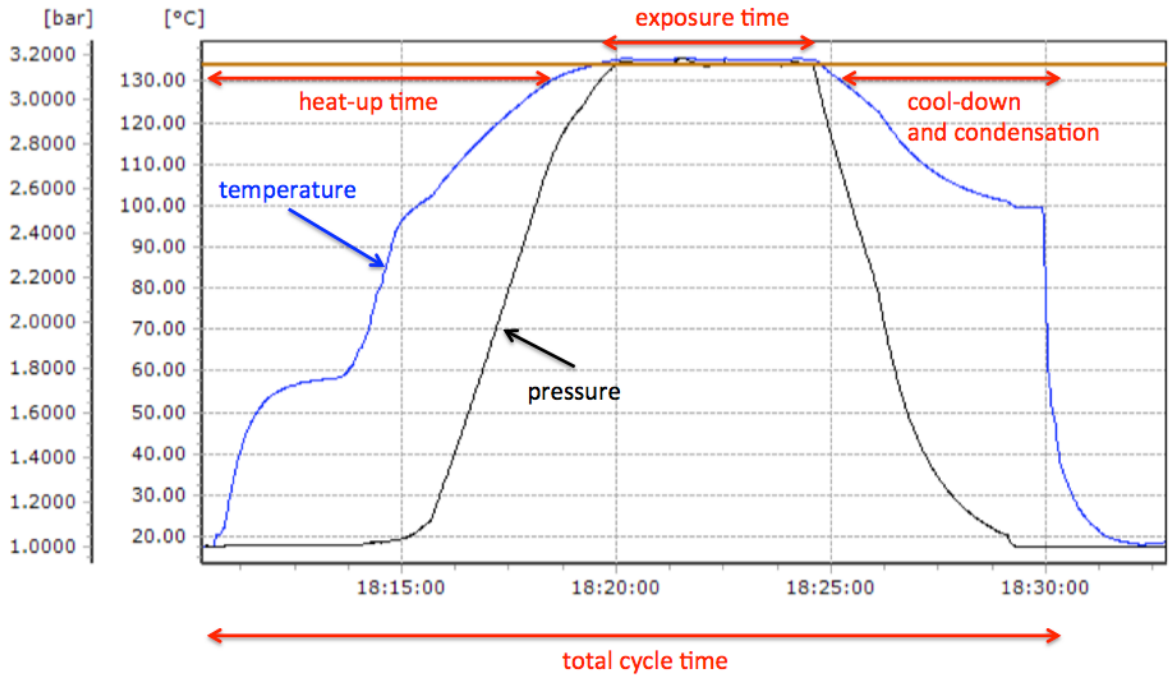

Figure 4-1 Example of a non-vacuum sterilization cycle

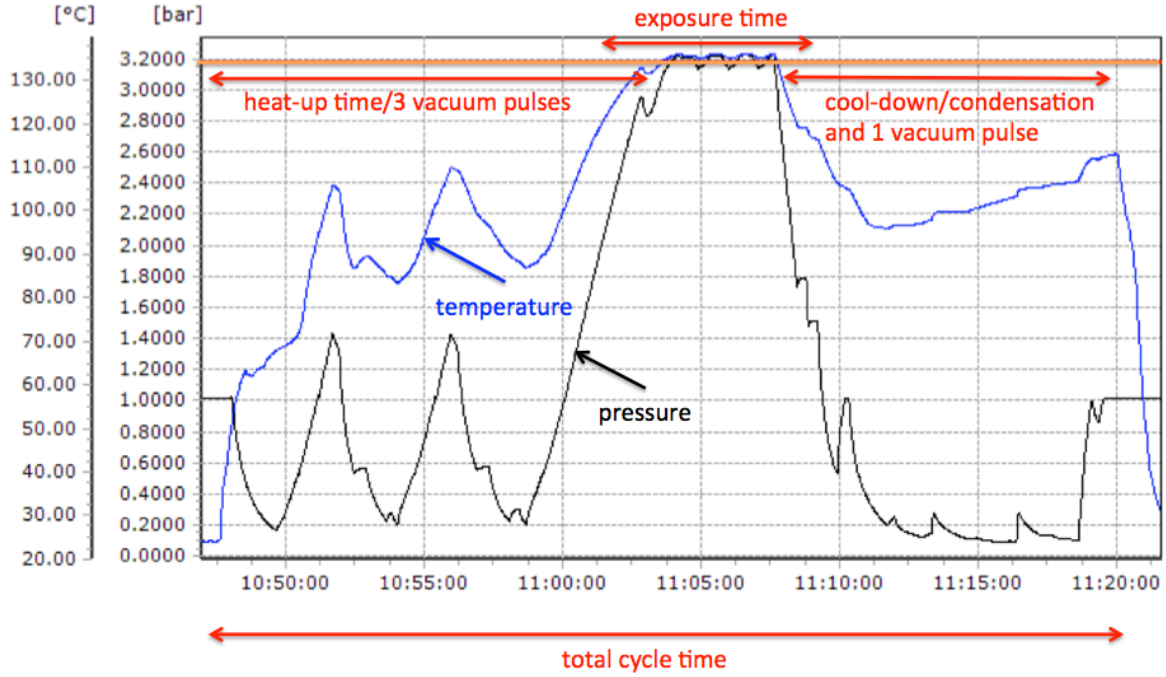

Figure 4-2 Example of a vacuum sterilization cycle with 3 pre-vacuum pulses and one post-vacuum pulse

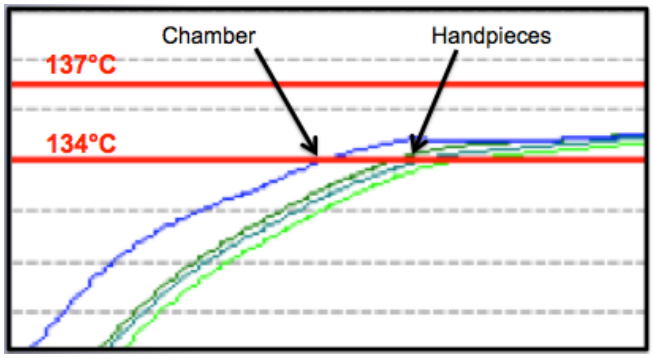

Figure 4-3 Example of measurement target: time difference between the chamber and the inside of the handpieces reaching sterilization temperature

4.2.1 Preliminary experiments

Preliminary experiments were performed in triplicate to determine whether there was a difference in temperature detectable in the handpieces (TA-98 C LED, W&H, Austria) as well as in the chamber of a Little Sister 3 non-vacuum sterilizer (Eschmann) using a warm up cycle before sterilization.

4.2.2 Type T thermocouples (cross section 2 mm x 1 mm)

Every ten cycles thermocouples (TC: Type T, Class 1 IEC, Flat Twin) were calibrated using a hot block (Ametek) and the pressure sensor was calibrated using a pressure calibrator (Druck). Both instruments have been validated by the United Kingdom Accreditation Service (UKAS). A data logger (Anville 825) and EaziVal SE software (Anville) were used to record and analyse the acquired data. New TC ends were made every three cycles and recordings were saved as pdf files.

Three dental turbines (TA-98 C LED, W&H) were dismantled and type T TC were carefully placed in different positions (A, B and C) along the drive air channel (Figure 4-4). After reassembling, handpieces were put through a non-vacuum sterilization cycle (Little sister 3, Eschmann). Small loads and full loads, where the small load was 0.5 kg and the full load was set up as per manufacturers' instructions (5 kg), were compared by using dental instruments, such as probes, mirrors and forceps. Experiments were performed in triplicate.

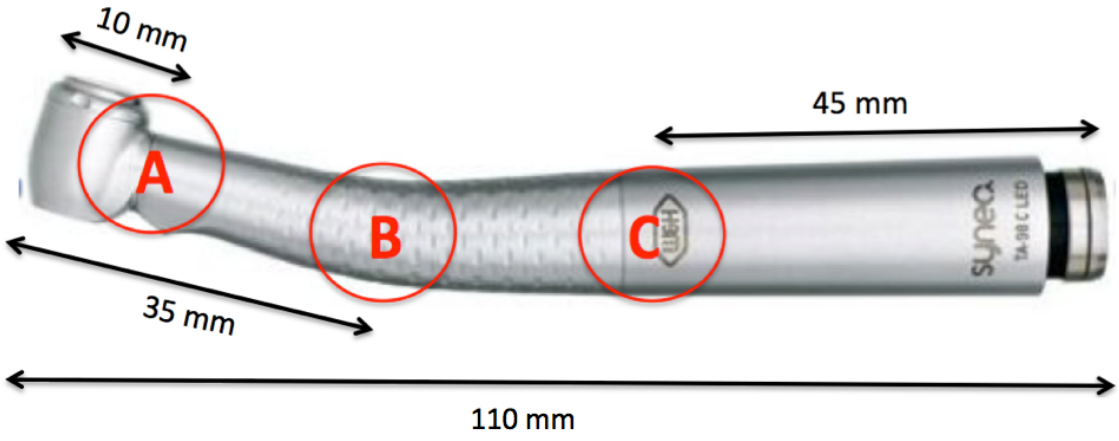

Figure 4-4 Assessed positions in TA-98; A-behind the turbine blade; B-inside the air channel (metal, d=2.3 mm, l=80 mm); C-inside the air channel (plastic)

The Bowie and Dick test pack (BDT) and the helix process challenge device (Browne Ltd.) were used as controls. The BDT was carefully opened using a scalpel and TC were placed in three positions (top, centre and bottom with 100 paper sheets between locations, as shown in figure 4-5. The test pack was re-sealed again using autoclave tape (3M). A non-vacuum sterilization cycle was performed.

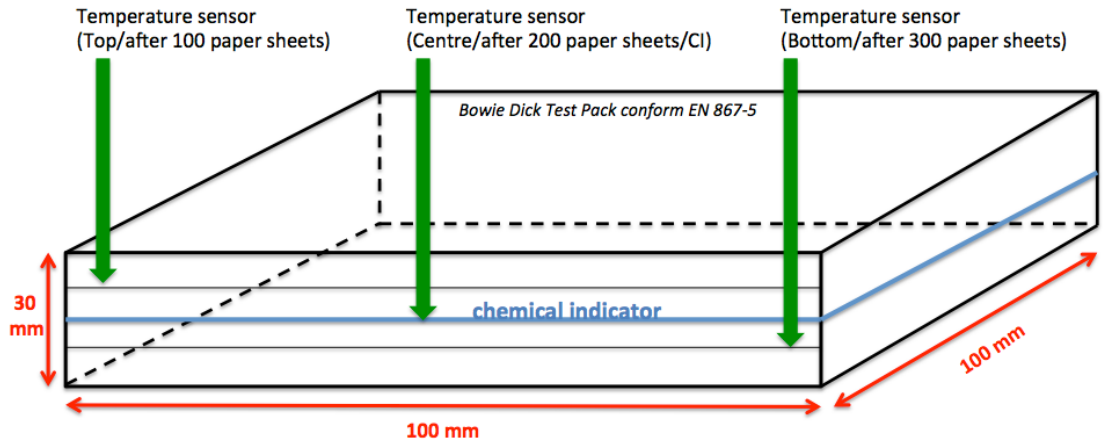

Figure 4-5 Schematic compositions of BDT and thermocouple positioning

4.2.3 Thin type T thermocouples (D = 0.8 mm)

Experiments described in section 4.2.2 were repeated using thinner TCs in order to record temperature in the air channel (D=2.3 mm). Additionally, three handpieces were used to measure temperature in location C of the spray channels (D=0.9). A comparison of the different TC and channels inside the turbines used are shown in figure 4-6 and 4-7. Calibration, recording and analysis were performed using the equipment listed in section 4.2.2.

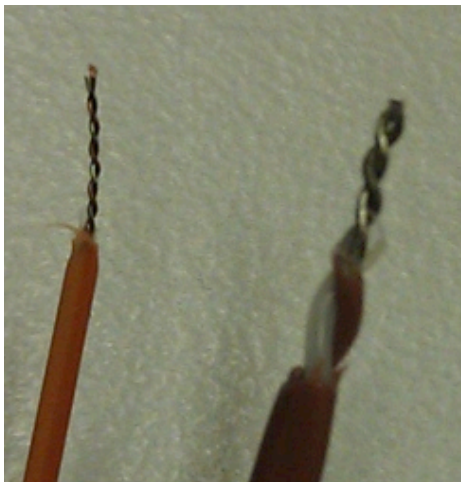

Figure 4-6 Comparison of type T thermocouples; thin type T (D=0.8) left and type T (2 mm x 1 mm) right

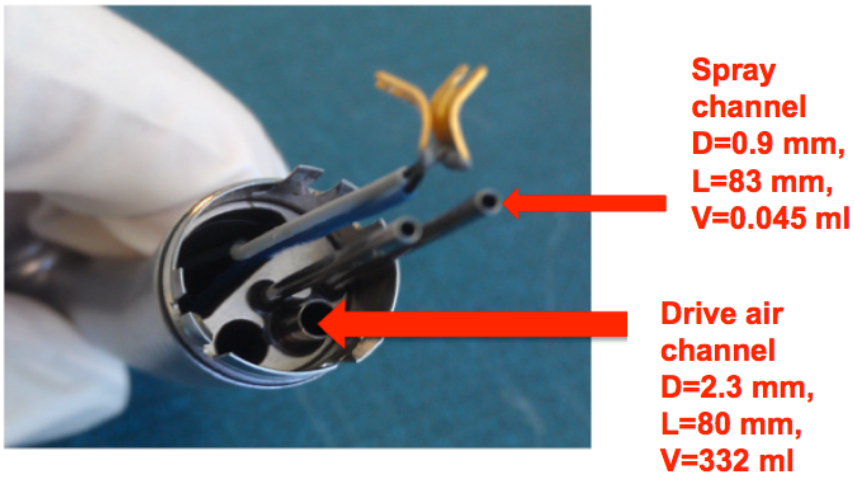

Figure 4-7 Inside of dental handpiece with channel dimensions

As controls the BDT and helix PCD were used, as well as a type T TC (described in section 4.2.2) for comparison of the differents TC. Experiments were performed in triplicate.

4.2.4 Data loggers (Ellab, D=2 mm, Teflon)

4.2.4.1 Dental air turbine

Experiments described in sections 4.2.2 and 4.2.3 were repeated using wireless data loggers (Ellab), shown in figure 4-8, which were validated annually at Ellab in Denmark. The data loggers were used to monitor temperature and pressure in different locations of the turbine (drive air channel) in a vacuum sterilization process (Lisa 517, W&H, Austria) as a comparison to the non-vacuum process. Ellab's ValSuit Basic software was used for analysing the recorded data. Reports were saved as pdf files. As a control the BDT was used and experiments were performed in triplicate.

Figure 4-8 Ellab Tracksense Pro data loggers in docking station Teflon and metal temperature sensors top row, pressure sensor bottom row

4.2.4.2 Other handpieces

Two different types of turbines (CROMA Bien Air, W&H Alegra) and two motors (KaVo Powertorque, W&H toplight) each in sets of three were used to investigate steam penetration into lumens as determined by thermometric measurements (ellab data loggers) using non-vacuum sterilization (Little Sister3, Eschmann).

4.2.5 Chemical indicator study

Three different types of dental handpieces were used

- Air turbines (TA-98 C LED, W&H, Austria)
- Slow speed motors (WA-56, W&H, Austria)
- Surgical handpieces (S11, W&H, Austria)

Chemical indicator strips (Browne, class 2), usually used for detection of time/temperature/presence of steam in the Browne helix PCD were cut into small pieces (l= 22 mm), as shown in figure 4-9, and placed into 3 different locations inside the different handpieces. Handpieces were reassembled and placed in a type N sterilizer (LittleSister3, Eschmann). As a control, the helix PCD (Browne) was placed in the chamber of the sterilizer. These CI are designed to change colour from yellow to dark blue in the presence of saturated steam at 134°C for 3 min (867-4:2001, BS EN).

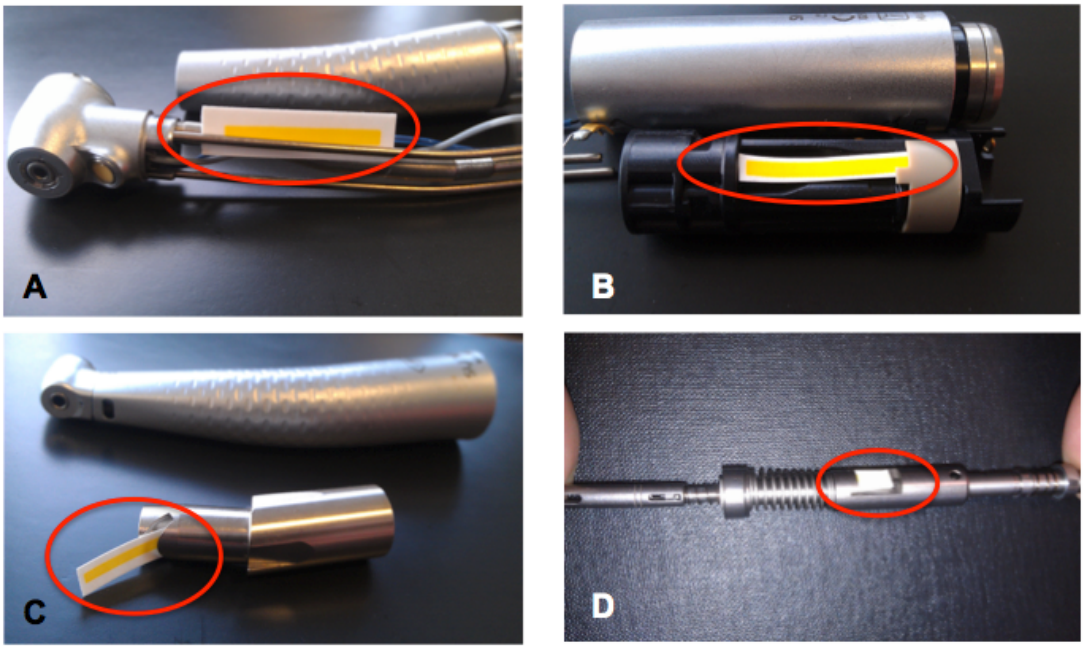

Figure 4-9 Chemical indicator strips in high-speed turbine head (TA-98) (A), back of high-speed turbine (TA-98) (B), slow-speed motor WA-56 (C) and gear of S11 surgical handpiece (D)

Additionally, CI strips were cut into small strips (dimensions can be found in figure 4-10) and placed inside the channels and the head of 12 air turbines. After a non-vacuum sterilization cycle (Little Sister 3, Eschmann), CI were removed from the handpieces and visually assessed for colour change.

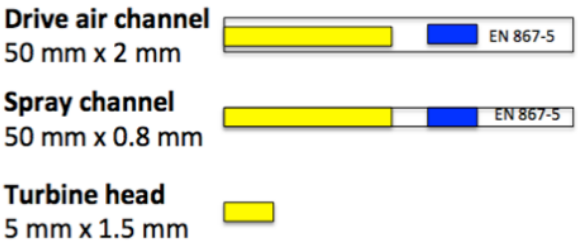

Figure 4-10 Chemical indicator strips with dimensions for channels and turbine head

4.2.6 Biological indicator study

4.2.6.1 Vegetative bacteria

In a separate set of experiments *Staphylococcus aureus, Streptococcus mutans* and saliva were used as biological indicators due to the fact that researchers found these contaminants on dental handpieces in *in-vivo* investigations after patient treatment (Dreyer and Hauman, 2001, Smith et al., 2014).

4.2.6.1.1 Dental air turbine

Circular filter paper discs (Whatman filter paper) were cut (D=0.5 cm) and submerged into bacterial suspensions of *S. aureus* (ATCC 25923) and *S. mutans* (NCTC 10449) in tryptone soy broth (TSB, Sigma), which were prepared as per chapter 2, section 2.1.4.2, dried for 30 minutes and placed into the back cap of the turbines. 14 TA-98 turbines were contaminated with 10^2 cfu/ml of *S. aureus*, 20 handpieces were contaminated with 10^6 cfu/ml of *S. aureus* and 24 handpieces were contaminated with 10^6 cfu/ml *S. mutans*. Concentrations of bacteria in broth were prepared by serial diluting 24 h cultures and plating them onto TSA (tryptone soy agar, Oxoid) and blood agar (Prestige Medical, Omega Media) in order to get initial cfu counts. The turbines were reassembled and placed in a Little Sister 3 (Eschmann). All handpieces were processed using a non-vacuum sterilization cycle. While 28 handpieces subjected to the sterilization process accompanied with a drying step, where the sterilizer door was kept locked for an additional 10 min, 32 handpieces were processed using the same cycle without drying. After the sterilization process, the filter paper discs were transferred into 1 ml TS broth (tryptone soy broth, Sigma) and incubated for 24 h at 37°C. The samples containing *S. aureus* were incubated in air, the samples with *S. mutans* in 5% CO_2.

24 high-speed TA-98 turbines were contaminated with 10^7 cfu/ml of *S. mutans* by pipetting 100 μl of the bacterial suspension directly into the back caps of the turbines; caps were left for drying for 60 minutes at room temperature. After reassembling the handpieces were processed using a type non-vacuum sterilization cycle (LittleSister3, Eschmann). One half of the handpieces were processed with drying conditions, the other half without drying. Once the

sterilization process was finished, the back caps of the turbines were transferred into 1 ml TS broth (tryptone soy broth, Sigma) and incubated over night at 37°C in 5% CO_2.

100 µl of suspension (10^8 cfu/ml) were injected into each spray channel of 24 high-speed air turbines (TA-98 C LED, W&H, Austria). 12 turbines were processed using a type N sterilization process with drying, 12 turbines were processed without drying (LittleSister3, Eschmann). Once the sterilization process was finished, the spray channels were flushed with 1 ml TSB each and incubated for 24 h at 37°C. As a recovery control, the spray channels of three handpieces were contaminated and flushed with TSB. Flushing the spray channels of three uncontaminated handpieces represented a negative control.

Filter paper strips (Whatman) were cut (1 x 0.5 cm) and submerged into 1 ml unstimulated saliva (collected from laboratory staff, 7 x 10^7 cfu/ml) and placed into the metal part of the drive air channel (front part of handpiece) and in the plastic part (back part of handpiece) of 9 high-speed air turbines (TA-98 C LED, W&H, Austria). 100 µl of saliva were pipetted around the turbine blade and injected into every spray channel. The handpieces were reassembled and processed using a non-vacuum sterilization cycle without drying (LittleSister3, Eschmann). After attempts at sterilization, all filter paper strips and turbine blades were transferred into 1 ml TSB and incubated at 37°C, 5% CO_2. Spray channels were flushed with 1 ml TSB (22 times the volume of channels).

4.2.6.1.2 Process challenge device

Circular filter paper discs (Whatman) were cut (D=0.5 cm) and submerged into bacterial suspensions of *S. mutans* and *S. aureus*, dried for 30 minutes and placed into the capsule of a helix PCD (Browne). One helix PCD was contaminated with 10^2 cfu/ml of *S. aureus*; a process challenge device was contaminated with 10^6 cfu/ml of *S. mutans* (repeated 6 times) and 10^6 cfu/ml of *S. aureus* (repeated 4 times). The helix was placed in a non-vacuum sterilizer (LittleSister3, Eschmann). Six cycles with drying and 5 cycles without drying were used. After the sterilization process, the filter paper discs were transferred

into 1 ml TS broth (tryptone soy broth, Sigma) and incubated aerobically for 24 h at 37°C (*S. aureus*) and 5% CO_2 for 24 h (*S. mutans*).

4.2.6.1.3 Influence of handpiece oil on bacterial inactivation

The turbine blades of 16 high-speed air turbines were contaminated with 10^5 cfu/ml of *S. mutans* and the turbine blades of 72 turbines were contaminated with 10^7 cfu/ml of *S. mutans* by submerging them into the bacterial suspensions; turbine blades were left for drying for 60 minutes. After drying the blades of 36 air turbines were submerged in 5 ml f1 handpiece oil (W&H, Austria). After reassembling, 12 handpieces with and 12 without oil were placed in a hot air oven at 80°C for 10 minutes. 16 handpieces contaminated with 10^5 cfu/ml, 24 handpieces contaminated with 10^7 cfu/ml and 24 handpieces contaminated with 10^7 cfu/ml and covered in oil were processed in a non-vacuum sterilizer (LittleSister3, Eschmann). One half of the handpieces were processed with drying, the other half without drying. Once the sterilization process was finished, the turbine blades were transferred into 1 ml TS broth (tryptone soy broth, Sigma) and incubated over night at 37°C with 5% CO_2.

4.2.6.2 *Geobacillus stearothermophilus* spores

Biological indicator strips (Excelsior), with approx. 2.5 x 10^6 spores of *G. stearothermophilus* per strip (2 mm x 10 mm, D_{121}=2.2 min), were placed inside the turbine head, the metal and the plastic part of the drive air channel of four dental air turbines (TA-98 C LED, W&H, Austria). Processing in a non-vacuum sterilizer (Little Sister 3, Eschmann) followed. As a control, biological indicator strips were placed in the Browne helix PCD as well as two sealed glass bottles (Schott), which were processed in the same way. As a positive control, a biological indicator strip was placed in 10 ml TSB without being processed for sterilization, incubated at 56ºC for 8 days.

After attempts at sterilization, handpieces were dismantled and the indicator strips were transferred into 2 ml TSB and incubated at 56ºC. Growth was checked every 24 h for 8 days. Controls underwent the same procedure. The experiment was repeated three times.

4.2.7 Handpiece test load experiments

For this investigation four different non-vacuum sterilizers (3x MS22 (W&H), 3x Alpha (Prestige) and 3x Kronos (Newmed) and 3x Little Sister 3 (Eschmann)) were tested and compared to a vacuum sterilizer (3x Lisa, W&H).

For each sterilization cycle a standard test load, as shown in figure 4-11, was used (refer to chapter 2, section 2.1.8). For each sterilizer a BDT was used as a control.

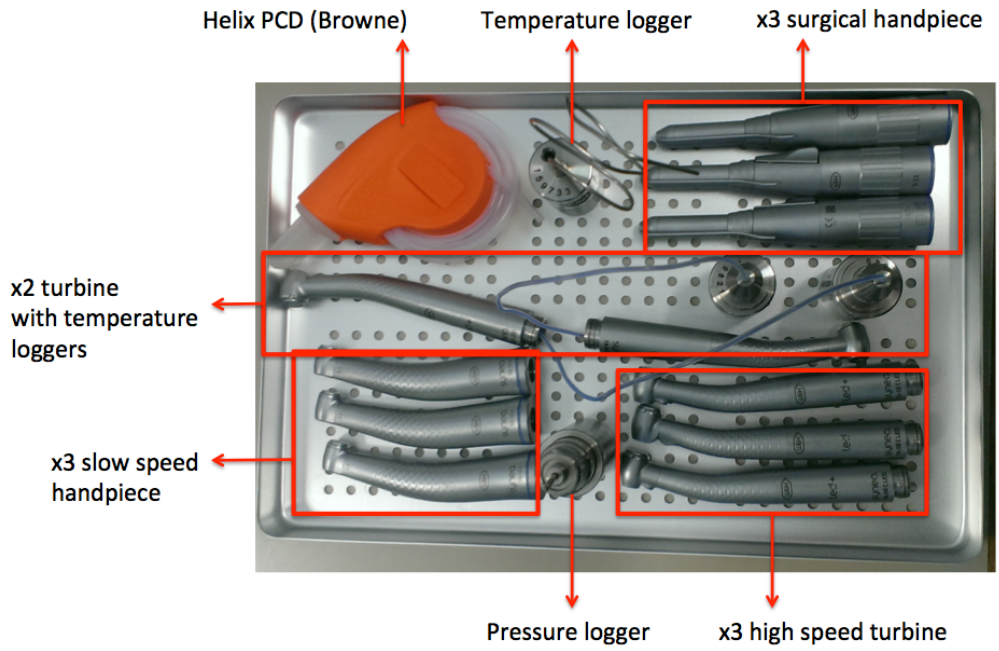

Figure 4-11 Standard test load with 3x dental air turbine TA-98 C LED (W&H, Austria), 3x straight surgical handpiece S11 (W&H, Austria), 3x slow speed motor WA-56 (W&H, Austria), 1x helix PCD (Browne), 2x temperature data logger inside dental air turbine (TA-98 C LED (W&H, Austria), 1x temperature data logger "free space" and 1x pressure data logger (all data loggers Ellab), total weight 0.5 kg

Small loads and full loads (as per manufacturer's instructions) were compared and experiments were performed in triplicate at least. Data analysis from data loggers was performed as described in section 4.2.4.1. According to Perkins the heat up time should bring all of the load up to sterilization temperature (Perkins, 1983). In the standard for small steam sterilizers a tolerable time

difference between the chamber and the load is 15 sec (13060:2014, BS EN), while the Scottish Health Technical Memorandum established a temperature lag of 2°C from the point where the chamber reaches 134°C compared to the load as tolerable (SHTM, 2010).

In order to take all three theories into account, time delays of 3 sec (based on results from vacuum cycle testing), 15 sec and a temperature lag of 2°C were established as thermometric fails and compared.

4.3 Results

4.3.1 Preliminary experiments

No differences were observed between temperature recordings inside the handpieces and the chamber when comparing a cold or pre-heated sterilizer chamber, as shown in figures 4-12 and 4-13, respectively.

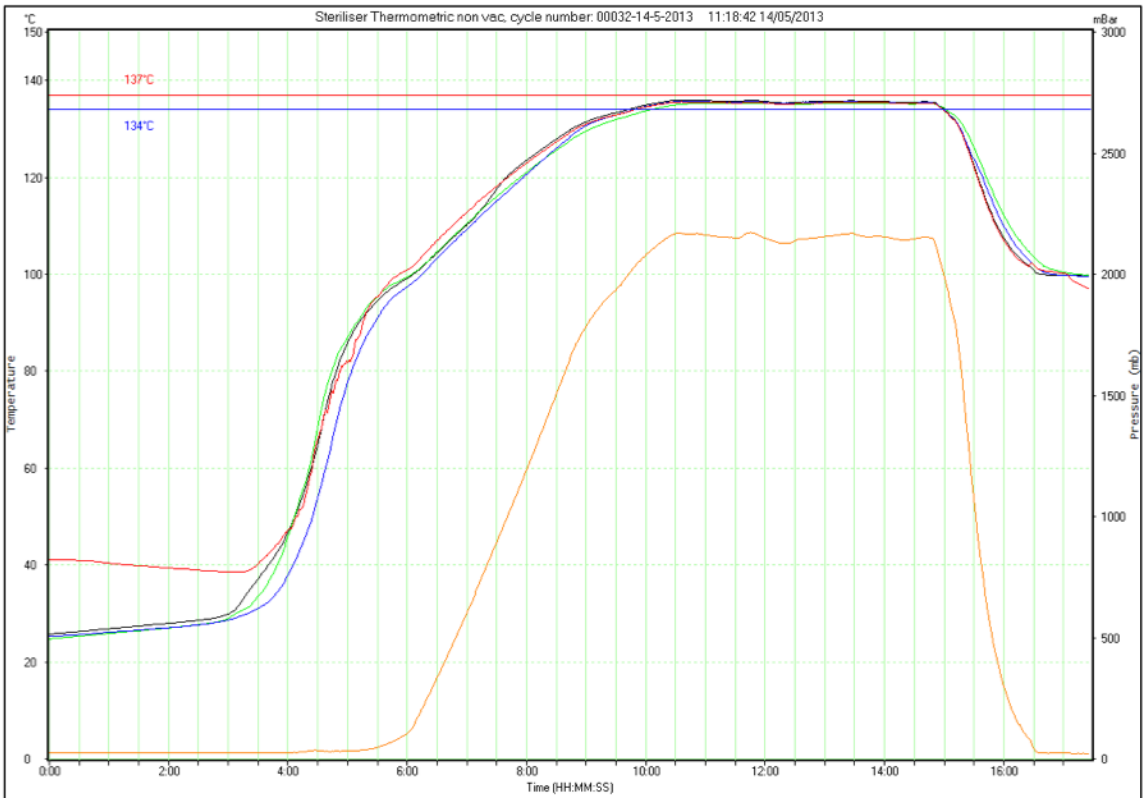

Figure 4-12 Example of non-vacuum sterilization cycle from cold chamber (orange=pressure, red=free chamber space, black, green, blue=inside handpiece) the horizontal blue and red lines indicate sterilization temperature range of 134 - 137°C

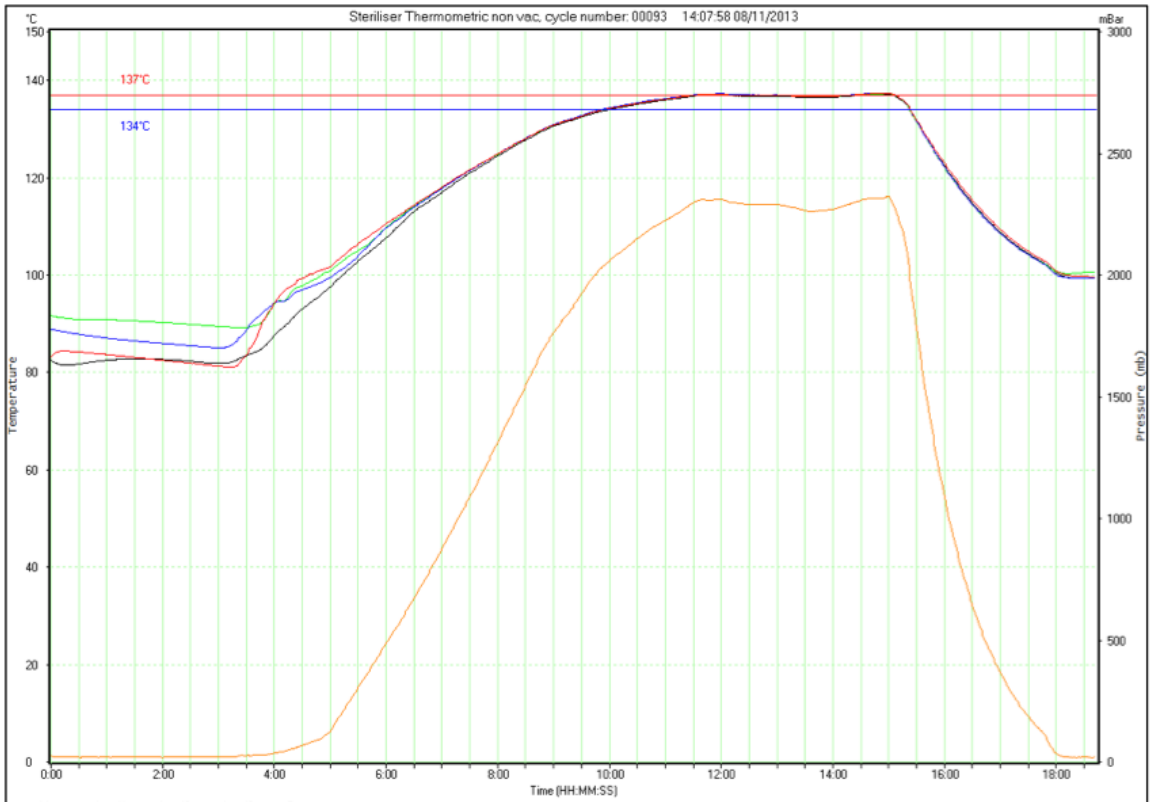

Figure 4-13 Example of non-vacuum sterilization cycle from pre-heated chamber (orange=pressure, red=free chamber space, black, green, blue=inside handpiece) the horizontal blue and red lines indicate sterilization temperature range of 134 - 137°C

4.3.2 Type T thermocouples (2 mm x 1 mm)

Results of temperature traces at different positions using non-vacuum and vacuum sterilization cycles are shown in close up charts of the time at plateau temperature (Fig 4-14 – 4-16). The results show that it takes longer to achieve sterilizing temperature (134-137°C) in handpieces . Position A shows a temperature lag of -4 - 24 sec compared to the chamber temperature, while sterilizing temperature in positions B and C was delayed by -1 - 14 and -1 - 150 sec, respectively. However, the temperature at all positions in the handpieces reached 134°C before the display on the sterilizer announced “S”, indicating that the “sterilizing” period was under way, which lasted 3 min and 15 sec after equilibration (time to ensure temperature above 134°C) in this model of sterilizer. In contrast, the Bowie and Dick test pack (positions “Bottom BDT” and “Centre BDT”) did not reach sterilizing temperature during plateau time at 134°C (Figure 4-17) in the non-vacuum sterilization cycle. Full loads were not

investigated in the Little Sister 3, because each cycle with a load of 5 kg failed before sterilization temperature was achieved.

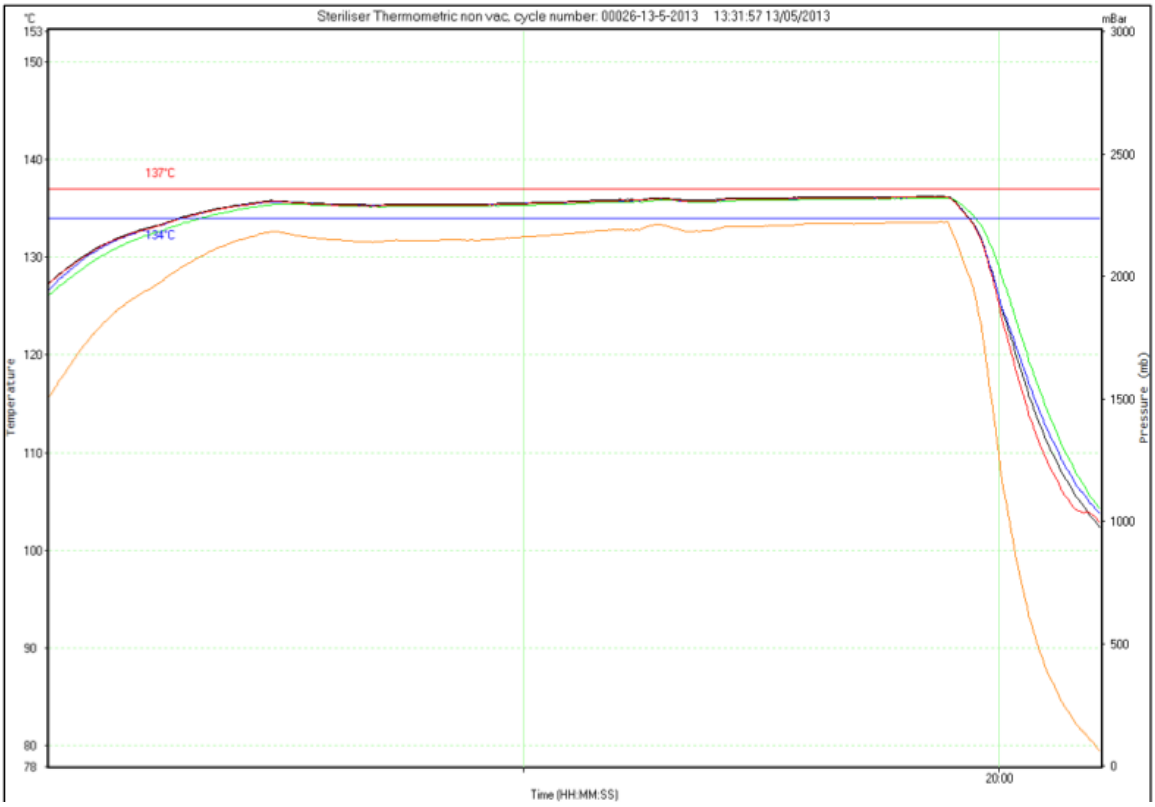

Figure 4-14 Demonstrating thermocouple type T results of experiments assessing position A in the handpiece (magnification of plateau period) using a non-vacuum sterilization cycle (orange=pressure, red=free chamber space, black, green, blue=inside handpiece) the horizontal blue and red lines indicate sterilization temperature range of 134 - 137°C

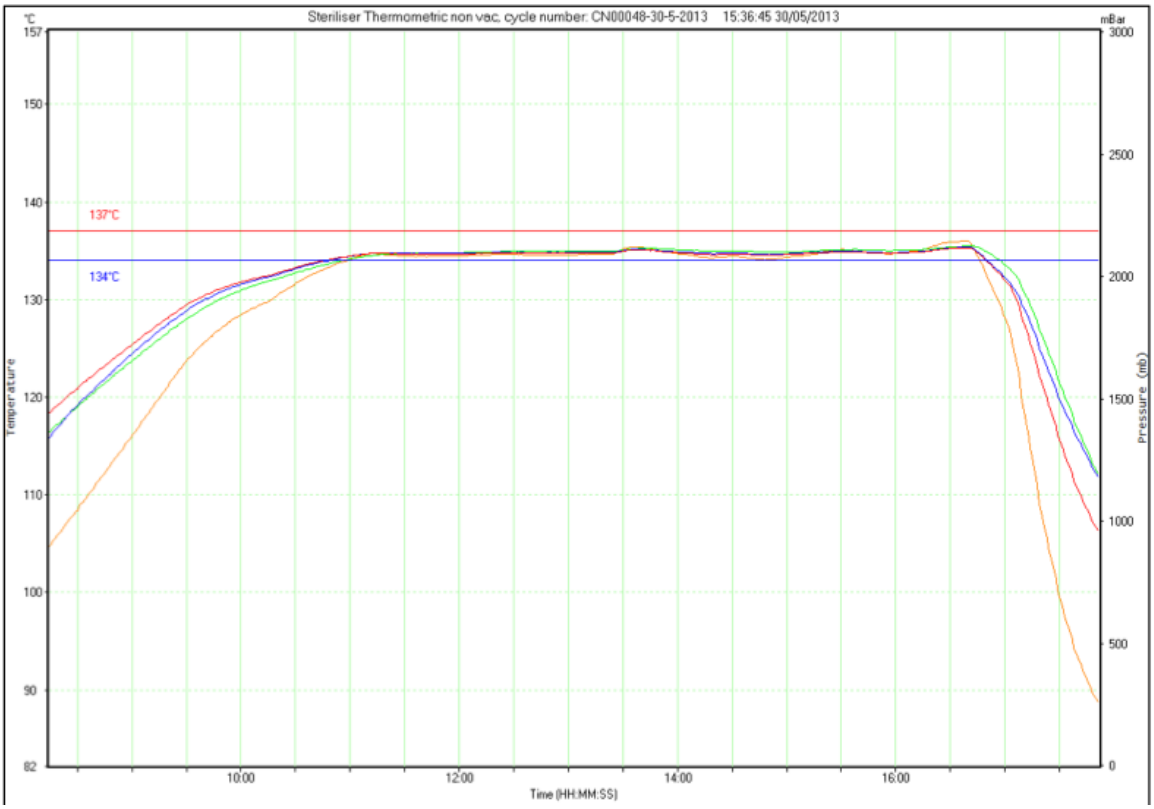

Figure 4-15 Demonstrating thermocouple type T results of experiments assessing position B in the handpiece (magnification of plateau period) using a non-vacuum sterilization cycle (orange=pressure, red=free chamber space, black, green, blue=inside handpiece) the horizontal blue and red lines indicate sterilization temperature range of 134 - 137°C

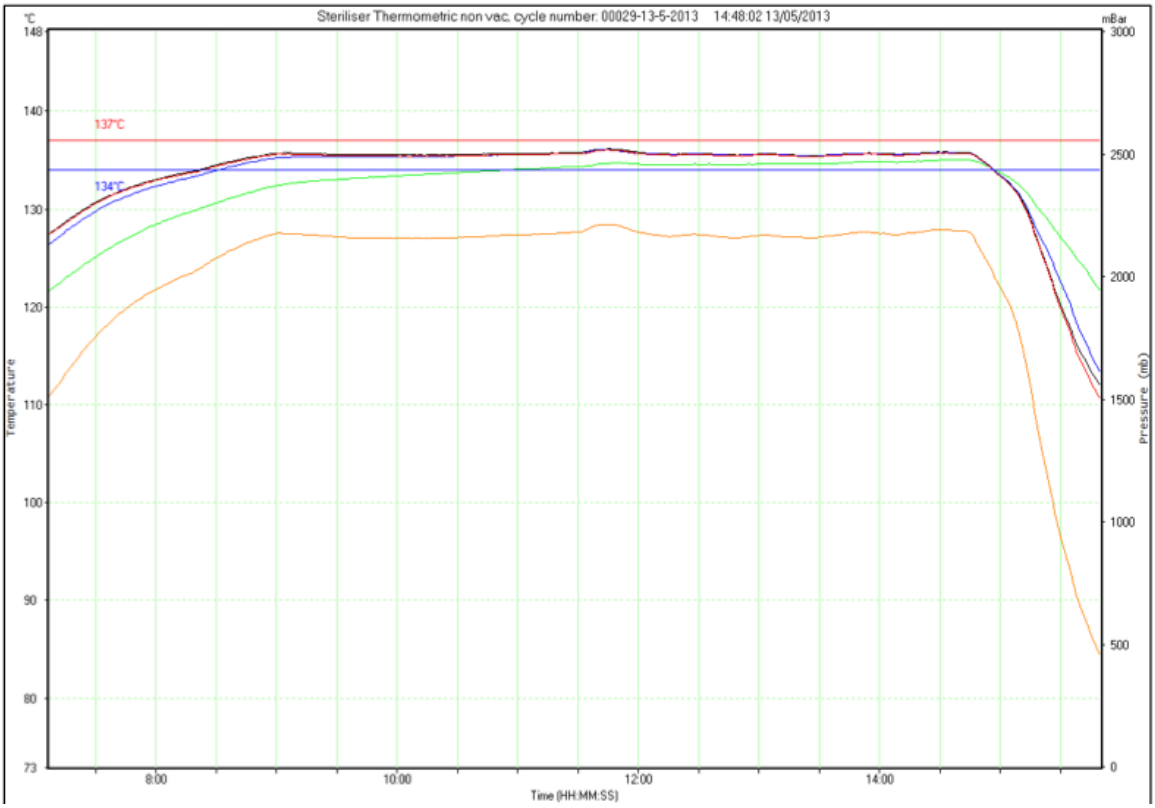

Figure 4-16 Demonstrating thermocouple type T results of experiments assessing position C in the handpiece (magnification of plateau period) using a non-vacuum sterilization cycle (orange=pressure, red=free chamber space, black, green, blue=inside handpiece) the horizontal blue and red lines indicate sterilization temperature range of 134 - 137°C

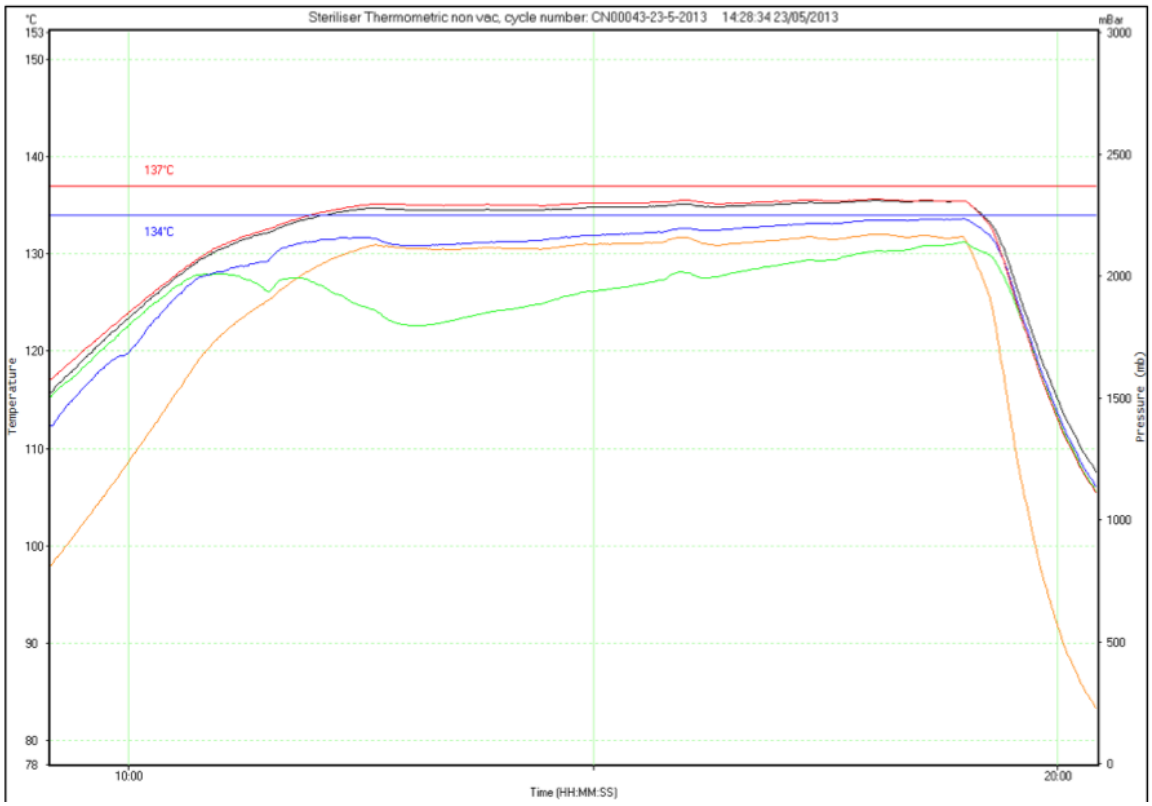

Figure 4-17 Demonstrating thermocouple type T results of experiments assessing BDT (magnification of plateau period) using a non-vacuum sterilization cycle (orange=pressure, red=free chamber space, black=top BDT, blue=bottom BDT, green=centre BDT) the horizontal blue and red lines indicate sterilization temperature range of 134 - 137°C

4.3.3 Thin type T thermocouples (D = 0.8 mm)

Recordings using the thin type T TC in different locations of the handpieces showed similar results to those described in section 4.5.1.2, where the temperature lag inside the handpiece ranged from -7 – 66 sec. Temperature traces from inside the spray channels showed a temperature lag of -1 - 74 sec compared to the chamber shown in figure 4-18. Type T and thin type T TC were in line with each other. The BDT traces showed that the centre of the test pack did not reach sterilization temperature.

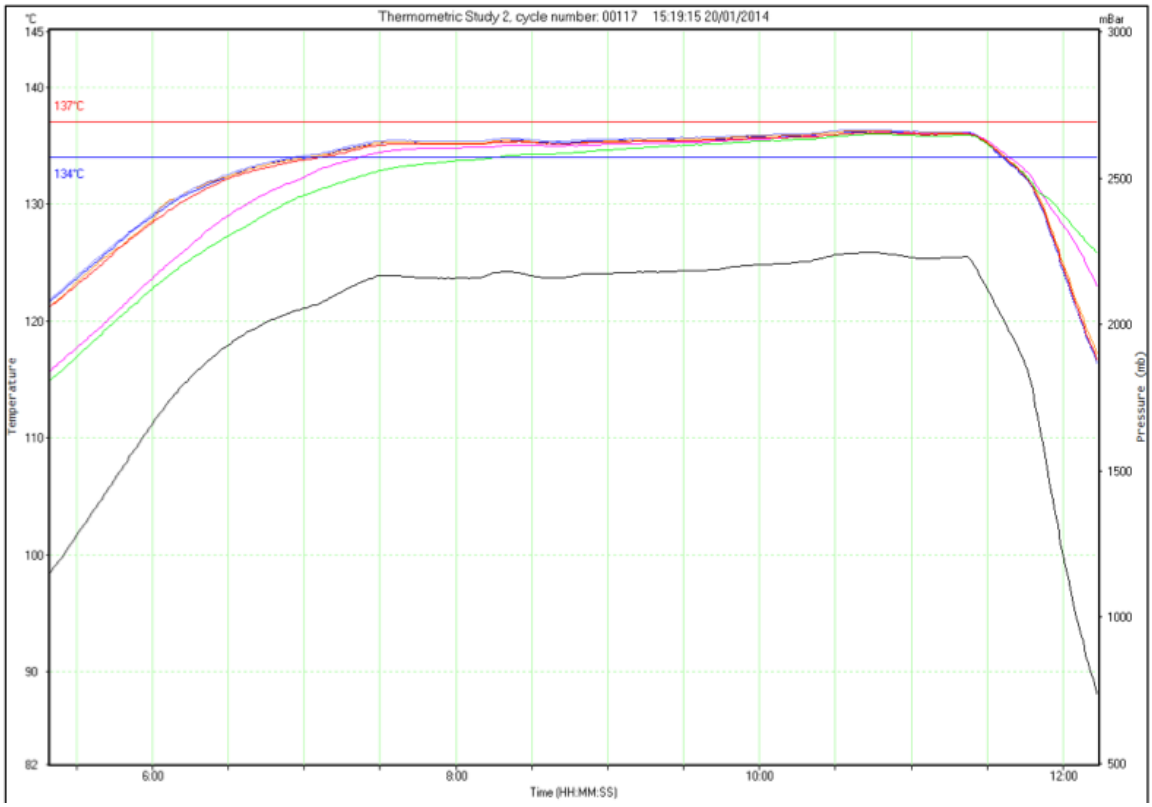

Figure 4-18 Demonstrating thermocouple thin type T results of experiments assessing spray channels in the handpiece (magnification of plateau period) using a non-vacuum sterilization cycle (black=pressure, blue=free chamber space, red, magenta, green=inside spray channels) the horizontal blue and red lines indicate sterilization temperature range of 134 - 137°C

4.3.4 Data loggers

4.3.4.1 Air turbine TA-98 C LED (W&H, Austria)

Thermometric results using data loggers did not differ from the results acquired with thermocouples in the non-vacuum sterilization cycle. Handpieces showed a 15 – 100 sec delay in reaching the same temperature as the chamber. Using a vacuum cycle (Lisa, W&H) thermometric measurements showed a time difference of -1 - 3 sec between the inside of handpieces compared to the chamber of the sterilizer. No differences were observed between locations A, B and C (Fig 4-19 – 4-21). The temperature traces from within the BDT shows that all positions achieved sterilization temperature (Fig 4-22).

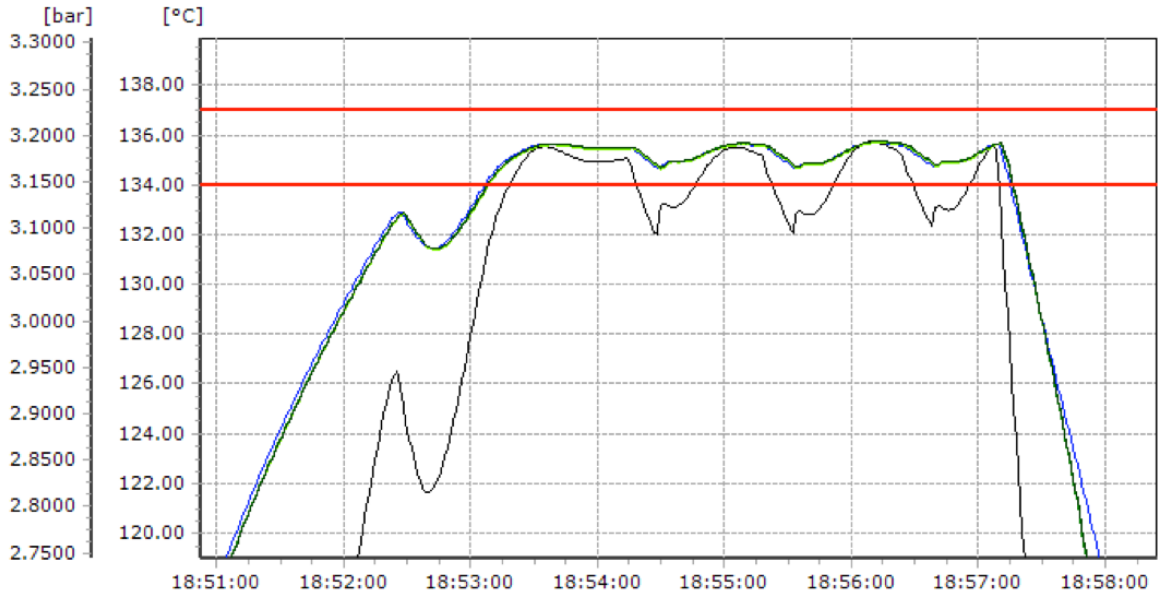

Figure 4-19 Demonstrating data logger results of experiments assessing position A in the handpiece (magnification of plateau period) using a vacuum sterilization cycle (black=pressure, blue=free chamber space, light green, green, dark green=inside handpiece), the horizontal red lines indicate sterilization temperature range of 134 - 137°C

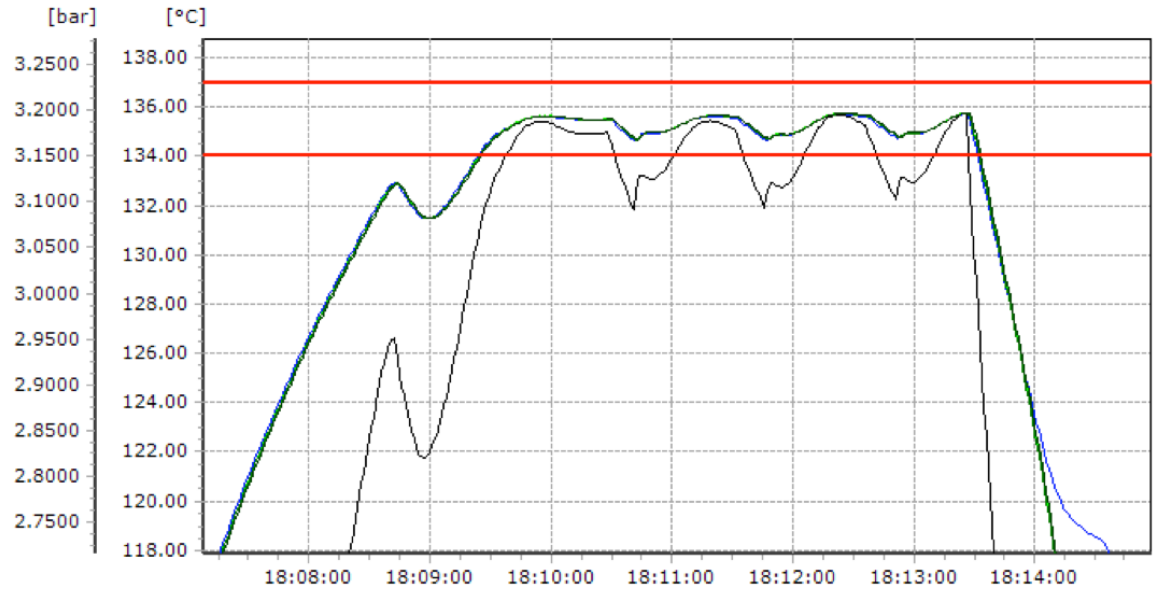

Figure 4-20 Demonstrating data logger results of experiments assessing position B in the handpiece (magnification of plateau period) using a vacuum sterilization cycle (black=pressure, blue=free chamber space, light green, green, dark green=inside handpiece), the horizontal red lines indicate sterilization temperature range of 134 - 137°C

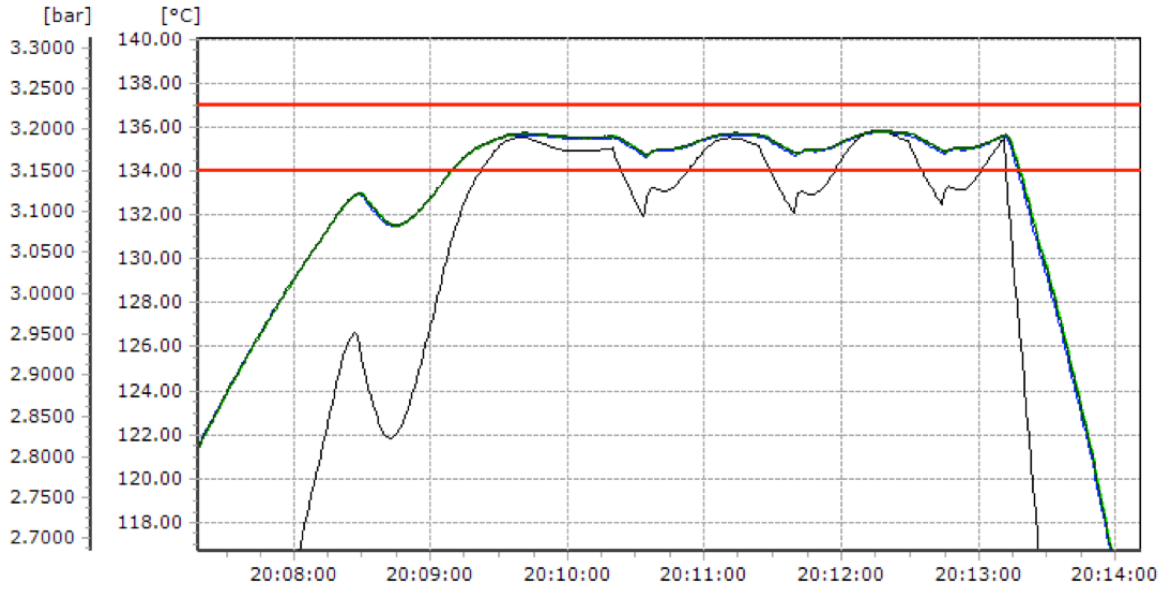

Figure 4-21 Demonstrating data logger results of experiments assessing position C in the handpiece (magnification of plateau period) using a vacuum sterilization cycle (black=pressure, blue=free chamber space, light green, green, dark green=inside handpiece), the horizontal red lines indicate sterilization temperature range of 134 - 137°C

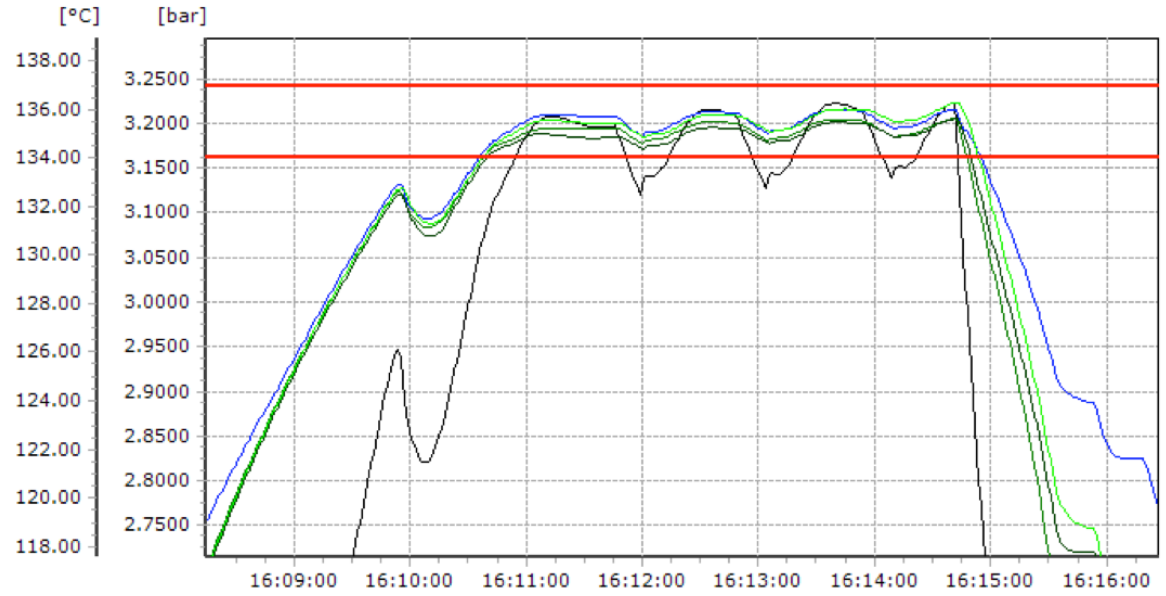

Figure 4-22 Demonstrating data logger results of experiments assessing BDT (magnification of plateau period) using a vacuum sterilization cycle (black=pressure, blue=free chamber space, light green=top BDT, green=bottom BDT, dark green=centre of BDT), the horizontal red lines indicate sterilization temperature range of 134 - 137°C

4.3.4.2 Other handpieces

Measuring temperature in different handpiece models resulted in a time difference of -1 to 147 sec between the chamber and the inside of the handpiece. Details are shown in table 4-1.

Table 4-1 Summary of time differences of inside other handpiece models compared to chamber temperature

Non-vacuum sterilization cycle (plateau time 6.5 min)	Croma Bien Air high speed turbine (N=9)	W&H Alegra high speed turbine (N=9)	Kavo Powertorque motor (N=9)	W&H Toplight 898 motor (N=9)
Time difference between chamber and handpiece to reach 134°C	23 - 80 sec	18 – 147 sec	13 – 38 sec	-1 – 8 sec

4.3.5 Chemical indicator study

Chemical indicators changed colour and indicated pass conditions in all tested locations and handpieces, as shown in figures 4-23 and 4-24, but failed in the helix PCD.

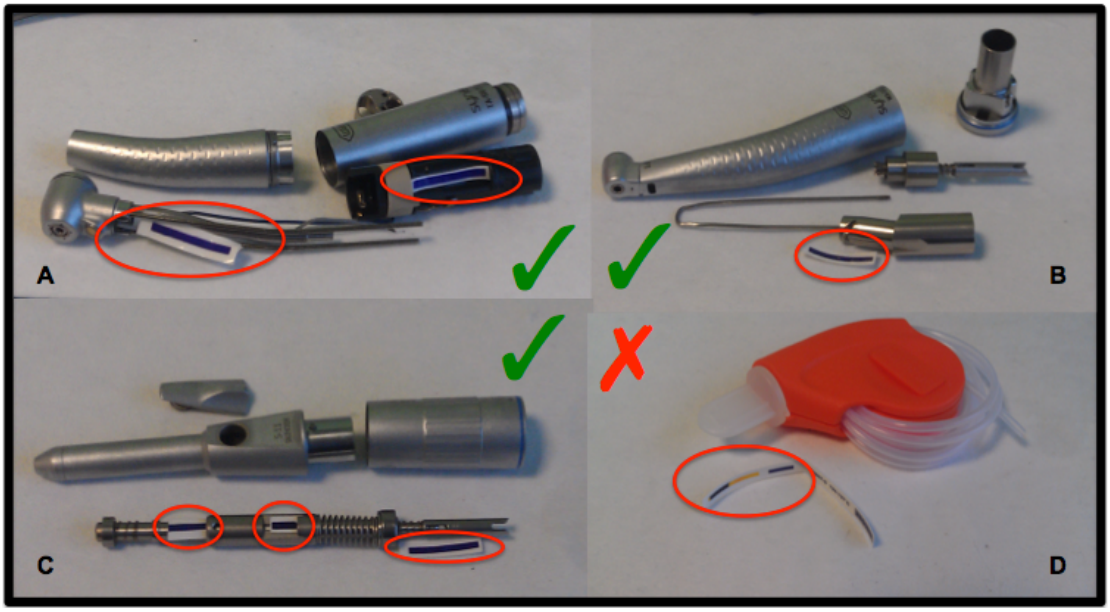

Figure 4-23 Indicator strips in a high-speed turbine (TA-98), B slow speed motor (WA-56) and C surgical handpiece (S11) show a pass, D (control helix PCD) shows a fail

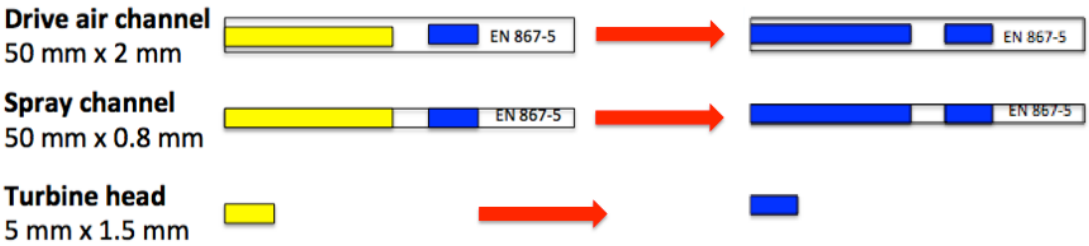

Figure 4-24 Indicator strips in drive air channel, spray channels and turbine head of TA-98 show passes

4.3.6 Biological indicator study

4.3.6.1 Vegetative bacteria

All negative control handpieces (TA-98 C LED, W&H, Austria; N=38) were sterilized in a type B sterilizer (Lisa, W&H, Austria). No growth was detectable on any of the 38 contaminated handpieces. Positive controls showed growth. The recovery control showed that $16x10^6$ cfu were recovered after contamination of stainless steel spray channels with 10^8 cfu S. *mutans*.

Non-vacuum sterilization processes (with and without drying) indicated no growth on all tested instruments, locations and concentrations of contamination, while the positive controls showed growth. Handpiece oil had no effect on the lethality of the sterilization process.

In contrast, use of 80°C hot air oven for 10 min: S. *mutans* showed growth in both cases (with and without oil), after transferring the filter paper into TSB and incubating at 37°C for 24 h, as well as controls.

4.3.6.2 Spores

No viable spores were recovered, as there was no growth of bacteria from strips taken from the instruments subjected to the non-vacuum cycle, while positive controls as well as the helix PCD showed growth of the test organisms.

4.3.7 Handpiece test load experiments

Three non-vacuum sterilizers MS22 (W&H, Austria) were used in this series of tests. Pressure traces show that the cycle performed 8 positive pressure pulses at 1.6 bar. The overall cycle time was 40 minutes with a plateau time of 4 min at 134°C (Figure 4-25). No BI fails and no CI fails were found in any of the 324 test samples (Tables 4-2 – 4-7). No differences were observed between the small load (0.5 kg) and the manufacturer's recommended full load (2 kg). Time difference between handpieces and chamber reaching sterilization temperature ranged from -1 – 3 sec. Therefore, all 36 handpieces tested performed

thermometric passes, using three different analysis methods, while CIs in the BDT passed and failed in the helix PCD.

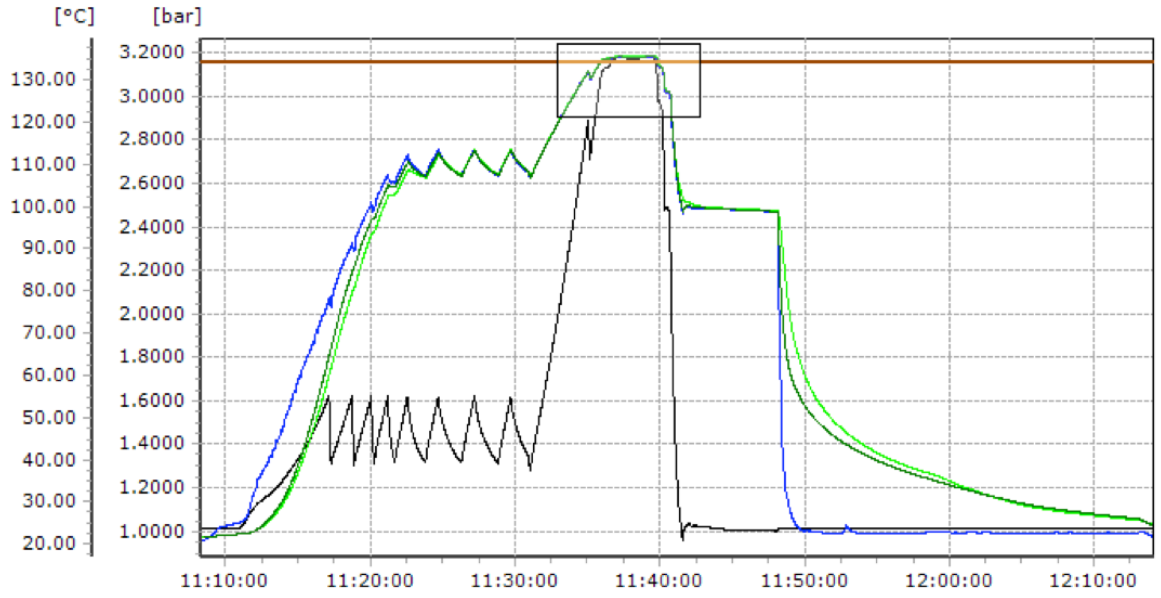

Figure 4-25 Cycle profile of non-vacuum sterilizer MS22 (W&H), total cycle time is 40 minutes with a plateau time of 4 min (black=pressure, blue=free chamber space, light green, dark green=inside handpiece), the horizontal orange line indicates sterilization temperature 134°C

Three non-vacuum Alpha (Prestige) were tested. The cycle profile did not show pressure pulses and the overall cycle time was 35 minutes with a plateau time of 3.5 min at 134°C (Figure 4-26). Out of 342 samples tested there were five BI fails and six CI fails (Tables 4-8 – 4-13). No differences were observed between small load (0.5 kg) and full load (6 kg as per manufacturer's instructions). Time difference between handpieces and chamber (N= 38) reaching the optimum (range = 25 – 40 sec) resulted in thermometric fails in addition the CI in the BDT and helix PCD failed.

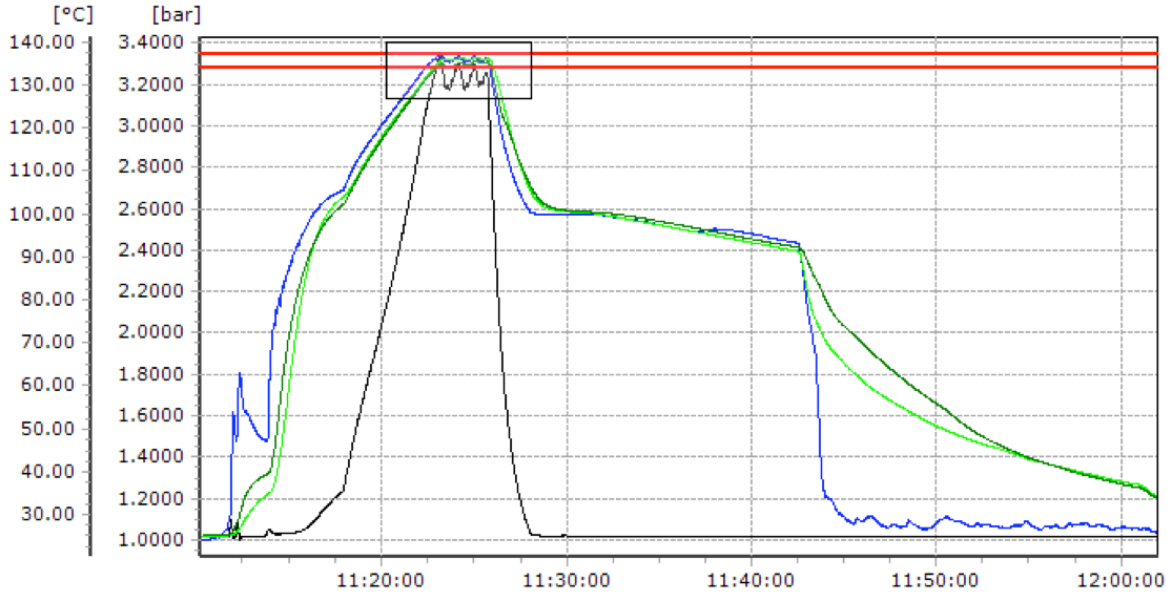

Figure 4-26 Cycle profile of non-vacuum sterilizer Alpha (Prestige), total cycle time is 35 minutes with a plateau time of 3.5 min (black=pressure, blue=free chamber space, light green, dark green=inside handpiece), the horizontal red lines indicate sterilization temperature range of 134-137°C

Three non-vacuum Kronos (Newmed) were tested. Pressure traces show steam injection (3 positive pressure pulses at 2 bar). The overall cycle time was 30 minutes with a plateau time of 6.5 min at 134°C (Figure 4-27). One BI failed but no CI fails were found in any of the 324 samples tested (Tables 4-14 – 4-19).

No differences were observed between the small load (0.5 kg) and the manufacturer's recommended 4 kg full load. Time difference between the 36 handpieces tested and chamber ranged from 25 – 39 sec. There were 36 thermometric fails and both the BDT and the helix PCD failed.

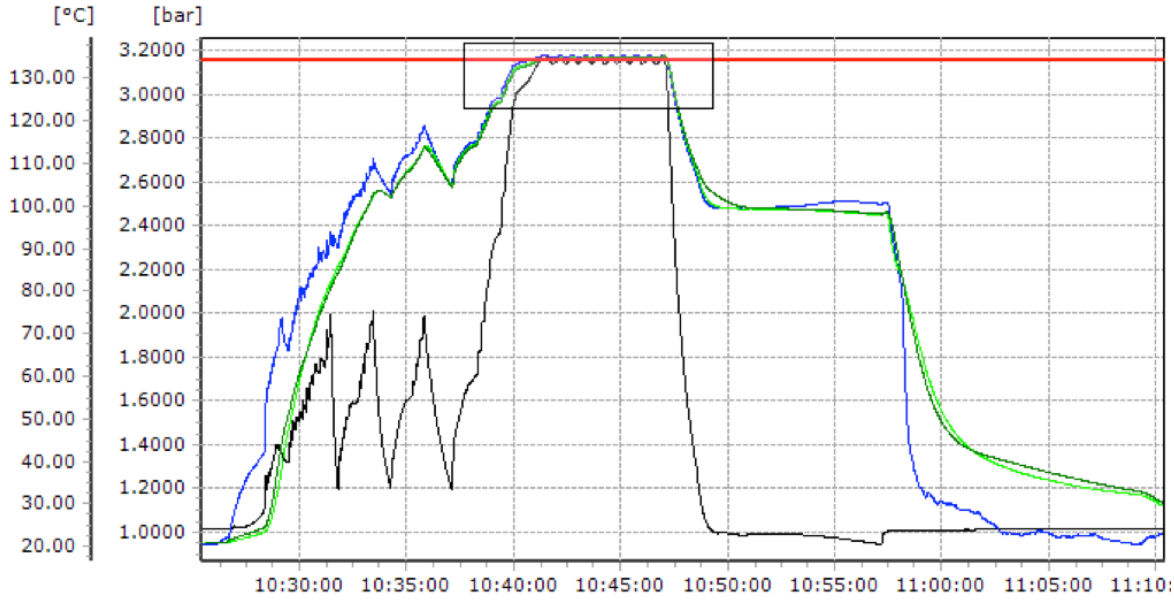

Figure 4-27 Cycle profile of non-vacuum sterilizer Kronos (Newmed), total cycle time is 30 minutes with a plateau time of 6.5 min (black=pressure, blue=free chamber space, light green, dark green=inside handpiece), the horizontal red line indicates sterilization temperature 134°C

Three non-vacuum Little Sister 3 (Eschmann) were tested. Pressure traces show no pressure pulses .The overall cycle time was 17 - 20 minutes with a plateau time of 3.5 - 6.5 min at 134°C (Figure 4-28). Seven BI fails and two CI fails were found in 162 samples (Tables 4-20 – 4-22). A full load of 5 kg (as per manufacturer's instructions) was not tested because the sterilizers failed the cycle with full loads. The time difference between 18 handpieces and chamber ranged from 15 – 100 sec and resulted in 18 thermometric fails according to Perkins and BS EN 13060:2014 and 16 fails according to SHTM 2010. Furthermore BDT and helix PCD also failed.

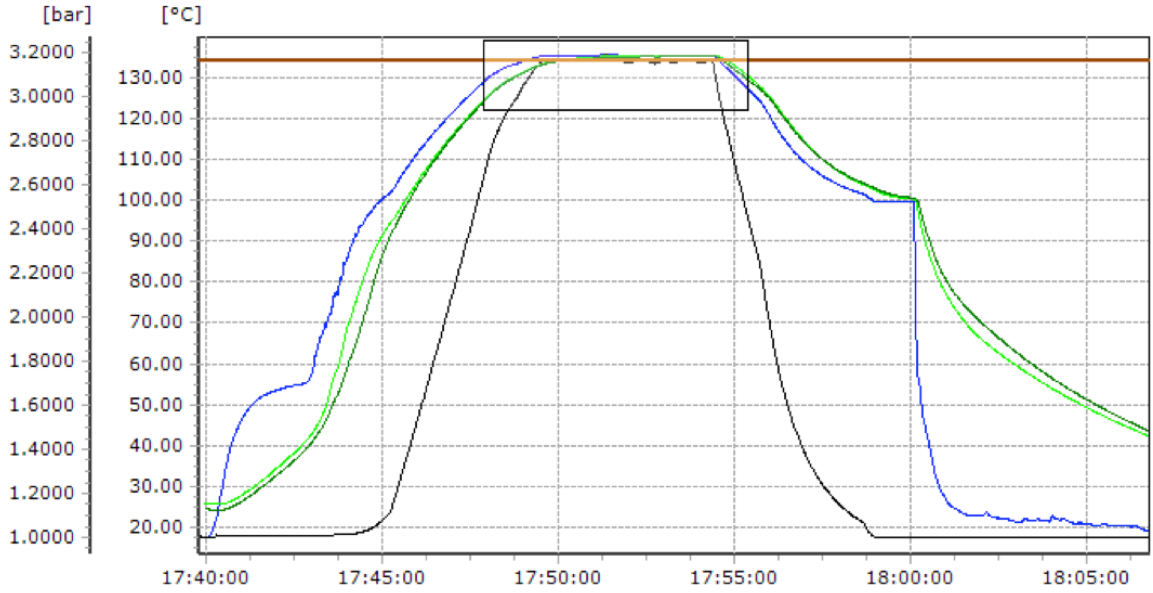

Figure 4-28 Cycle profile of non-vacuum sterilizer Little Sister 3 (Eschmann), total cycle time is 17-20 minutes with a plateau time ranges from 3.5 – 6.5 min (black=pressure, blue=free chamber space, light green, dark green=inside handpiece), the horizontal orange line indicates sterilization temperature 134°C

Three vacuum Lisa (W&H, Austria) were tested. Pressure traces show three vacuum pulses at 0.2 bar and the overall cycle time was 30 – 45 minutes with a plateau time of 4 min and 10 sec at 134°C (Figure 4-29). No BI fails and two CI fails were found in 342 samples (Tables 4-23 – 4-28). Small loads (0.5 kg) and recommended full load (4.5 kg) did not show differences. Time difference between handpieces and chamber ranged from 0 – 3 sec. All 36 tested handpieces constituted thermometric passes and BDT and helix PCD passed.

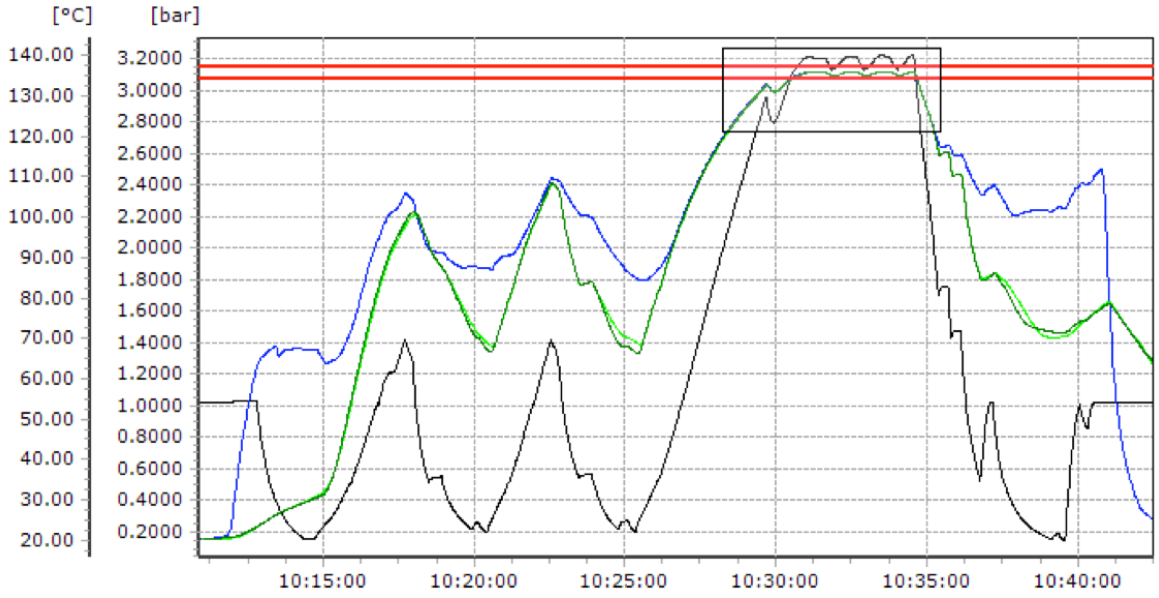

Figure 4-29 Cycle profile of non-vacuum sterilizer Lisa (W&H), total cycle time is 30-45 minutes with a plateau time of 4.5 min (black=pressure, blue=free chamber space, light green, dark green=inside handpiece), the horizontal red lines indicate sterilization temperature range of 134-137°C

Summaries of results are shown in tables 4-29 and 4-30.

Table 4-2 Result summary W&H N type sterilizer 1 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (small load/tray only)					
Model: MS22	00333		00334		00335	
SN: 113512	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-3 Result summary W&H N type sterilizer 1 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (full load/2 kg)					
Model: MS22	00336		00337		00338	
SN: 113512	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-4 Result summary W&H N type sterilizer 2 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (small load/tray only)					
Model: MS22	00010		00011		00012	
SN: 122223	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-5 Result summary W&H N type sterilizer 2 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (full load/2 kg)					
Model: MS22	00013		00014		00015	
SN: 122223	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-6 Result summary W&H N type sterilizer 3 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (small load/tray only)					
Model: MS22	00009		00010		00011	
SN: 122224	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-7 Result summary W&H N type sterilizer 3 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (full load/2 kg)					
Model: MS22	00012		00013		00014	
SN: 122224	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-8 Result summary Prestige N type sterilizer 1 small load; growth in 2/72, CI fail in 5/72, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Prestige	Cycle Number (small load/tray only)							
Model: Alpha	0004		0005		0006		0007	
SN: 14070405	CI	BI	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✗	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓	✓	✗
	✗	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
S11 Back	✗	✓	✗	✗	✗	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗	✗	✗

Table 4-9 Result summary Prestige N type sterilizer 1 full load; growth in 3/54, CI fail in 1/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Prestige	Cycle Number (full load/6 kg)					
Model: Alpha	0008		0009		0010	
SN: 14070405	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✗
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✗	✓	✗
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✗
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-10 Result summary Prestige N type sterilizer 2 small load; growth in 0/54, CI fail in 1/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Prestige	Cycle Number (small load/tray only)					
Model: Alpha	0004		0005		0006	
SN: 14082608	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✗	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-11 Result summary Prestige N type sterilizer 2 full load; growth in 1/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Prestige	Cycle Number (full load/6 kg)					
Model: Alpha	0007		0008		0009	
SN: 14082608	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✗
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-12 Result summary Prestige N type sterilizer 3 small load; growth in 1/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Prestige	Cycle Number (small load/tray only)					
Model: Alpha	0004		0005		0006	
SN: 14082609	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✗	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-13 Result summary Prestige N type sterilizer 3 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Prestige	Cycle Number (full load/6 kg)					
Model: Alpha	0007		0008		0009	
SN: 14082609	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-14 Result summary Newmed N type sterilizer 1 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Newmed	Cycle Number (small load/tray only)					
Model: Kronos N18	00008		00009		00010	
SN: UKN18D0450	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-15 Result summary Newmed N type sterilizer 1 full load; growth in 1/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Newmed	Cycle Number (full load/4 kg)					
Model: Kronos N18	00011		00012		00013	
SN: UKN18D0450	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✗	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-16 Result summary Newmed N type sterilizer 2 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Newmed	Cycle Number (small load/tray only)					
Model: Kronos N18	00017		00018		00019	
SN: UKN18D1604	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-17 Result summary Newmed N type sterilizer 2 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Newmed	Cycle Number (full load/4 kg)					
Model: Kronos N18	00020		00021		00022	
SN: UKN18D1604	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-18 Result summary Newmed N type sterilizer 3 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Newmed	Cycle Number (small load/tray only)					
Model: Kronos N18	00015		00017		00018	
SN: UKN18D1605	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-19 Result summary Newmed N type sterilizer 3 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Newmed	Cycle Number (full load/4 kg)					
Model: Kronos N18	00019		00020		00021	
SN: UKN18D1605	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-20 Result summary Eschmann N type sterilizer 1 small load; growth in 4/54, CI fail in 2/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Eschmann	Cycle Number (small load/tray only)					
Model: Little Sister 3	00188		00189		00190	
SN LCC6B1520	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✗	✗	✓	✗	✓	✓
	✓	✗	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✗	✗	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-21 Result summary Eschmann N type sterilizer 2 small load; growth in 3/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Eschmann	Cycle Number (small load/tray only)					
Model: Little Sister 3	06191		06192		06193	
SN LSK0E3582	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✗	✓	✓	✓	✓
	✓	✗	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✗
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-22 Result summary Eschmann N type sterilizer 3 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer Eschmann	Cycle Number (small load/tray only)					
Model: Little Sister 3	W4242		W4243		W4244	
SN LCB8D1031	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 4-23 Result summary W&H B type sterilizer 1 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (small load/tray only)					
Model: Lisa 517	00338		00339		00340	
SN 09-0602	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✓	✓	✓	✓	✓	✓

Table 4-24 Result summary W&H B type sterilizer 1 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (full load/4.5 kg)					
Model: Lisa 517	00342		00343		00344	
SN 09-0602	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✓	✓	✓	✓	✓	✓

Table 4-25 Result summary W&H B type sterilizer 2 small load; growth in 2/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (small load/tray only)					
Model: Lisa 517	00422		00423		00424	
SN 07-1079	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✗	✓
	✓	✓	✗	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✓	✓	✓	✓	✓	✓

Table 4-26 Result summary W&H B type sterilizer 2 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (full load/4.5 kg)					
Model: Lisa 517	00425		00426		00427	
SN 07-1079	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✓	✓	✓	✓	✓	✓

Table 4-27 Result summary W&H B type sterilizer 3 small load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (small load/tray only)					
Model: Lisa 517	00007		00008		00009	
SN 121316	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✓	✓	✓	✓	✓	✓

Table 4-28 Result summary W&H B type sterilizer 3 full load; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer W&H	Cycle Number (full load/4.5 kg)					
Model: Lisa 517	00010		00011		00012	
SN 121316	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✓	✓	✓	✓	✓	✓

Table 4-29 Summary of BI, CI and thermometric fails in non-vacuum sterilizers (small and full loads)

Sterilizer N=3	BI fails	CI fails	Thermometric fails (>3 sec)*	Thermometric fails (>15 sec)*	Thermometric fails (>2°C)*
Newmed Kronos	1/324	0/324	36/36	36/36	9/36
Prestige Alpha	7/342	7/342	38/38	38/38	26/38
W&H MS22	0/324	0/324	0/36	0/36	0/36
Eschmann Little Sister3	7/162	2/162	18/18	18/18	16/18
Total	15/1152	9/1152	92/128	92/128	51/128
Total fails (%)	1	1	72	72	40

*Refer to section 4.2.7

Table 4-30 Summary of BI, CI and thermometric fails in vacuum sterilizers (small and full loads)

Sterilizer N=3	BI fails	CI fails	Thermometric fails (>3 sec)*	Thermometric fails (>15 sec)*	Thermometric fails (>2°C)*
W&H Lisa	0/324	2/324	0/36	0/36	0/36
Total fails (%)	0	1	0	0	0

*Refer to section 4.2.7

4.4 Discussion

These investigations of steam penetration into lumens clearly showed that saturated steam penetrates lumens more successfully in vacuum cycles (J P C M van Doornmalen, 2013, Kaiser U., 1998) and that non-vacuum processes are unreliable (non-vacuum processes showed 15/1152 BI fails, 9/1152 CI fails and 51-92/128 thermometric fails, while vacuum processes showed 0/324 BI fails, 2/324 CI fails and 0/36 thermometric fails). Preliminary experiments recording temperature traces from inside the handpieces determined that it made little difference whether chambers were preheated or not to the delay for handpieces to reach the same temperature as the chamber interior. Thermometric measurements showed that the plateau time for the Little Sister 3 (Eschmann), which was used for most non-vacuum cycles, lasted over 6 min because the chamber did not start 'sterilizing' phase until the chamber reached 135.5°C (personal conversation Dave Whiteford, Eschmann). Both types of thermocouples as well as data loggers showed that the time lag is greatest in position C (50-100 sec), which is located in the plastic component of the handpiece. This suggests that heat conductivity may play a role in this particular case. The fact that data loggers and thermocouples show identical readings provided an independent method of validation for each device. Measurements from inside the spray channels showed a lag of up to 74 sec. This suggested, that all lumens inside a high-speed turbine pose a challenge for steam penetration. Two additional high-speed turbines and two slow-speed motors were assessed, which showed a time difference compared to the chamber ranging from -8 – 147 sec. As a control, temperature was recorded in three locations within the BDT, which is designed to challenge air removal. Temperatures recorded in the centre and the bottom of the pack indicated that they did not achieve sterilization parameters in a non-vacuum sterilization process. Experiments with the manufacturers recommended 5 kg full load, for Little Sister 3 (Eschmann) were unsuccessful, due to the fact that the cycle failed to reach the appropriate temperature and the process was aborted while the maximum recommended weight was still in the chamber. Recordings taken at different locations in the handpieces and the BDT using the non-vacuum cycle were compared to a vacuum sterilization process, which showed time differences of -1 - 3 sec in the handpieces compared to the chamber in all tested locations. The time difference observed in the non-vacuum

cycle may indicate that residual air inside the handpieces delayed the increase in temperature. This result indicates that it is inadvisable to process handpieces in a non-vacuum sterilizer. Measuring temperature and pressure only in order to investigate presence of saturated steam was found to be insufficient (J.P.C.M. van Doornmalen, 2014).

In order to determine a more suitable method to investigate what happens inside a handpiece during sterilization processes, CI and BI were used inside different handpieces. Using a non-vacuum sterilization cycle, all CI within the different handpieces performed a colour change indicating pass conditions, while CI in PCD (helix) failed to change colour, indicating unsuccessful sterilization conditions. These results suggest successful steam penetration into handpieces, however, results reported in chapter 3 on the response and sensitivity of chemical indicators showed that these findings should be interpreted with care, i.e. occurrence of false positives. No literature on the use of CI in dental handpieces has been found.

Vegetative bacteria were identified in dental instruments after patient treatment Dreyer and Hauman, 2001, Kellett and Holbrook, 1980 and Larsen et al., 1997 found that bacteria and endospores survived in dental air turbines after type N sterilization processes (Dreyer and Hauman, 2001, Kellett and Holbrook, 1980, Larsen et al., 1997). For these reasons human saliva, *S. aureus* and *S. mutans* were used as contaminants in high-speed turbines in-vitro. The non-vacuum cycle resulted in inactivation of these contaminants. The fact that the non-vacuum process used inactivated BI in turbines but not in the PCD (helix, BDT) suggests that the lumens of dental handpieces were successfully sterilized. The BI results indicate only a 10^4 – 10^5 reduction of spores. Therefore, a sterility assurance level (SAL) is not achieved. However, CI and BI results did not agree with thermometric measurements. Therefore, four different non-vacuum sterilizers were tested using a test set up including different handpiece types with CI, BI, and data loggers and these tests were repeated in vacuum process for comparison purposes. BI and CI fails were found in three out of four non-vacuum sterilizers. One type of non-vacuum sterilizer was capable of inactivation of spores in all tests and showed pass conditions in all CI. However, three out of four non-vacuum models resulted in thermometric fails for all handpieces tested. The different non-vacuum cycles used showed a variation in

time at plateau temperature at 134°C, ranging from 3.5 to 6.5 min, which may impact on microbial inactivation. While a single non-vacuum sterilizer passed the BDT and showed no thermometric fails, the CI and BI that had been placed in helix PCDs failed in all four non-vacuum models. The vacuum sterilization cycle used as a control successfully inactivated all BI and passed all, except two CI (incl. helix PCD). This agrees with findings by Andersen et al., who found that different small non-vacuum sterilizers show different results (Andersen et al., 1999). It was also shown that a vacuum cycle inactivates spores of *G. stearothermophilus* (Skaug and Kalager, 1986).

The wide range of time required for the inside of different handpieces to reach sterilization temperature is probably caused by differences in construction of different handpiece models. The evidence shows not all handpieces can be sterilized successfully in all non-vacuum cycles and that vacuum cycles should be used in order to achieve a SAL of 10^{-6} (556-1:2001, BS EN, 13060:2014, BS EN). In addition, the advantage observed by using data loggers to record temperature inside handpieces is that the sensing ends are insulated to make sure that the metal end does not have contact with metal surfaces, which may interfere with the recording. The use of data loggers is more efficient in terms of time, due to the fact that the use of thermocouples requires breaking seals of sterilizers and sensing ends of thermocouples are more prone to breakage, which requires re-calibration. Therefore, data loggers were considered more practical for further in-vitro investigations such as those performed in General dental practice (GDP) and described in chapter 6.

Chapter 5 - Investigating steam penetration into lumens under controlled conditions

5.1 Introduction

Dental handpieces have been classed as medical devices by the Medical Devices Directive (MDD, 1993). Several standards detail conditions for sterilizing medical devices (556-1:2001, BS EN), design and requirements for large and small steam sterilizers (285:2006+A2:2009, BS EN, 13060:2014, BS EN), chemical indicators (11140-1:2009, BS EN ISO), biological indicators (11138-1:2006, BS EN ISO) and process challenge devices (867-4:2001, BS EN, 867-5:2001, BS EN). Recommendations for the requirements to reprocess medical devices, published in 2012 by the Robert Koch-Institut (RKI, 2005) in cooperation with the Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM), dental handpieces were described as “semicritical/critical A” (assessment, treatment) or “semicritical/critical B” (invasive treatment, operation, endoscopy). The Centers for Disease Control and prevention (CDC) published "Guidelines for Infection Control in Dental Health-Care Settings” in 2003 (CDC, 2003), where dental handpieces were classed as “semi-critical”, which is defined as a medical device, which touches the mucus membrane but does not penetrate soft tissue and therefore poses a lower risk of transmitting diseases than “critical” items. These documents, as well as requirements by the British Dental Association (BDA) and guidelines in the Health Technical Memorandum “Decontamination in primary care dental practices” address the requirement for dental handpieces to be decontaminated and maintained in order to avoid cross infections and provide health and safety of patients and staff in dental practice (BDA, 2013, SHTM, 2010). However, cleaning as well as steam sterilization of dental handpieces is challenging, due to their multiple internal components and lumens, which are difficult to be accessed by steam. Critical parameters for steam sterilization are 134°C for 3 minutes (based on Perkins, 1956 and the 1st MRC report, published in 1959) at a chamber pressure of 2.2 bar, as described in BS EN 556-1:2001 “Sterilization of medical devices – Requirements for medical devices to be designed “sterile”” (Perkins, 1983, MRC, 1959, 556-1:2001, BS EN).

As stated above, several researchers have shown in their investigations that steam penetration of lumens is difficult to achieve. They have used infrared spectroscopic measurements (van Doornmalen and Kopinga, 2009, J P C M van Doornmalen, 2013) and have used PCD with metal lumens using CI

(J P C M van Doornmalen, 2013, J.P.C.M. van Doornmalen, 2013, J.P.C.M. van Doornmalen, 2012). Most studies were performed with lumens with one open and one blind end. However, dental handpieces have two open ends. Kaiser et al. 1998 compared blind ended lumens to lumens with two open ends in steam sterilization processes and found that a blind ended lumen behaves like a lumen with two open ends double the length (Kaiser U., 1998), while others established that current PCD for hollow loads are not a realistic challenge in terms of length and diameter (S. Esen, 2012a).

In addition to the challenge of sterilizing hollow devices, little is known about the effect of handpiece maintenance oil on the D value of microorganisms and therefore the efficacy of the steam sterilization process. However, Halleck et al. found that oil (Mobil DTE 25, Mobile Oil Corp., New York, NY) increases the D value of *Geobacillus stearothermophilus* from 2.5 to 5.6 at a temperature of 250°F (121°C) and from 0.2 to 0.7 at 270°F (132°C) (Halleck et al., 1979). The choice of BI carrier has great impact on their performance as well. The carrier of the BI should not affect or be affected by the sterilization process, which means that if the carrier itself reacts to the process, it might change conditions for the BI. Filter paper is not an ideal choice, due to the tendency of celluloses to overheat when exposed to steam, which changes conditions for the BI. Even the smallest overheating of the carrier can alter the BI performance. Therefore, glass fibre fleece is a more promising carrier.
In terms of reaction of different spores to the parameters it can be said that *Bacillus stearothermophilus* showed a lower sensitivity to the choice of carrier and level of saturation of steam than *Bacillus subtilis*. However, using a BI with a lower sensitivity towards levels of saturation of steam might result in an "oversight" of a problem. An ideal BI should have the heat resistance of *Bacillus stearothermophilus* and possess the sensitivity to different conditions of *Bacillus subtilis* (Spicher et al., 1999).

Maintenance of dental handpieces includes, according to the manufacturers' instructions, internal lubrication prior sterilization. An example of the protective effect of oils on microorganisms has been published by Senhaji and Loncin in "The protective effect of fat on the heat resistance of bacteria" in 1977, where

they describe that heat resistance of Bacillus subtilis is greatest in the presence of oil and the absence of water (Senhaji, 1977).

Although there are several legislations, standards, guidelines and recommendations (134°C, 3 min, 2.2 bar), many different cycle profiles for steam sterilization processes exist. Rutala et al., 2008 reported steam sterilization at 134°C for 4 minutes using a vacuum sterilization process (vacuum depth not specified) to be effective for sterilizing surgical instruments lubricated with refined mineral oil (98%) (Rutala et al., 2008), while Hegna et al., 1978 reported a sterilization process at 134°C for 8 min in a large vacuum sterilizer (vacuum depth not specified) to be sufficient to sterilize lubricated and non-lubricated dental handpieces. Intraspray (Kavo) was used to lubricate the handpieces (Hegna et al., 1978).

Halleck et al., 1979 recommended vacuum sterilization processes for lumen devices. However, most dental practices still use a non-vacuum sterilization process (Smith et al., 2009a), which raised the research question of whether handpiece oil influences steam sterilization using non-vacuum sterilization.

The aim of this study was to investigate the influence of handpiece lubricating oil on *G. stearothermophilus* spore inactivation on stainless steel surfaces and in dental handpieces during steam sterilization processes. In order to investigate different sterilization cycle profiles, a biological indicator evaluation resistometer (BIER vessel) was used, to individually program exposure time, number and depth of vacuum pulses. Furthermore, steam penetration into lumens of different material, length and diameter using non-vacuum sterilization cycles with different times at plateau temperature was investigated by using CI, BI and performing temperature measurements.

5.2 Material and Methods

5.2.1 Monitoring steam penetration into lumens - different materials, diameters and times at plateau temperature in non-vacuum sterilization using thermometric measurements, CI and BI

This section attempts to answer the research question whether material, diameter and length of lumens influence internal temperature during sterilization process in a non-vacuum sterilizer. This question was addressed by several approaches. Silicone and plastic models of handpieces with a different mass and thermal conductivity were compared to handpieces during a non-vacuum sterilization cycle. Tubes of common process challenge device (Helix) were cut into different lengths and used for investigating temperature measurements and CI assessment. Three different handpiece types were used in non-vacuum sterilization cycles with different plateau periods in order to assess CI and BI behaviour in different locations within the different handpiece types.

5.2.1.1 Plastic and silicone handpieces

Type T thermocouples (for calibration and general details see chapter 4, section 4.2.2) were inserted into the centre of the lumens (d = 2.3 mm) of two different silicone handpiece models (18g) (Figure 5-1), one open on one end (green) and one open on both ends (yellow), made from a vinylpolysiloxane type 3 low consistency light-body impression material (Kerr Extrude), and a dental handpiece (TA-98 C LED, W&H, Austria) (42 g). Non-vacuum sterilization using a Little Sister 3 (Eschmann) followed and temperature traces were compared to chamber temperature.

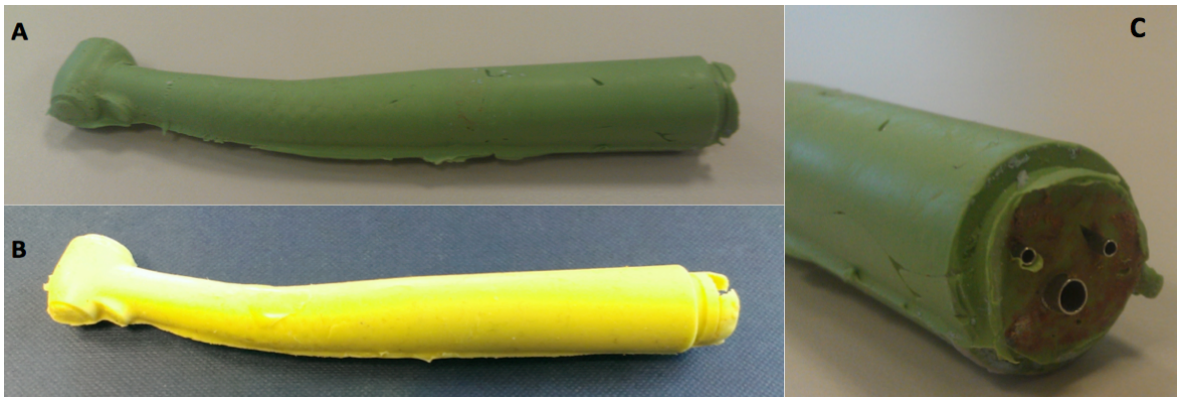

Figure 5-1 Silicone handpiece A open on one end and B open on both ends, back of silicone handpiece showing one air channel (D=2.3 mm) and two spray channels (D=0.9 mm)

The same experimental setup was performed with two 3D printed plastic models of handpieces (Figure 5-2) with the same thermal conductivity as a handpiece (provided by W&H, Austria), which have the same mass as the handpiece (42 g). Again the temperature traces were compared to a TA-98 C LED and chamber temperature.

Figure 5-2 3D printed plastic handpiece x2 open on one end channel diameter of 0.9 mm

5.2.1.2 Helix process challenge device

Common PCDs for small steam sterilizers (867-5:2001, BS EN) were used for these experiments. The helix PCD (Albert Browne Ltd., UK) consists of a PTFE

tube (l=1500 mm, d=2 mm) and a capsule on one end (Figure 5-3), which accommodates a chemical indicator strip.

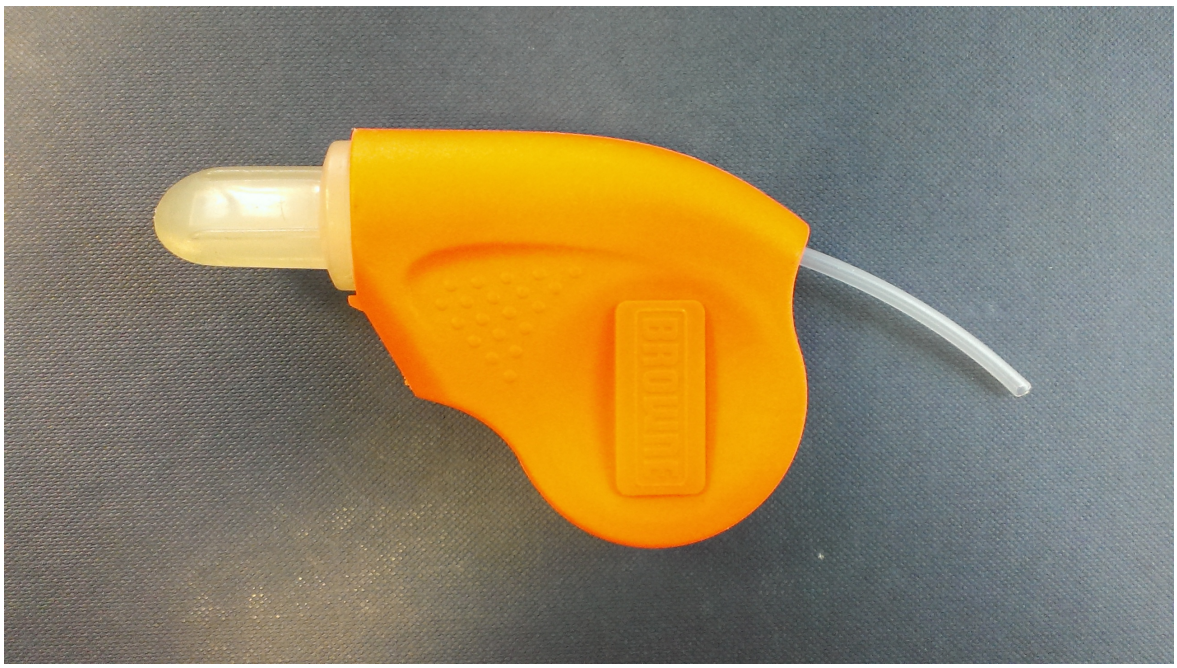

Figure 5-3 Helix pcd with tube cut down to 94 mm

Thermocouples were inserted 30 cm into the tubes of two helix PCDs (Browne). This PCD complies with the standard in terms of dimensions (867-5:2001, BS EN):

- Wall thickness: (0,5 ± 0,025) mm;
- Internal diameter: (2,0 ± 0,1) mm
- Length: (1 500 ± 15) mm
- Capsule mass: (10,0 ± 0,1) g
- Free capsule volume: (6 ± 1) % of the total internal volume minus the capsule volume
- Material of construction: Polytetrafluorethylene (PTFE).

21 non-vacuum cycles were performed in a Little Sister 3 (Eschmann, UK). After six cycles the tubes of both PCDs were cut from 1500 mm to a length of 750 mm. Four cycles in the N type sterilizer were performed before cutting the tubes down to 375 mm. After three runs the tubes were cut to a length of 188 mm and nine cycles followed. At a length of 94 mm, which equals the length of a handpiece, another three cycles were performed. After each cycle, chemical indicators from inside the PCD's capsules were visually assessed. Temperature traces were recorded and compared to the chamber temperature. The reason for the inconsistency in the number of cycles is that the thermocouples and/or ports of the logger broke. In order to obtain thermocouple readings however, experiments had to be repeated. The chemical indicators were assessed, with and without the temperature traces.

5.2.1.3 Sterilization at shortened plateau times

The standard test load for handpiece sterilization was used for these experiments (for details refer to chapter 2, section 2.1.8). Chamber controls of BI (mini spore strips, D_{121}=2.2 min, Excelsior) and CI (helix CI, Browne) as well as two Browne TST CI for non-vacuum sterilization cycles (reacting to presence of steam, temperature and time, according to the manufacturer) were included in each cycle. Non-vacuum cycles with plateau times of 0, 0.5, 1, 2 and 3 min were performed using a Little Sister 3, (Eschmann). As soon as the display on the sterilizer indicated 134°C, a stopwatch was started and after 0, 0.5, 1, 2 or 3 min the cycle was aborted. Experiments were performed in triplicate.

5.2.2 Effect of handpiece oil on inactivation of spores

In this section, the following questions were investigated in order to determine the influence of handpiece oil on spore inactivation using different steam sterilization cycles in a BIER/CIER vessel:

What effects do different steam sterilization cycle profiles have on the survival of *Geobacillus stearothermophilus* spores, which had been dried on stainless steel wires then inserted into spray channels of dental high-speed turbines and covered in f1 service oil?

What effect does an exposure time of 3 min at 134°C have on the survival of *Geobacillus stearothermophilus* spores dried and covered in f1 service oil on stainless steel tokens with different surface finishes?

5.2.2.1 Preparation

Cleaning of stainless steel wires (stainless steel 316, d=0.2 mm, l=122 cm, Cadence Science, Baltimore, MD, USA): The wires were soaked in enzymatic soap solution (Metrizyme, Metrex, CA, USA) for at least 1 min at room temperature (RT), wiped clean using a cotton cloth until no residues were visible on the cloth. This was followed by a hot water rinse and three rinses using distilled water. Wires were left to dry for 30 – 60 min in a laminar flow hood.

Cleaning of stainless steel tokens: Tokens were soaked in enzymatic soap (Metrizyme, Metrex, CA, USA) solution for at least 1 min, sonicated for 20 min (Branson 2200, CA, USA), followed by a hot water rinse and three rinses using distilled water. Tokens were left to dry for 30 – 60 min in a laminar flow hood.

Preparation of spore solution: Tenfold dilutions of spore solution in water (spore crop ID “S718”, 3M, St. Paul, MN, USA) harvested from cultures on agar, provided by 3M, St. Paul, MN, USA) with a D-value of D_{121}= 2.2 min, were performed and plated onto TSA (tryptic soy agar, Remel, Lenexa, KS, USA) plates in duplicate and incubated at 66°C for up to 24 h (Mechanical Convection Incubator, Precision). Plates with colonies between 30 and 300 colony forming units (cfu) were counted and the stock solution was calculated to have a spore

population of 2.3 x 10^8 spores/ml. However, according to the standard (EN ISO 11138-1:2006), the spore population can range from 50% to 300% from the spore count, due to potential aggregation of the spores. Therefore the range of the stock spore solution was determined to be between 1.15 x 10^8 - 6.9 x 10^8 spores/ml. The stock solution was diluted in 20% Ethanol in Millipore H_2O solution, to a range of 5.8 x 10^7 - 3.5 x 10^8 spores/ml, in order to dry on stainless steel wires over night. The standard inoculum was 10 μL of spore solution (range: 5.8 x 10^5 - 3.5 x 10^6 spores).

5.2.2.2 Effect of steam on spores inoculated into dental handpiece lumens and coated with oil

10 μL of *G. stearothermophilus* spores (range: 5.8 x 10^5 - 3.5 x 10^6 spores) was inoculated, using gel loading pipette tips, onto stainless steel wires (d=0.2 mm), tightly screwed to a metal rack, and dried over night at room temperature (Figure 5-4). Stainless steel wires were cut to a length of 82 mm each and inserted into spray channels (d=0.9 mm, l=80 mm, V=50.8 mm^3) of three handpieces (Figure 5-5), followed by inoculation of 10 μL handpiece oil (synthetic hydrocarbon oil ester oil, f1 service oil, W&H, Austria) directly into each spray channel using a pipette and 200 μL tips. A negative control comprising one handpiece with a wire in each spray channel without spores and a positive control comprising inoculated wire without sterilization were included and repeated three times.

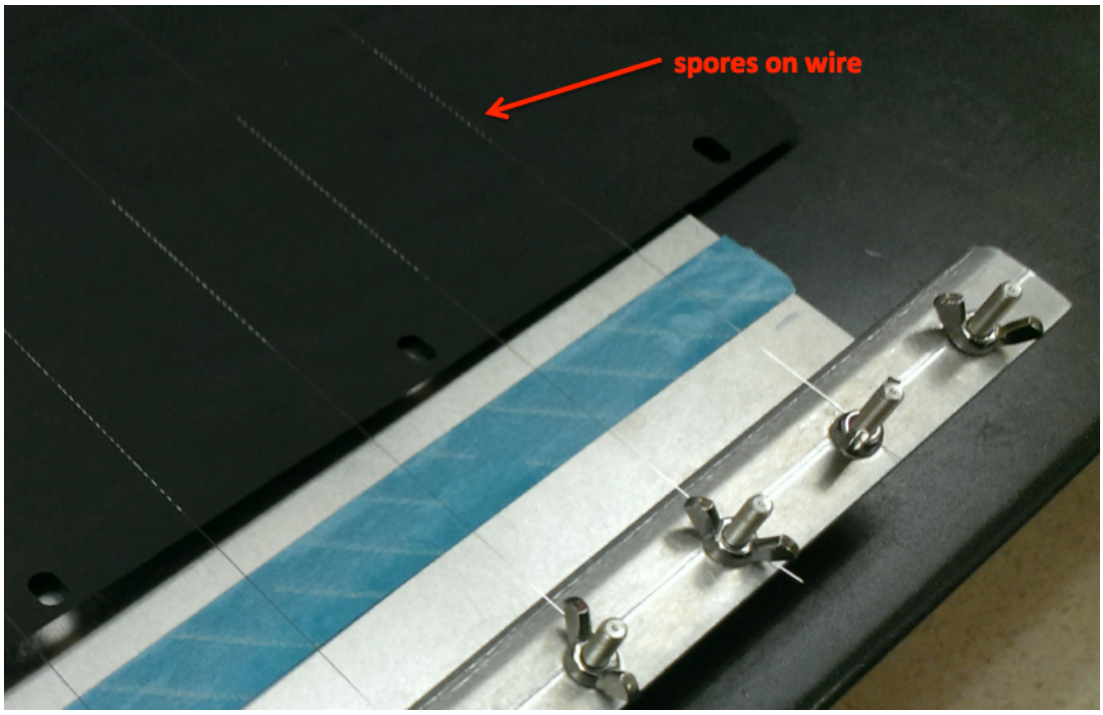

Figure 5-4 Wires on rack inoculated with spores

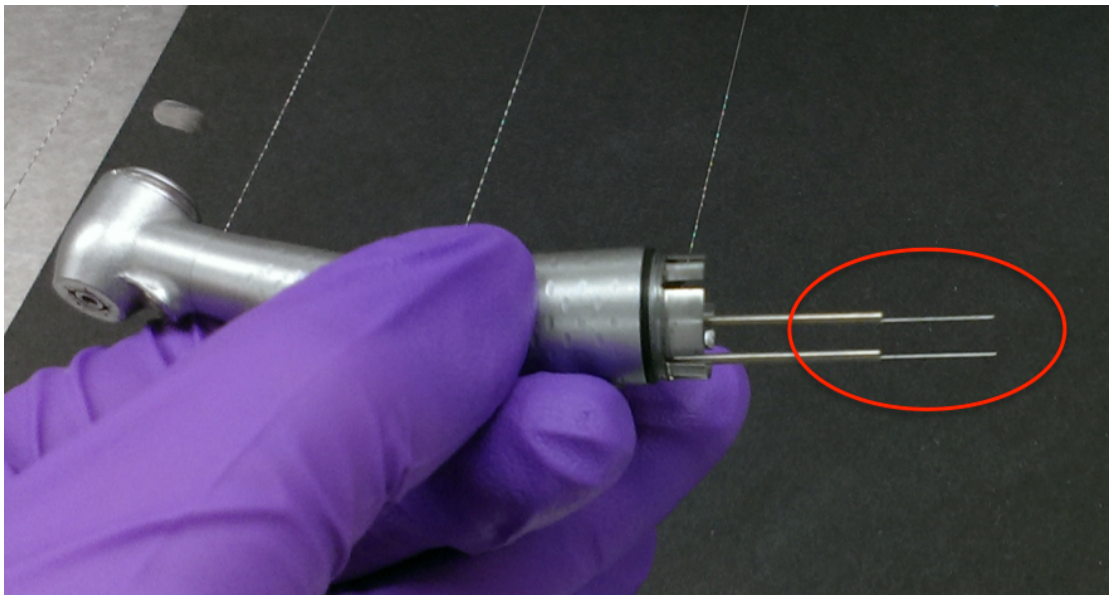

Figure 5-5 Dental turbine spray channels inoculated with spore impregnated wires

Three different sterilization cycles were programmed using a BIER vessel (3M) and compared (Figures 5-6 to 5-8). The following equation was used to used to calculate integrated lethality (F_0)

$F_0=t\ (10^{((T-121.1)/z)})$,

where t is the time interval of points of measurement; T is the temperature at the point of measurement; z is the z-value, assumed to be 10.

Further calculation of log reduction of the microbial population is performed by F_0/D_{121} (Pharmacopoeia, 2014).

Cycle 1: Non-vacuum cycle, no pre-vacuum, 3 min at 134°C, no post-vacuum, F_0=114 (52 log reduction)

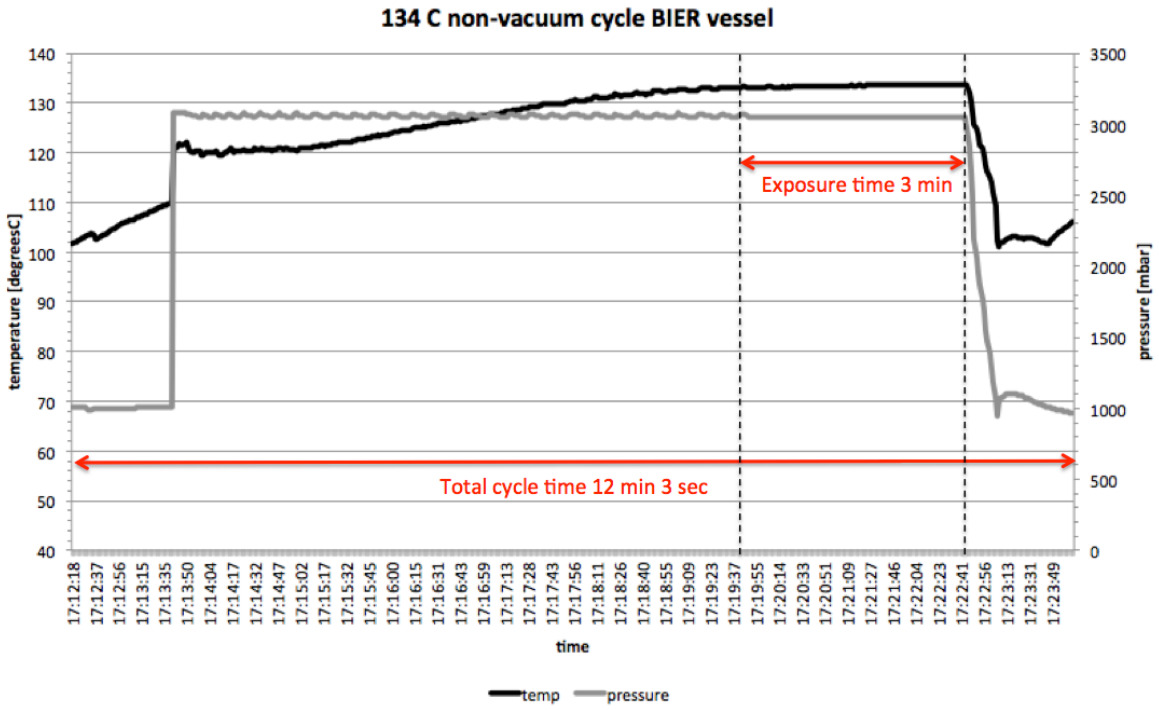

Figure 5-6 Non-vacuum cycle resistometer (H&W) (total cycle time shown 12 min 3 sec)

Cycle 2: Vacuum cycle 1, one 45 mbar pre-vacuum pulse, 3 min at 134°C, one 65 mbar post-vacuum pulse, F_0=60 (27 log reduction)

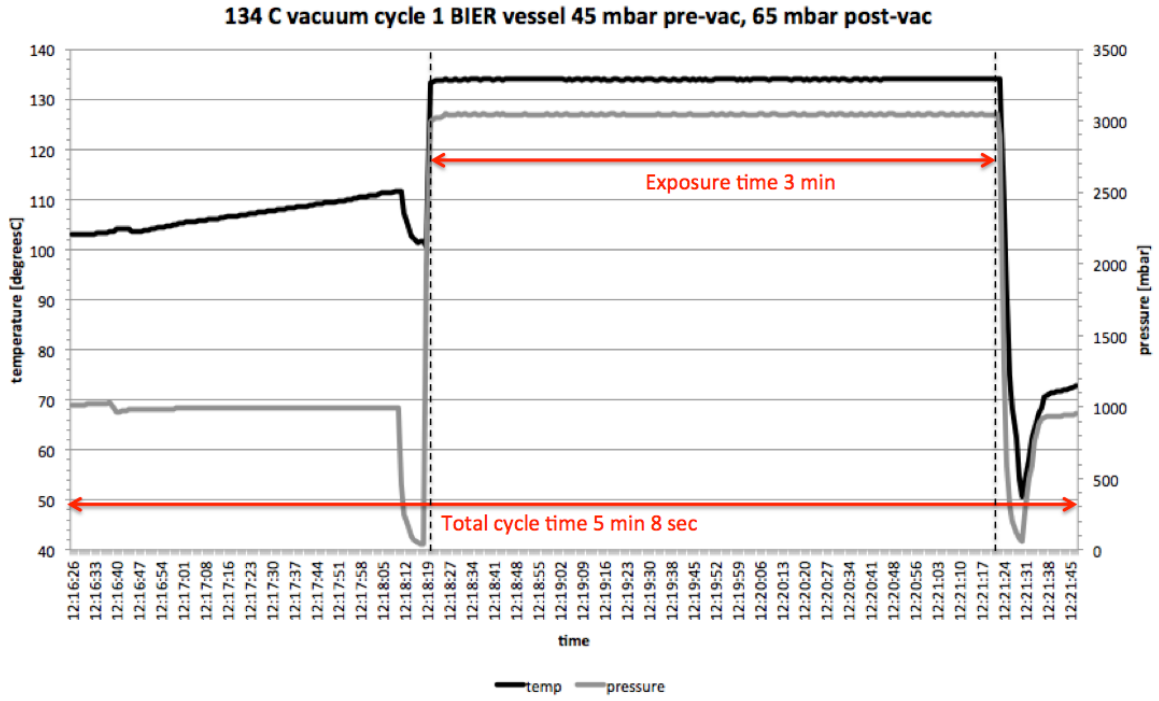

Figure 5-7 Vacuum cycle 1 with 1 pre-vacuum pulse (total cycle time shown 5 min 8 sec)

Cycle 3: Vacuum cycle 2, ten 900 mbar pre-vacuum pulses, 3 min at 134˚C, one 65 mbar post-vacuum pulse, F_0=60 (27 log reduction)

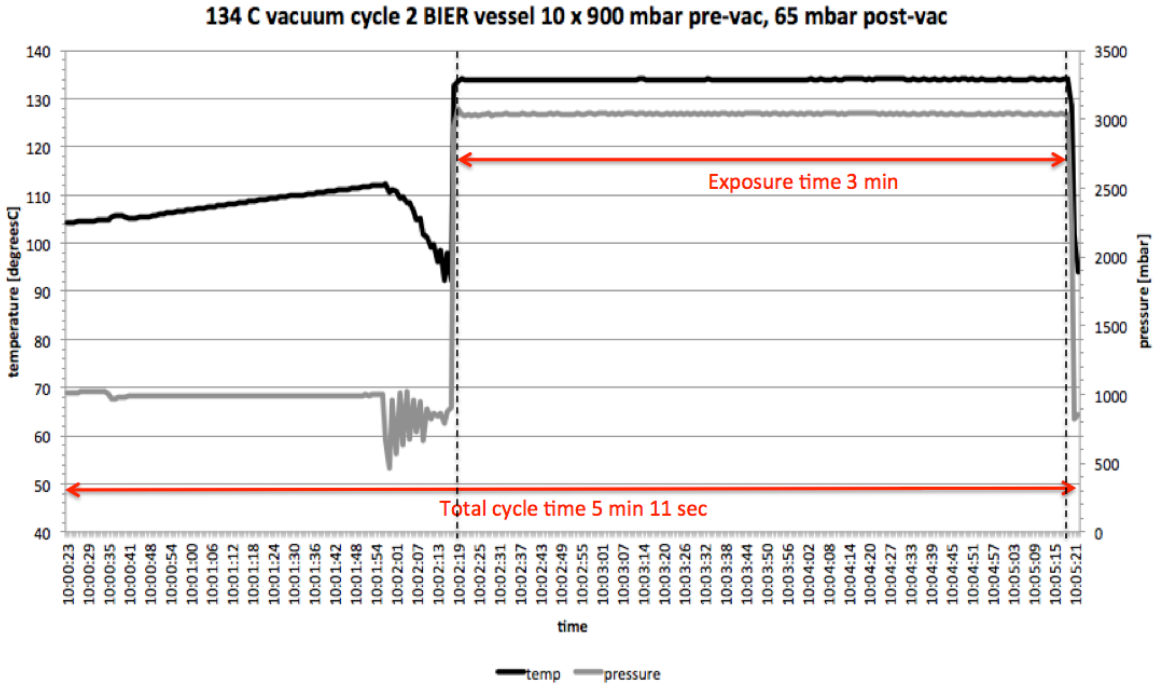

Figure 5-8 Vacuum cycle 2 resistometer 10 x 900 mbar pre-vacuum pulses (total cycle time shown 5 min 11 sec)

Cycles with an empty chamber were performed to investigate whether the load influenced the heat up time. Recovery of *G. stearothermophilus* was performed by transferring both wires into centrifuge tubes containing 50 ml of phosphate buffer saline (PBS) + 0.1% Tween 80), followed by 20 min sonication (Branson 2200, CA, USA), filtration (Micro Funnel filter unit, GN-6 membrane 0.45 µm, Pall Life Science, USA) with two wash steps, using 50 ml of buffer, and placing the filter paper onto tryptic soy agar (TSA; Remel, Lenexa, KS, USA). Incubation was performed at 66°C for up to 48 h (Mechanical Convection Incubator, Precision), followed by counting colony forming units (cfu). Experiments were performed in triplicate and included negative controls (two cleaned wires and two cleaned spray channels (flushing with 2 ml of PBS + 0.1% Tween 80)) and positive controls (six contaminated wires, no sterilization). The sterilization processes were simulated using a biological indicator evaluation resistometer (H&W) at 3M (St. Paul, Minnesota, USA).

5.2.2.3 Effect of steam on spores inoculated onto tokens and coated with oil

Examination of the thermal death characteristics of *G. stearothermophilus* spores covered in a thin film of f1 service oil (W&H, Austria) was undertaken by applying 10 µl of spores (range 5.8×10^5 to 3.5×10^6) to each stainless steel token with four different surface finishes: mirror, intermediate, rough and indented (Figures 5-9 and 5-10). After drying over night at 37°C (Mechanical Convection Incubator, Precision), 10 µl of f1 service oil were applied using a pipette with 200 µl tips. A typical resistometer steam sterilization cycle was performed (3 min exposure at 134°C, one 45 mbar pre-vacuum pulse, 3 min at 134°C, one 65 mbar post-vacuum pulse, F_0=61 (28 log reduction)). Recovery of *G. stearothermophilus* spores was performed by transferring each token into 50 ml PBS + 0.1% Tween 80, and sonicating for 20 min (Branson 2200, CA, USA), the eluate was then filtered (Micro Funnel filter unit, GN-6 membrane 0.45 µm, Pall Life Science, USA) and filtrate washed twice using 50 ml PBS. Filter papers were transferred onto TSA (tryptic soy agar, Remel, Lenexa, KS, USA) and incubation was performed at 66°C for up to 48 h (Mechanical Convection Incubator, Precision). Plates with between 30 and 300 colonies were counted for analysis.

Each cycle included three tokens of each type inoculated with spores and oil, one token of each type inoculated with spores only. As positive controls, three contaminated tokens of each type without oil and two contaminated tokens of each type with oil were assessed without sterilization. As negative controls: two cleaned tokens of each type were assessed. Experiments were performed in triplicate. The sterilization process was programmed using a resistometer (H&W) at 3M (St. Paul, Minnesota, USA).

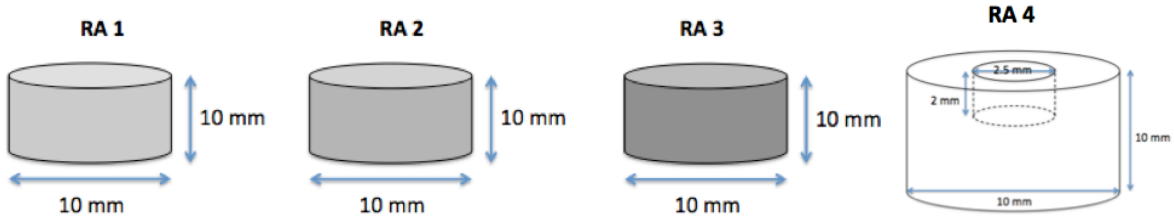

Figure 5-9 Four different stainless steel tokens; RA1 = mirror finish, RA2 = intermediate roughness surface finish, RA3 = rough surface finish, RA4 = indented surface

Figure 5-10 Stainless steel tokens inoculated with spores; row A = mirror finish; row B = intermediate finish; row C = rough finish; row D indented

5.3 Results

5.3.1 Plastic and silicone handpieces

Compared to the chamber the time difference for in all tested silicon handpieces to reach the sterilization temperature ranged from -2 – 10 sec, while all tested plastic handpiece showed a time difference of 0 – 7 sec.

5.3.2 Helix process challenge device

The time difference between the chamber and the PCD over the entire length (1500 mm) of the tube was 9 sec – n/a (the temperature never reached 134°C) and all other lengths of helix tubes (table 5-1) ranged from -1 – 203 sec, while all the handpieces tested showed a time difference of -2 – 150 sec compared to the chamber. Table 5-1 shows CI results of helix experiments. No trend or significant difference was observed between the individual lengths of helix tube.

Table 5-1 CI result summary for helix experiments exposed to non-vacuum process

Length of tube (helix)	Number of cycles performed (two helixes per cycle)	Passes/assessed helix CI	Fails/assessed helix CI
1500 mm	6	8/12 (66%)	4/12
750 mm	4	6/8 (75%)	2/8
375 mm	3	3/6 (50%)	3/6
188 mm	9	7/18 (38%)	11/18
94 mm	3	4/6 (66%)	2/6

5.3.3 Sterilization at shortened plateau times

Graphs for cycles with plateau times of 0-3 min for a non-vacuum process are shown in figures 5-11 to 5-15 and CI and BI results can be found in table 5-2. Both, CI and BI tend to pass at lower times at plateau temperature in high-speed turbines compared to surgical handpieces or slow-speed motors. BI chamber controls started to pass (i.e. sterilization conditions met) after 0.5 min at the sterilization temperature 134°C, while CI chamber controls passed after 1 min. Positive BI controls, which were not included in the sterilization process showed growth. TST CI for non-vacuum sterilization processes showed constant passes at 1 min and longer at 134°C (Figure 5-16).

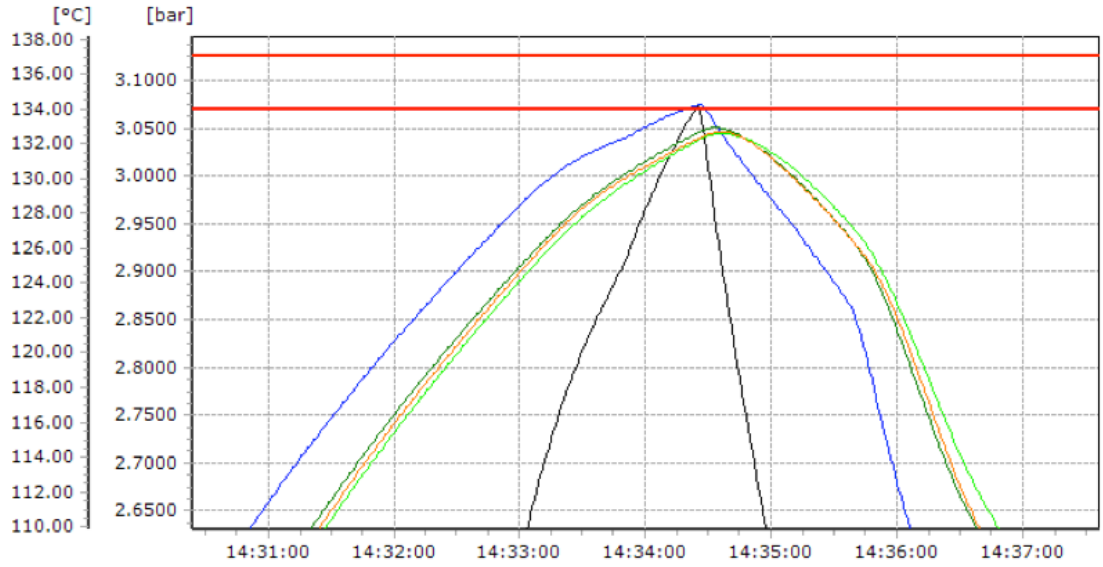

Figure 5-11 Magnified graph of plateau period 0 min (blue=free chamber space, black=pressure, green, light green, orange=temperature inside the handpieces) the horizontal red lines indicate sterilization range of 134-137°C, x4 CI fails/x2 CI passes

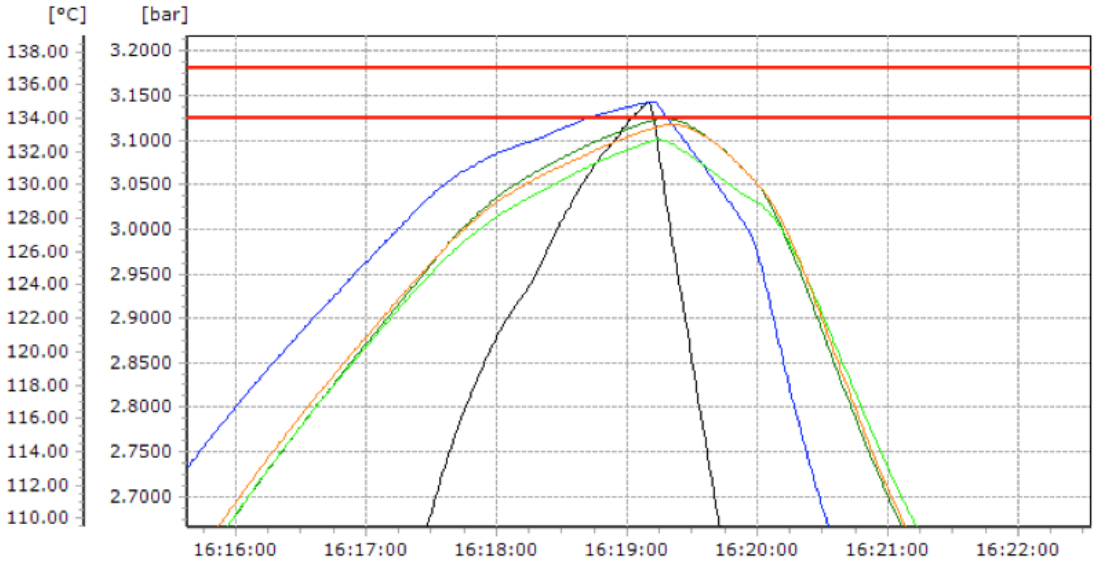

Figure 5-12 Magnified graph of the plateau period 0.5 min (blue=free chamber space, black=pressure, green, light green, orange=temperature inside the handpieces) the horizontal red lines indicate sterilization range of 134-137°C x1 CI fails/x5 CI passes

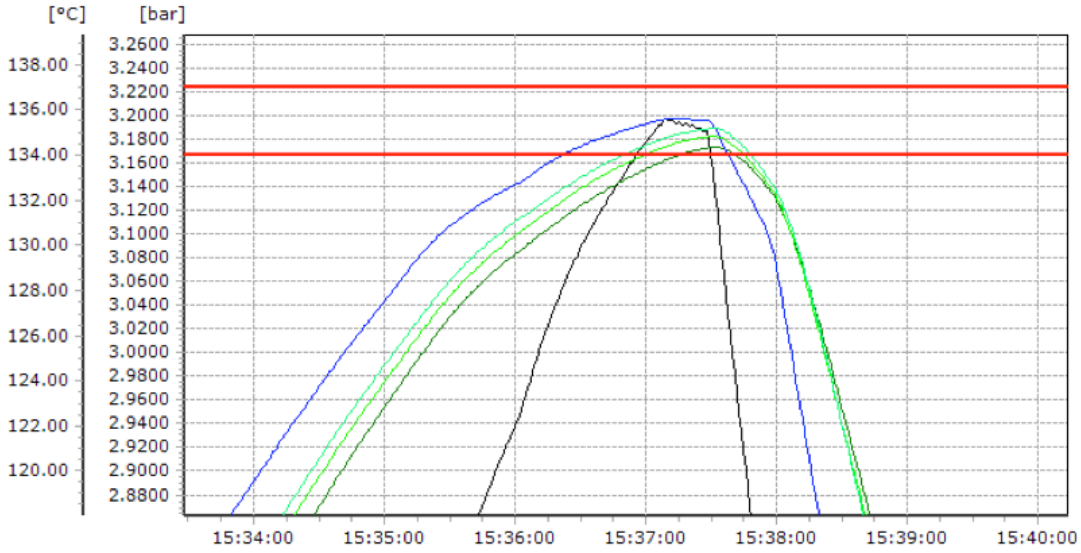

Figure 5-13 Magnified graph of the plateau period 1 min (blue=free chamber space, black=pressure, green, light green, dark green=temperature inside the handpieces) the horizontal red lines indicate sterilization range of 134-137°C, x0 CI fails/x6 CI passes

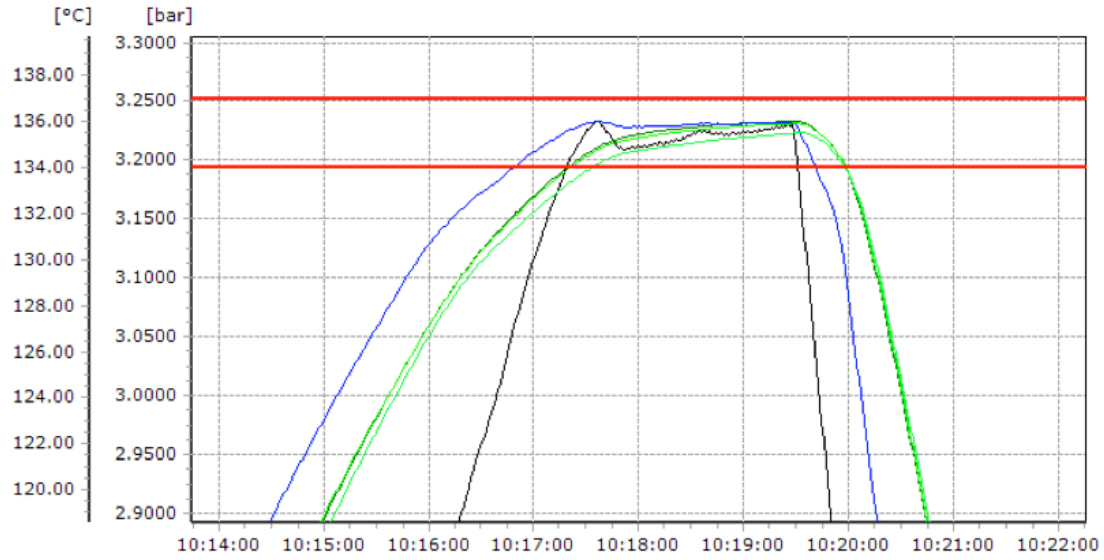

Figure 5-14 Magnified graph of the plateau period 2 min (blue=free chamber space, black=pressure, green, light green=temperature inside the handpieces) the horizontal red lines indicate sterilization range of 134-137°C, x0 CI fails/x6 CI passes

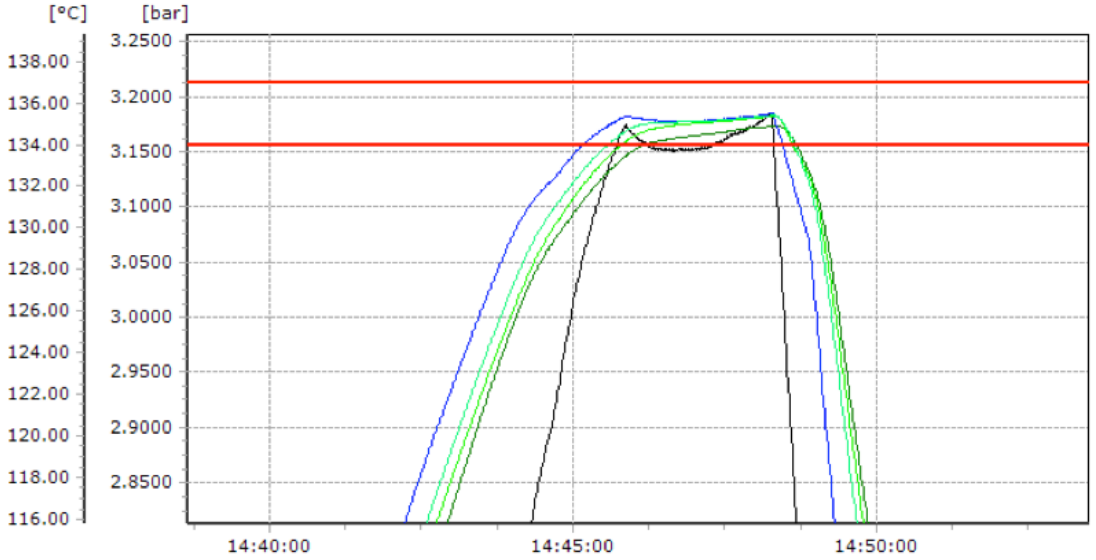

Figure 5-15 Magnified graph of the plateau period 3 min (blue=free chamber space, black=pressure, green, light green, dark green=temperature inside the handpieces) the horizontal red lines indicate sterilization range of 134-137°C, x0 CI fails/x6 CI passes

Table 5-2 Summary of CI and BI results in different handpieces at shortened plateau periods (✓= pass, ✗= fail)

LS3 TS1 SN LCC6B15020	Holding Time [min]									
	0		0.5		1		2		3	
	CI	BI	CI	BI	CI	BI	CI	BI	CI	BI
TA-98 Head	✓✗✗	✗✗✗	✗✗✗	✗✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✗✓✓
	✓✗✗	✗✗✗	✓✗✗	✓✓✓	✓✓✗	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✗✓✓
	✓✗✗	✓✗✗	✓✗✗	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
TA-98 Air Channel	✓✗✗	✗✗✗	✗✗✗	✗✗✗	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
	✓✗✗	✗✗✗	✓✗✗	✓✗✗	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
	✓✗✗	✓✗✗	✓✗✗	✓✗✗	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✓✓✓	✗✗✗	✓✓✓	✓✓✗	✓✓✓
	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✓✓✓	✗✗✓	✓✓✓	✓✗✗	✓✓✓
	✗✗✗	✓✗✗	✗✗✗	✓✗✗	✗✗✗	✓✓✓	✗✗✓	✓✓✓	✗✓✓	✓✓✓
S11 Chuck Lever	✗✗✗	✗✗✗	✓✗✗	✗✗✗	✓✗✓	✓✗✗	✓✓✗	✗✓✓	✓✓✓	✓✓✗
	✗✗✗	✗✗✗	✗✗✓	✗✗✗	✓✓✗	✗✓✗	✓✓✓	✓✓✓	✓✓✓	✓✗✓
	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✓✓✗	✗✗✗	✓✓✓	✓✓✗	✓✓✓	✓✓✗
S11 Back	✗✗✗	✓✓✓	✗✗✗	✓✓✓	✓✗✗	✓✓✗	✓✓✓	✓✓✓	✓✓✓	✓✓✓
	✗✗✗	✓✓✓	✗✗✗	✓✓✓	✗✗✓	✓✗✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
	✗✗✗	✓✓✓	✗✗✗	✓✓✓	✓✗✗	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
WA-56 Inside	✗✗✗	✓✗✗	✗✗✗	✗✗✗	✗✗✗	✓✗✗	✓✗✗	✓✓✗	✓✓✓	✓✓✓
	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✓	✓✓✗	✓✗✓	✓✓✓	✓✓✓
	✗✗✗	✗✗✓	✗✗✗	✗✗✗	✗✗✗	✓✓✗	✓✗✗	✓✓✓	✓✓✓	✓✓✓
Helix (Browne)	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗	✗✗✗
Chamber co	✓✗✗	✓✓✓	✓✗✗	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
+ co (BI)	Growth		Growth		Growth		Growth		Growth	
TST Chamber	x 2 ✗✗✓		x 2 ✓✓✓		x 2 ✓✓✓		x 2 ✓✓✓		x 2 ✓✓✓	

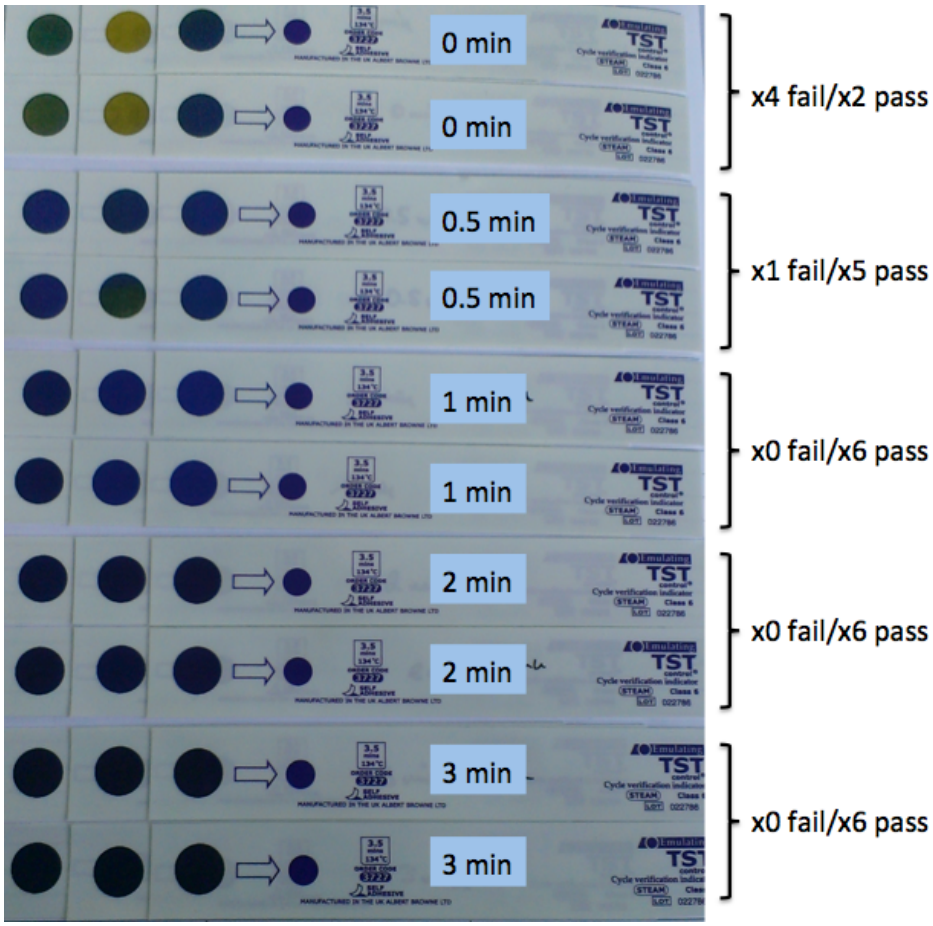

Figure 5-16 Summary of TST CI in shortened plateau period experiments

5.3.4 Effect of handpiece oil on inactivation of spores

5.3.4.1 Effect of steam on spores inoculated into dental handpiece lumens and coated with oil

A total number of nine cycles were performed, comprising four handpieces inoculated with spores on stainless steel wires in spray channels. Spray channels in three handpieces were also inoculated with 10 μL handpiece oil. In total 80 wires were assessed. No growth was detected in the negative controls (clean spray channels x2 and cleaned wires 2x). The mean recovery range of contaminated wires without oil (N=6) was 3.4×10^6 - 2.5×10^7. Using the non-vacuum cycle, the mean time to reach sterilization temperature was 9 min 14 sec in an empty chamber and 11 min 47 sec in a chamber with experimental load. The same comparison for empty chamber and chamber with load was performed for vacuum cycle 1, which resulted in 2 min 4 sec and 1 min 50 sec, respectively to reach sterilization temperature. For vacuum cycle 2 the heat up time was 2 min 58 sec empty and 4 min 56 sec with load. Spores could only be recovered from processed handpieces in vacuum cycle 1 with pre-vacuum (1 oil control, 3 samples) (Tables 5-3a to 5-3c).

Table 5-3a Results of handpiece experiments in non-vacuum cycle (Hp = handpiece)

Growth of *G. stearothermophilus* spores on wires from handpieces in non-vacuum cycle (cfu/number of wires)											
Run 1 (total cycle time 12 min 44 sec)				**Run 2 (total cycle time 12 min 7 sec)**				**Run 3 (total cycle time 27 min 11 sec)**			
Hp 1 +oil	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**	**Hp 1 +oil**	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**	**Hp 1 +oil**	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**
0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

Table 5-3b Results of handpiece experiments in vacuum cycle 1 (Hp = handpiece)

Growth of *G. stearothermophilus* spores on wires from handpieces in vacuum cycle 1 (cfu/number of wires)											
Run 1 (total cycle time 6 min 28 sec)				**Run 2 (total cycle time 6 min 44 sec)**				**Run 3 (total cycle time 6 min 11 sec)**			
Hp 1 +oil	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**	**Hp 1 +oil**	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**	**Hp 1 +oil**	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**
2/2	0/2	0/2	1/2	0/2	3/2	0/2	0/2	0/2	0/2	2/2	0/2

Table 5-3c Results of handpiece experiments in vacuum cycle 2 (Hp = handpiece)

Growth of *G. stearothermophilus* spores on wires from handpieces in vacuum cycle 2 (cfu/number of wires)											
Run 1 (total cycle time 5 min 44 sec)				**Run 2 (total cycle time 5 min 9 sec)**				**Run 3 (total cycle time 6 min 57 sec)**			
Hp 1 +oil	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**	**Hp 1 +oil**	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**	**Hp 1 +oil**	**Hp 2 +oil**	**Hp 3 +oil**	**Hp -oil**
0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

5.3.4.2 Effect of steam on spores inoculated onto tokens and coated with oil

Three cycles were performed and 55 tokens were assessed. The recovery method did not influence the recovery of survivors, however, the recovery method was less efficient in removing spores from indented tokens than from all other surfaces (Table 5-4).

Table 5-4 Results of controls and recovery method validation

Items		Recovery range (mean)
Smooth surface	- Oil (N=3)	1.8×10^6 – 1.1×10^7
	+ Oil (N=2)	3.4×10^6 – 2×10^7
Intermediate surface	- Oil (N=3)	2.2×10^6 – 1.3×10^7
	+ Oil (N=2)	1.8×10^6 – 1.1×10^7
Rough surface	- Oil (N=3)	3.1×10^6 – 1.8×10^7
	+ Oil (N=2)	2×10^6 – 1.2×10^7
Indented surface	- Oil (N=3)	1.1×10^6 – 6.5×10^6
	+ Oil (N=2)	3×10^5 – 1.8×10^6

The mean heat up time for vacuum cycle 1 was 1 min 52 sec. Spores were recovered from three indented tokens with oil and one indented token without oil, as well as from one rough token without oil and one smooth token without oil (Tables 5-5a to 5-5d), however, the spore on the smooth token might have been transferred from an indented token during transport in the tray.

Table 5-5a Results of smooth token experiments in vacuum cycle 1 (Tk = token)

Growth of *G. stearothermophilus* spores on smooth tokens in vacuum cycle 1 (cfu)											
Run 1 (total cycle time 5 min 6 sec)				**Run 2 (total cycle time 6 min 2 sec)**				**Run 3 (total cycle time 5 min 18 sec)**			
Tk 1 +oil	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**	**Tk 1 +oil**	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**	**Tk 1 +oil**	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**
0	0	1	0	0	0	0	0	0	0	0	0

Table 5-5b Results of intermediate token experiments in vacuum cycle 1 (Tk = token)

Growth of *G. stearothermophilus* spores on intermediate tokens in vacuum cycle 1 (cfu)											
Run 1 (total cycle time 5 min 6 sec)				**Run 2 (total cycle time 6 min 2 sec)**				**Run 3 (total cycle time 5 min 18 sec)**			
Tk 1 +oil	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**	**Tk 1 +oil**	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**	**Tk 1 +oil**	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**
0	0	0	0	0	0	0	0	0	0	0	0

Table 5-5c Results of rough token experiments in vacuum cycle 1 (Tk = token)

Growth of *G. stearothermophilus* spores on rough tokens in vacuum cycle 1 (cfu)											
Run 1 (total cycle time 5 min 6 sec)				**Run 2 (total cycle time 6 min 2 sec)**				**Run 3 (total cycle time 5 min 18 sec)**			
Tk 1 +oil	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**	**Tk 1 +oil**	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**	**Tk 1 +oil**	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**
0	0	0	1	0	0	0	0	0	0	0	0

Table 5-5d Results of indented token experiments in vacuum cycle 1 (Tk = token)

Growth of *G. stearothermophilus* spores on indented tokens in vacuum cycle 1 (cfu)											
Run 1 (total cycle time 5 min 6 sec)				**Run 2 (total cycle time 6 min 2 sec)**				**Run 3 (total cycle time 5 min 18 sec)**			
Tk 1 +oil	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**	**Tk 1 +oil**	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**	**Tk 1 +oil**	**Tk 2 +oil**	**Tk 3 +oil**	**Tk -oil**
9	0	0	1	0	8	0	0	0	3	0	0

5.4 Discussion

The results presented in this chapter demonstrate that, contrary to expectations, temperature measurements in silicone and plastic handpieces did not show significant differences to measurements recorded from inside high-speed turbines, which suggests that the lumens were free draining. However, condensate can accumulate in the lumen if it is in a horizontal position and water logging can take place, resulting in blockage, as described by van Doornmalen et al. (van Doornmalen, 2015). PCD results did not show a trend towards better steam penetration into shorter lumens. The fact that CI in the 1500 mm tube did not indicate a pass in the absence of the thermocouples, but did pass when thermocouples were present, which suggests that the thermocouple facilitated the steam penetration of the tube. The inconsistent results reported using CI raises the question of whether CI are an adequate indicator for steam sterilization/steam penetration or the results are a method effect. The TC were introduced through the tube, rather than through the body of the capsule. Other researchers came to a similar conclusion, stating that current PCD for hollow loads are not valid steam penetration tests (S. Esen, 2012b). According to the ISO 11140-1:2005 standard, chemical indicators should be comparable to biological indicators. These results show that this is not the case. Biological indicators were inactivated after 1 min at plateau temperature 134-137°C at all tested locations, while chemical indicators still failed when located inside spray channels at 3 min at the plateau temperature. TST CI for non-vacuum processes however started performing colour changes from 0 – 0.5 min at 134-137°C, even though they claim to indicate pass conditions at 134°C after 3 min, which suggests that CI results do not necessarily prove that the load has been exposed to 134°C for 3 min and should be therefore interpreted with care. However, a trend was found in CI and BI results, indicating that surgical handpieces and slow-speed motors are more difficult to sterilize than high-speed handpieces due to either their mass (high-speed handpieces are the lightest in weight) or the construction, which would mean that the outcome of non-vacuum sterilization processes differs between all types and models of handpieces. Both, Rutala (1996) and van Doornmalen (2012) found CI to provide inconsistent results and may be too sensitive and give false positive

results (Rutala et al., 1996, J.P.C.M. van Doornmalen, 2012). BI show more consistent results, however, BI spore strips exposed to non-vacuum cycles with shortened controlled plateau periods were inactivated at 0-0.5 min and failed in handpieces even after 3 min in some locations, which suggests that BI might be dependent on the challenge/device they are located in. BI testing by Rutala et al. (1996) showed more consistent results compared to CI testing, which agrees with the results from this study (Rutala et al., 1996). Moreover, the results in this study suggest that investigating steam penetration using CI may depend on the location of the CI within the lumen. Taking the CI results from chapter 3 into account, where it was shown that CI do not only react to dry saturated steam, but to the quantity of water molecules in the environment as well, the possibility that pass conditions of CI in dental handpieces may not prove successful penetration of steam into lumens must be considered.

In complex devices, such as dental handpieces, oil in lumens may block steam penetration. Another possibility is that coating the surfaces and the spores with oil prevent steam from penetrating through the layer of oil. Thus dry heat conditions exist, which makes time a critical factor for effective sterilization. The results show that, whilst survivors were recovered when oil was present, considerable log reduction occurred. Dry heat processes are carried out at higher temperatures and for longer times than moist heat e.g. 160°C for 2 hours (Russell, 1982). It would have been useful to determine the D value of the spores when suspended in oil. The mean heat up time in the non-vacuum cycles was 11 min 47 sec and 4 min 56 sec in the vacuum cycle 2 with ten small vacuum pulses. During this time, some of the spores will be inactivated even before exposure time (3 min) at 134°C, which adds up to a greater lethality than the vacuum cycle 1 (heat up time 1 min 50 sec), where surviving spores were found in 4 out of 12 handpieces. No surviving spores were found in the other cycles tested. Hegna et al. (1978) used a sterilization process with gravity air displacement and an exposure time of 8 min at 134°C to assess *G. stearothermophilus* inactivation in dental handpieces after lubrication. Eight minutes is more than required according to the first MRC report (1959), which is based on Perkins (1956) and delivers a higher thermal energy. No growth was found (Hegna et al., 1978).

These results correspond to the findings in the present study, although, only eight instruments were tested in the study by Hegna et al.

Edwardsson et al. (1983) tested handpiece lubrication with and without an antimicrobial agent and compared the results to handpieces that were sterilized without lubrication after contaminating the handpieces with a saliva suspension containing *G. stearothermophilus*. Sterilization processes used were 120 - 124°C for 20 min and 134 - 138°C for 10 min. Some *G. stearothermophilus* survived in handpieces without lubrication and lubrication without antimicrobial agent. Therefore the authors concluded that oil without antimicrobial agent impeded access for steam and the gravity cycles used failed to sterilize the lumens. Multiple experiments on 10 handpieces were performed (Edwardsson et al., 1983).

In 1999, Andersen et al. investigated the efficacy of four non-vacuum sterilizers and one vacuum benchtop sterilizer, working at 121°C for 20 minutes. 12 dental air turbines were cleaned before contamination with *G. stearothermophilus*. Growth was observed in all non-vacuum processes. The vacuum process resulted in no growth (Andersen et al., 1999). However, no lubrication was used. The presence of handpiece oil might block steam from accessing the channels of handpieces even in vacuum processes.

The majority of spores recovered originated from indented tokens, with and without oil. This suggests that oil in thin layers might not prevent spore inactivation on surfaces with different surface finishes if access for steam is provided. The more critical parameter appears to be the thickness of the layer of spores. The results show that a protective effect occurs to spores located at the bottom of the layer, which are therefore more resistant than spores located on top of the layer. This raises the question whether the same effect occurs in debris on instruments after patient treatment.

The investigations undertaken in this section did not look at a great variety of different diameters in lumens, which is reported to have an impact on steam penetration. Young, 1993 described that smaller blind-ended lumens (d=0.4 mm) are more dependent on position within a non-vacuum sterilizer and *Geobacillus*

stearothermophilus spore population recovered was ten times higher than in larger blind-ended tubes (d=1.7 mm) of the same length (9.4 cm), which suggests that especially for small diameter lumens, vacuum sterilization is required (Young, 1993). Additionally, Young et al. discovered that vertically positioned lumens show a “draining” effect and facilitate better steam penetration (Young, 1995).

Chapter 6 - In vivo study investigating steam sterilization in general dental practice

6.1 Introduction

General dental practices in the UK commonly use non-vacuum benchtop sterilizers (Smith et al., 2009c) for sterilizing instruments, including dental handpieces, after patient treatment, even though the European standard recommends vacuum sterilizers for lumen devices (13060:2014, BS EN). A survey, carried out in the US in 1996, that involved 1355 dental practices showed that 7% of dental practices did not sterilize their handpieces in steam sterilizers, and 49% of dental practices did not use BI as recommended. The overall conclusion of this survey was that the risk of cross infections of blood born viruses in dental practice should not be ignored, especially if handpieces are inadequately reprocessed and/or the sterilizer is not maintained and validated (Gurevich et al., 1996). Howie discussed the importance of a profound understanding of the sterilization process and education of staff in decontamination facilities in order to operate the sterilizer correctly and recognize and avoid problems, which could potentially lead to cross-infections (Howie, 1961). Some dental handpieces manufacturers specifically describe the requirement to use a vacuum or S type sterilization process (W&H, Sirona) and for packaging handpieces prior to vacuum sterilization where required. Such packaging is only performed in vacuum sterilizers (KaVo). NSK however does not specify the type of steam sterilization process. The most commonly used benchtop sterilizers for dental practices in the UK are shown in Table 1 (data provided by W&H, UK). Furthermore, a lack of training of dental staff and the necessity of quality management in local decontamination units (LDU) in dental practice has been reported (Smith et al., 2009b). An observational study, assessing 179 dental practices in Scotland, showed that poor record keeping of periodic testing for sterilizers according to the Scottish Health Technical Memorandum (SHTM) can be a quality control issue (SHTM, 2010, Smith et al., 2007).

Table 6-1 Most commonly used benchtop steam sterilizers in UK dental practice

N type sterilizers	
Model	**Manufacturer**
SES 2010 (11 L chamber)	Eschmann
Alpha N (16 L chamber	Prestige
MS	W&H
B type sterilizers	
Model	**Manufacturer**
SES 3000B	Eschmann
SES 225B	Eschmann
Optima B (16 L chamber)	Prestige
Advanced B (16 L chamber)	Prestige
Vacuklav 41B	Melag
Vacuklav 31B	Melag
Lisa	W&H

The aims of this chapter were to test non-vacuum and vacuum sterilizers in a general dental practice environment using CI, BI and dataloggers to monitor temperature and pressure during the sterilization cycles.

6.2 Methods

The sources of sterilizers were five different GDPs in Scotland, where we were invited to perform tests. In Scotland sterilizers undergo periodic testing according to the SHTM 2010. However, validation documentation was not inspected.

Non-vacuum and vacuum sterilization processes were compared. Three cycles were performed in each sterilizer using CI, BI and data loggers. For each cycle a "standard test tray", consisting of three of each of three different types of handpiece: Dental air turbine TA-98 C LED (W&H, Austria), straight surgical handpiece S11 (W&H, Austria), slow speed motor WA-56 (W&H, Austria) and a helix PCD (Browne) used as control (Figure 6-1). Three handpieces of each type were inoculated with CI and BI. Chemical indicators (class 2, Browne) were placed in three positions in the turbine (turbine head, drive air channel, spray channels), in two positions in the surgical handpiece (chuck lever, handpiece back) and in one position in the slow speed (inside sleeve). Biological indicators (mini spore strips, Excelsior, D_{121}= 1.8 – 2.5 min) were placed in three positions in the turbine (turbine head, drive air channel center, drive air channel back), in two positions in the surgical handpiece (chuck lever, handpiece back) and in one position in the slow speed (inside sleeve) (for handpiece test load details, see chapter 2, section 2.1.8). Handpieces for vacuum sterilization were placed in sealable sterilization pouches (Steris) before sterilization.

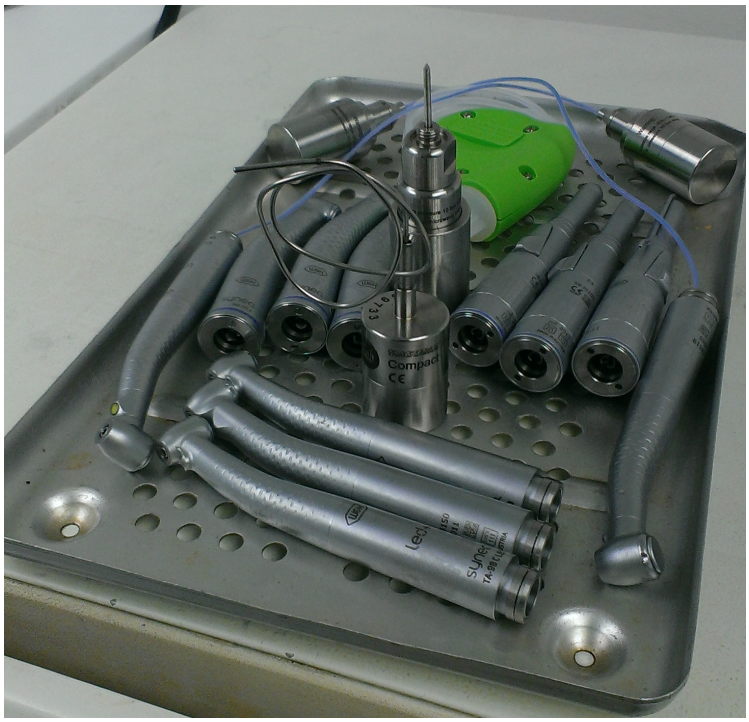

Figure 6-1 “Standard test tray” with 3x dental air turbine TA-98 C LED (W&H, Austria), 3x straight surgical handpiece S11 (W&H, Austria), 3x slow speed motor WA-56 (W&H, Austria), 1x helix PCD (Browne), 2x temperature data logger inside dental air turbine (TA-98 C LED (W&H, Austria), 1x temperature data logger “free space” and 1x pressure data logger (all data loggers ellab)

As controls a helix PCD (Browne) was inoculated with a chemical indicator and a spore strip (Excelsior) and two turbines were inoculated with data loggers (ellab, accuracy 0.05˚C) to trace temperature during the sterilization process. Additionally, a pressure sensor (ellab, accuracy 0.25%) and a temperature sensor were used to trace the sterilization cycle within the chamber. Humidity and room temperature in the LDU were recorded during the visits using a humidity/temperature sensor (ellab).

After the sterilization process chemical indicators were visually assessed for color change, while biological indicators were transferred into 2 ml TSB (Sigma) and incubated up to 8 days at 56˚C.

According to Perkins the heat up time should bring all of the load up to sterilization temperature (Perkins, 1983). In the standard for small steam sterilizers a tolerable time difference between the chamber and the load is 15 sec (13060:2014, BS EN), while the Scottish Health Technical Memorandum

established a temperature lag of 2°C from the point where the chamber reaches 134°C compared to the load as tolerable (SHTM, 2010).

In order to take all three theories into account, time delays of 3 sec (based on results from vacuum cycle testing), 15 sec and a temperature lag of 2°C were established as thermometric fails and compared.

Additionally, the "worst case" in thermometric measurements was used to calculate integrated lethality (F_0) and log reduction of the process (chamber compared to handpiece inside. Non-vacuum process 3 (N3) was selected. The following equation was used to used to calculate integrated lethality (F_0)

$F_0 = t\ (10^{((T-121.1)/z)})$,

where t is the time interval of points of measurement; T is the temperature at the point of measurement; z is the z-value, assumed to be 10.

Further calculation of log reduction of the microbial population is performed by F_0/D_{121} (Pharmacopoeia, 2014).

6.3 Results

6.3.1 Non-vacuum processes

Seven non-vacuum sterilizers were tested in GDP. A total of 34 BI fails and 25 CI fails were detected out of a total of 360 tests. Most fails of CI and BI were located in the chuck lever (S11, W&H) or the inside of the slow speed motors (WA56, W&H), while all but one CI and one BI located in turbines (TA98, W&H) passed. Sterilization cycle times ranged from 16 – 25 min, with plateau periods of 3.5 – 4.5 min at 134˚C. The period over which temperature differences between the sterilizer chamber and the inside of the handpieces occurred ranged from 0 sec – N/A, which meant that some handpieces did not achieve sterilization temperature during the whole cycle. There were 31 thermometric fails out of in the 40 handpieces tested. Only one non-vacuum sterilizer indicated no thermometric or BI fails but did have one CI fail in a surgical handpiece. In every cycle a helix PCD (Browne) was included as a control, where all CI and BI indicated failed sterilization conditions. BI and CI fails are displayed in tables 6-1 to 6-7 and cycle profiles including magnifications of charts of the plateau period are shown in figures 6-2 to 6-9. A summary of all CI, BI and thermometric results of non-vacuum sterilizers tested in GDP is shown in table 6-9. Thermometric traces of non-vacuum sterilizer 3, shown in figure 6-5, were used to calculate integrated lethality and log reduction (chamber compared to dental turbine 2).

Chamber; F_0=102.11, log red=51

Dental turbine 2; F_0=71.36, log red=36

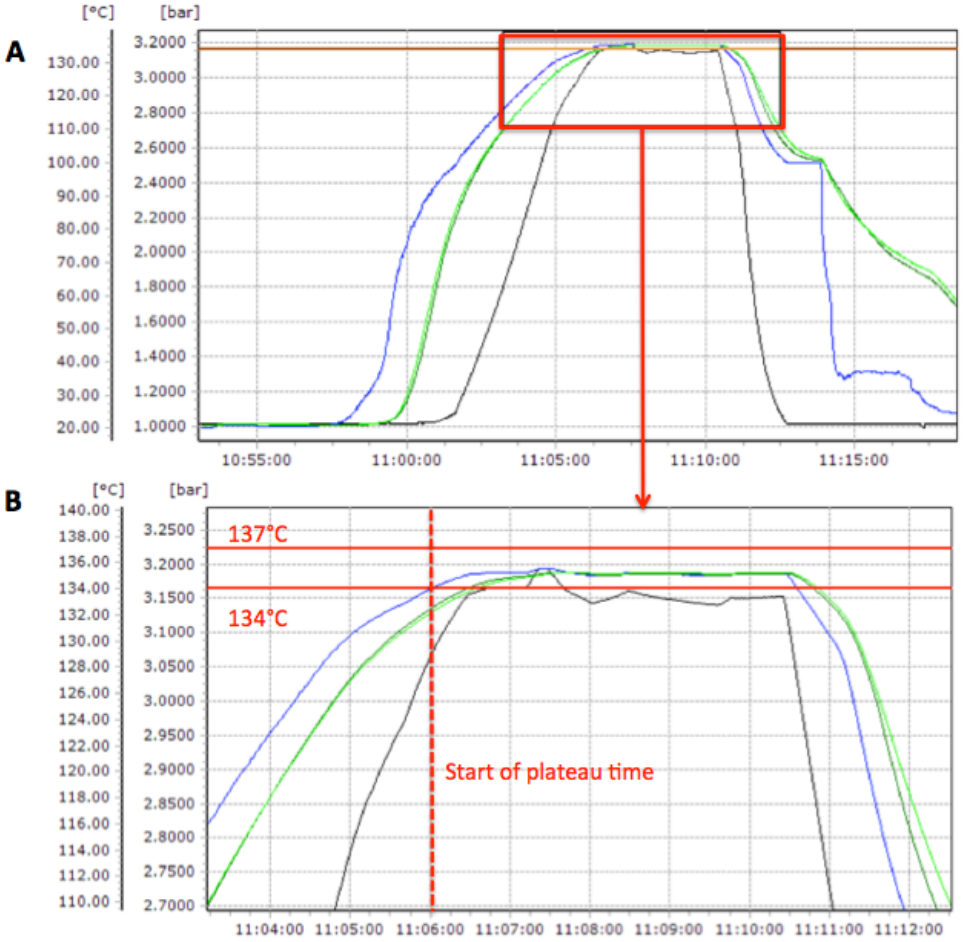

Figure 6-2 Temperature traces of N type sterilizer 1 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (6/6 thermometric fails)

Table 6-2 Result summary N type sterilizer 1; growth in 7/54, CI fail in 6/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (N1) SN LCC-7L-1154	Cycle					
Model: Eschmann Little Sister SES2010	1		2		3	
	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✗	✗	✓	✓	✓	✓
	✓	✓	✗	✗	✓	✓
	✓	✓	✗	✗	✗	✗
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✗	✗	✓	✓
	✓	✓	✗	✗	✓	✓
	✓	✓	✓	✓	✓	✗
Helix (Browne)	✗	✗	✗	✗	✗	✗

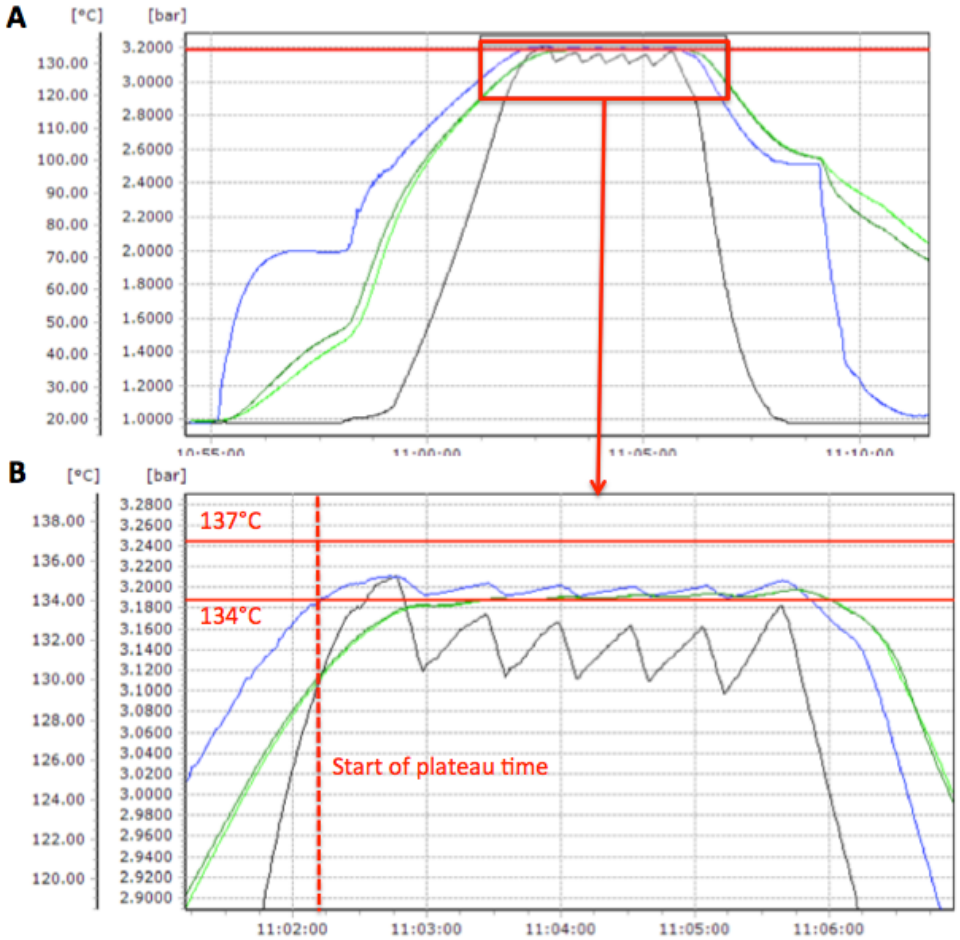

Figure 6-3 Temperature traces of N type sterilizer 2 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (6/6 thermometric fails)

Table 6-3 Result summary N type sterilizer 2; growth in 11/54, CI fail in 7/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (N2) SN SCB-5C-9084	Cycle Number					
Model: Eschmann SES 2000	1		2		3	
	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✗	✓	✓	✓	✓	✓
	✓	✓	✗	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✗	✗	✗	✗	✗	✗
	✗	✗	✗	✓	✓	✗
	✓	✗	✓	✓	✓	✓
S11 Back	✓	✗	✓	✓	✓	✗
	✓	✓	✓	✓	✓	✗
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✗	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✗
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

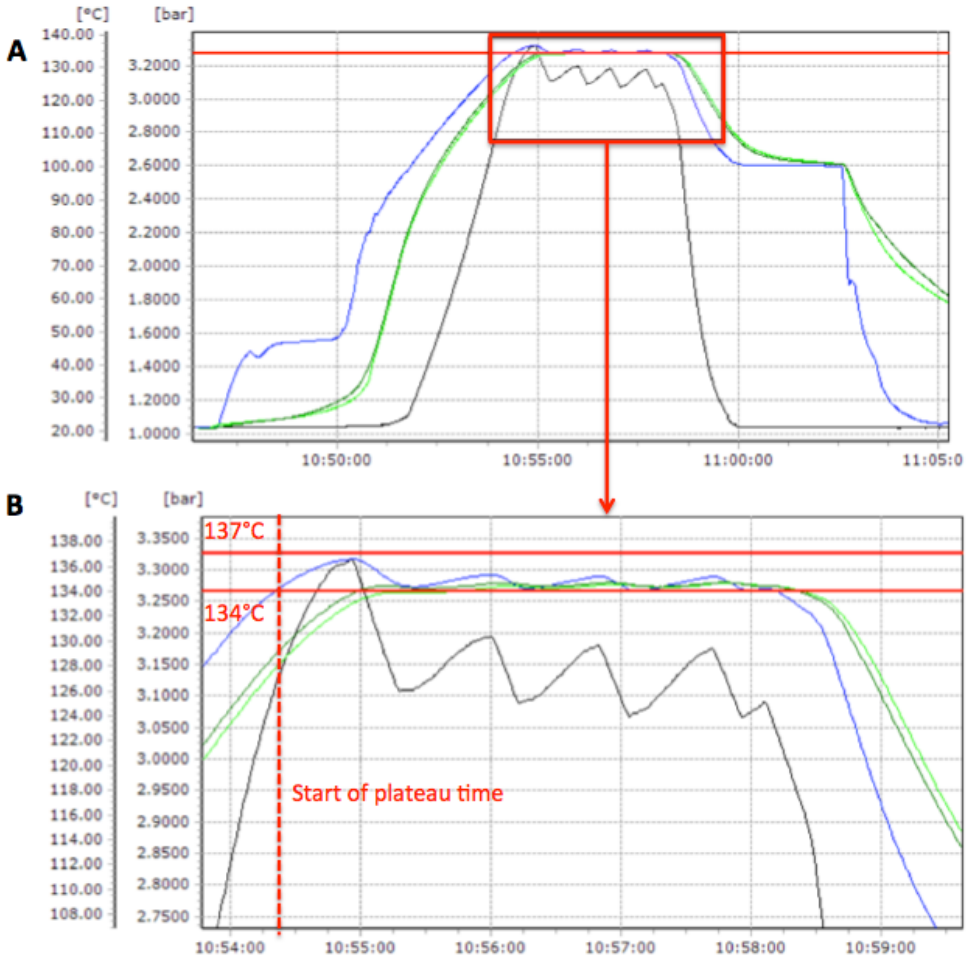

Figure 6-4 Temperature traces of N type sterilizer 3 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (6/6 thermometric fails)

Table 6-4 Result summary N type sterilizer 3; growth in 10/54, CI fail in 9/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (N3) SN SCB-4J-8437	Cycle					
Model: Eschmann SES 2000	1		2		3	
	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✗	✓	✓	✓
	✓	✓	✗	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✗	✓	✗	✓	✗
	✓	✗	✓	✗	✓	✗
	✓	✗	✓	✓	✓	✗
S11 Back	✓	✓	✗	✓	✗	✓
	✗	✓	✓	✓	✗	✓
	✗	✓	✓	✓	✓	✓
WA-56 Inside	✓	✗	✓	✗	✗	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✗	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

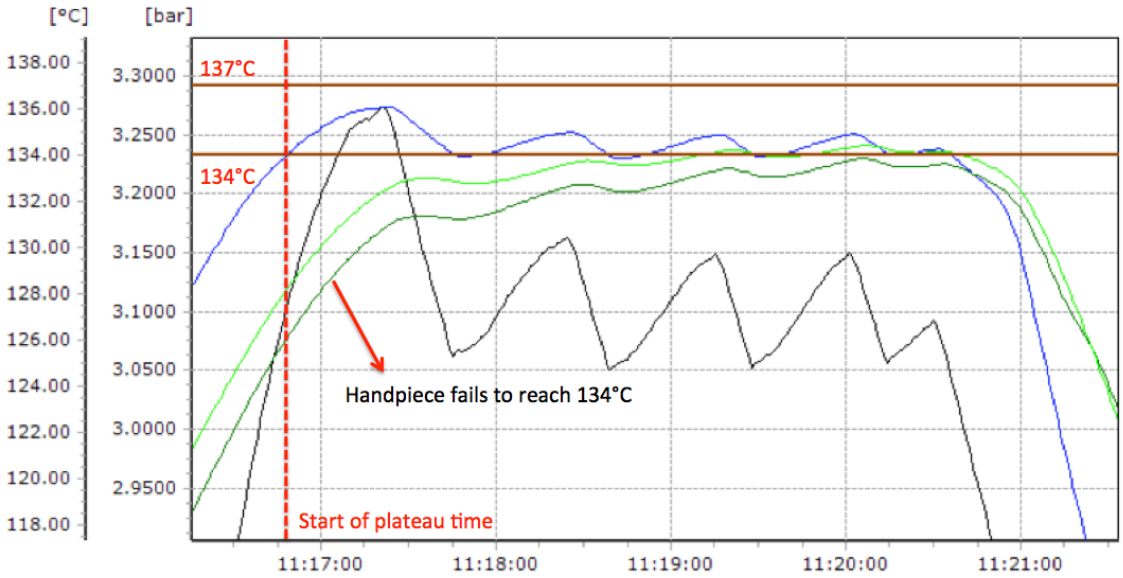

Figure 6-5 Temperature traces of N type sterilizer 3 (magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2, which never reaches sterilization temperature

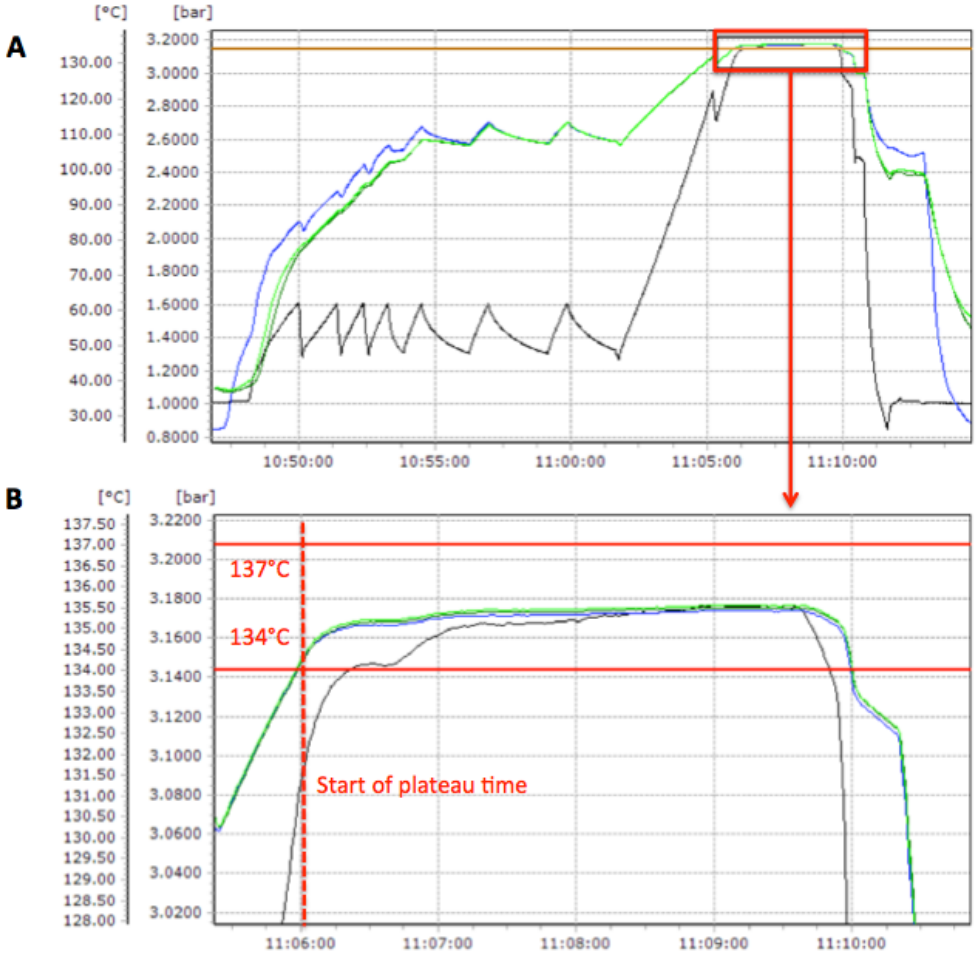

Figure 6-6 Temperature traces of N type sterilizer 4 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (0/4 thermometric fails)

Table 6-5 Result summary N type sterilizer 4; growth in 1/36, CI fail in 0/36, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (N4) SN 107957	Cycle					
Model: W&H MS 22	1		2		3 – FAIL (excluded)	
	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✗	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✓	✓

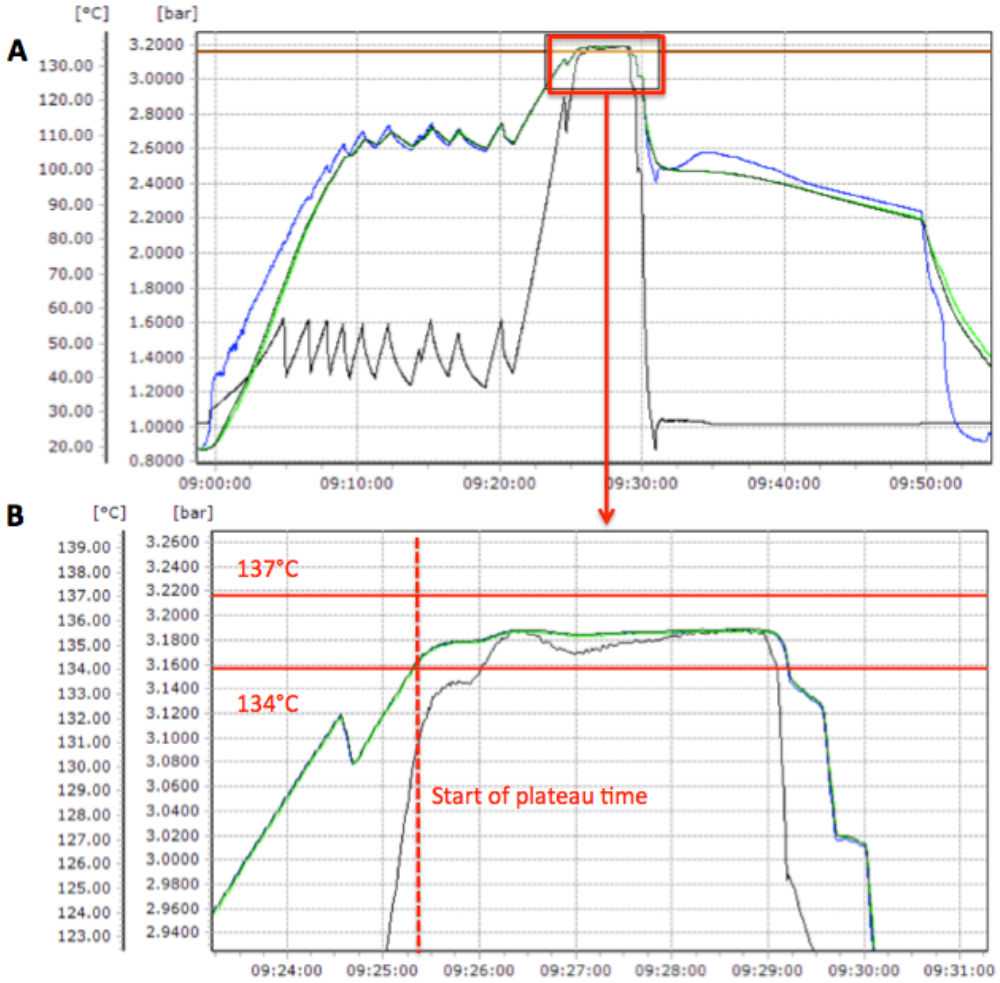

Figure 6-7 Temperature traces of N type sterilizer 5 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (6/6 thermometric fails)

Table 6-6 Result summary N type sterilizer 5; growth in 1/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (N5)* SN SCB-5G-9684	Cycle					
Model: Eschmann SES 2000	1		2		3	
	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✗	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

*Positive pressure pulses are regarded as “active air removal” (personal conversation with Brian Kirk, PhD, 3M, Loughborough, UK)

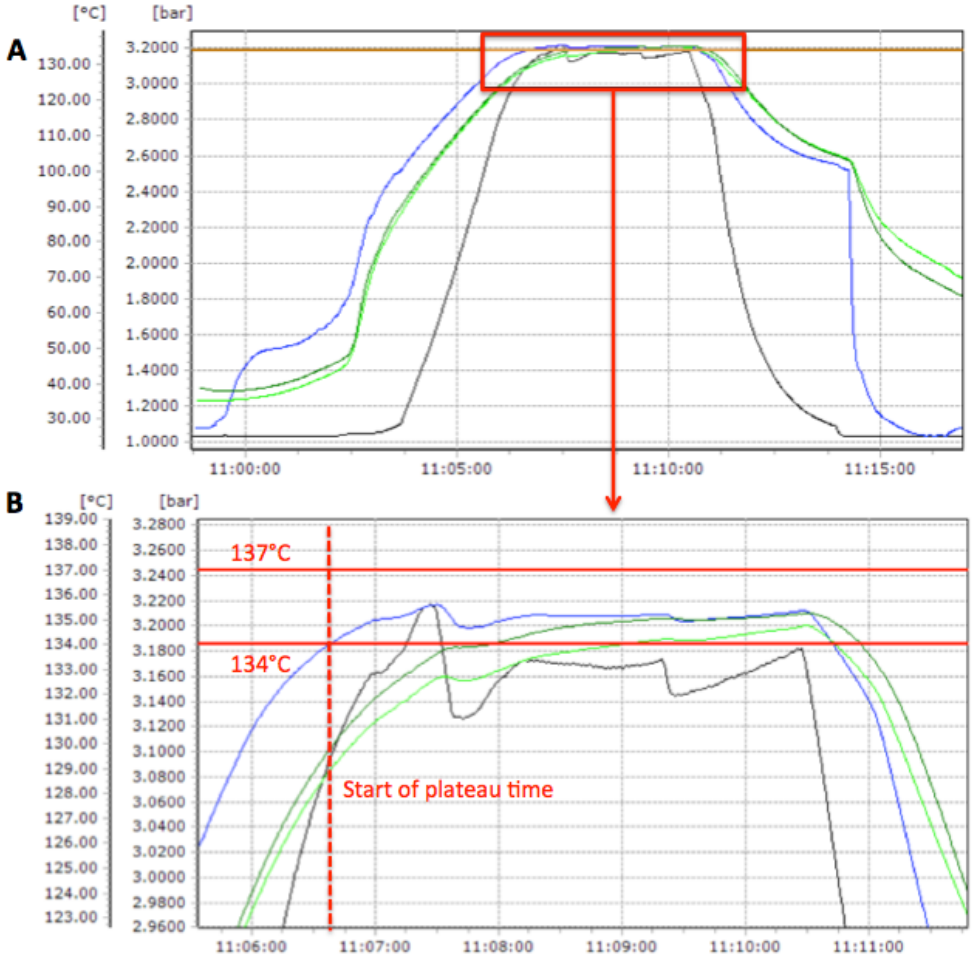

Figure 6-8 Temperature traces of N type sterilizer 6 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (6/6 thermometric fails)

Table 6-7 Result summary N type sterilizer 6; growth in 3/54, CI fail in 3/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (N6) SN LCB-3E-3706	Cycle					
Model: Eschmann Little Sister 3	1		2		3	
	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✗	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✗	✓	✓	✓
	✓	✓	✓	✓	✗	✓
S11 Chuck Lever	✓	✗	✓	✗	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✗	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

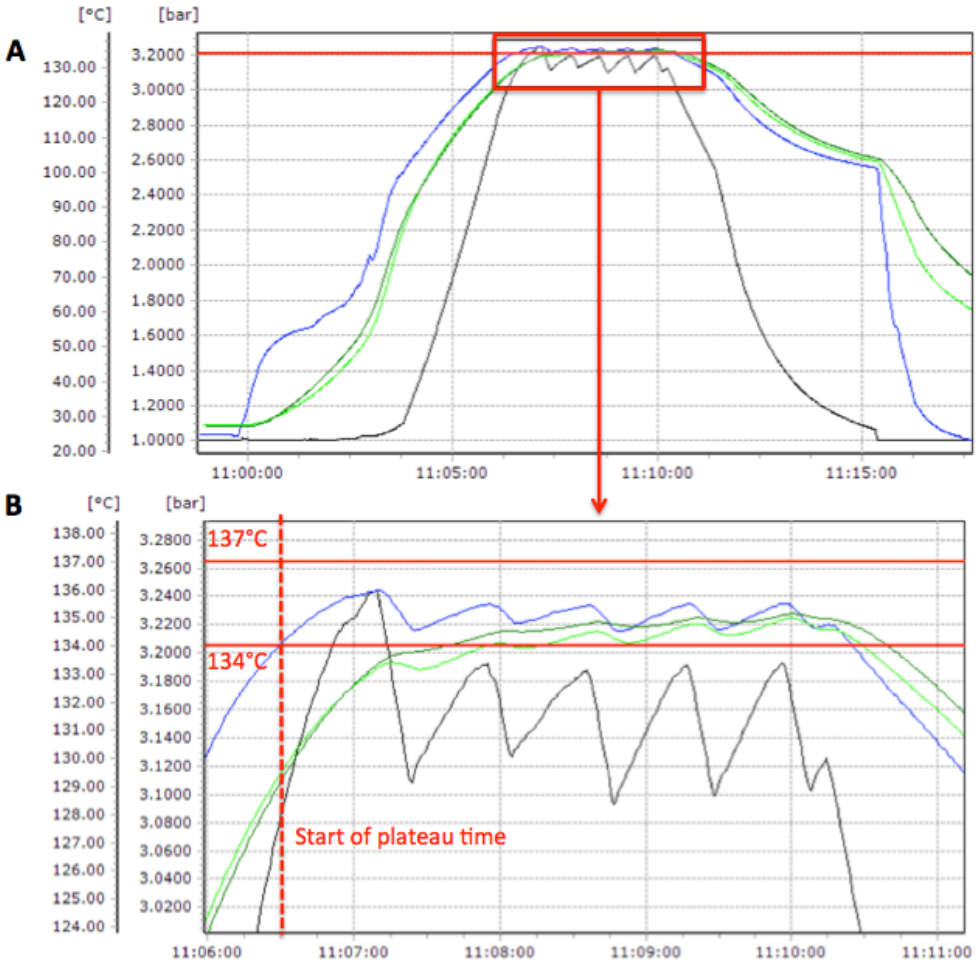

Figure 6-9 Temperature traces of N type sterilizer 7 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (1/6 thermometric fails)

Table 6-8 Result summary N type sterilizer 7; growth in 1/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (N7) SN 113512	Cycle					
Model: W&H	1		2		3	
MS22	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✗
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✗	✗	✗	✗	✗	✗

Table 6-9 Summary of BI, CI and thermometric fails in non-vacuum sterilizers tested in GDP

Sterilizer	BI fails	CI fails	Thermometric fails (>3 sec)*	Thermometric fails (>15 sec)*	Thermometric fails (>2°C)*
N1	7/54	6/54	6/6	6/6	4/6
N2	11/54	7/54	6/6	6/6	6/6
N3	10/54	9/54	6/6	6/6	6/6
N4	1/54	0/54	0/6	0/6	0/6
N5	1/54	0/54	6/6	6/6	6/6
N6	3/54	3/54	6/6	6/6	6/6
N7	1/54	0/54	1/6	0/6	0/6
Total	34/378	25/378	31/42	30/42	28/42
Total fails (%)	9	7	74	71	67

*Refer to section 6.2

6.3.2 Vacuum processes

Growth was detected in one out of 108 BI used in the handpieces processed in two vacuum sterilizers. There were also no fails in 108 CI placed in these devices (Tables 6-10 and 6-11). Cycle times were 60 – 70 min, with a holding time of 4 – 4.5 min at 134˚C (Figures 6-10 and Figures 6-11) and a temperature difference between the sterilization chamber and the inside of the handpieces of 0 – 1 sec. No thermometric fails and no CI fails of control helix PCD were seen, however, one BI fail in a helix was detected in vacuum sterilizer 2. A summary of all CI, BI and thermometric fails is shown in table 6-12.

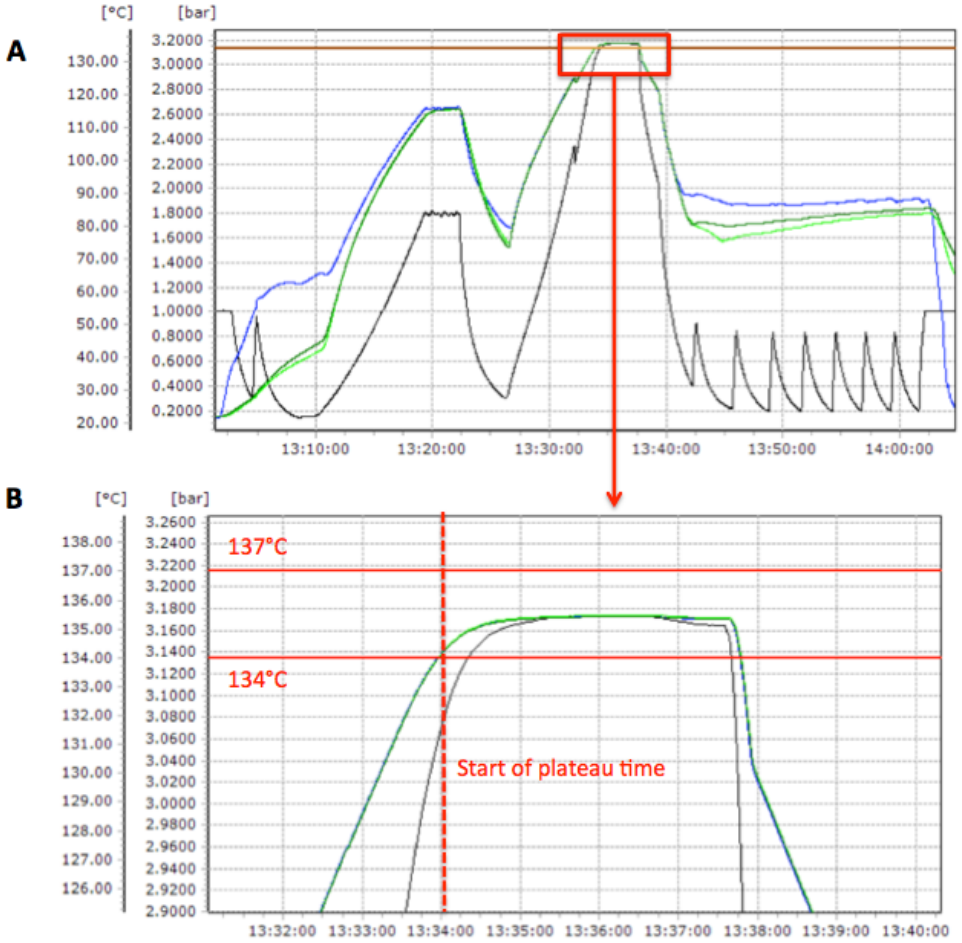

Figure 6-10 Temperature traces of B type sterilizer 1 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (0/6 thermometric fails)

Table 6-10 Result summary B type sterilizer 1; growth in 0/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (B1) SN L2DH3D 1997	Cycle					
Model: Eschmann Little Sister SES225B vacuum	1		2		3	
	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✓	✓	✓	✓	✓	✓

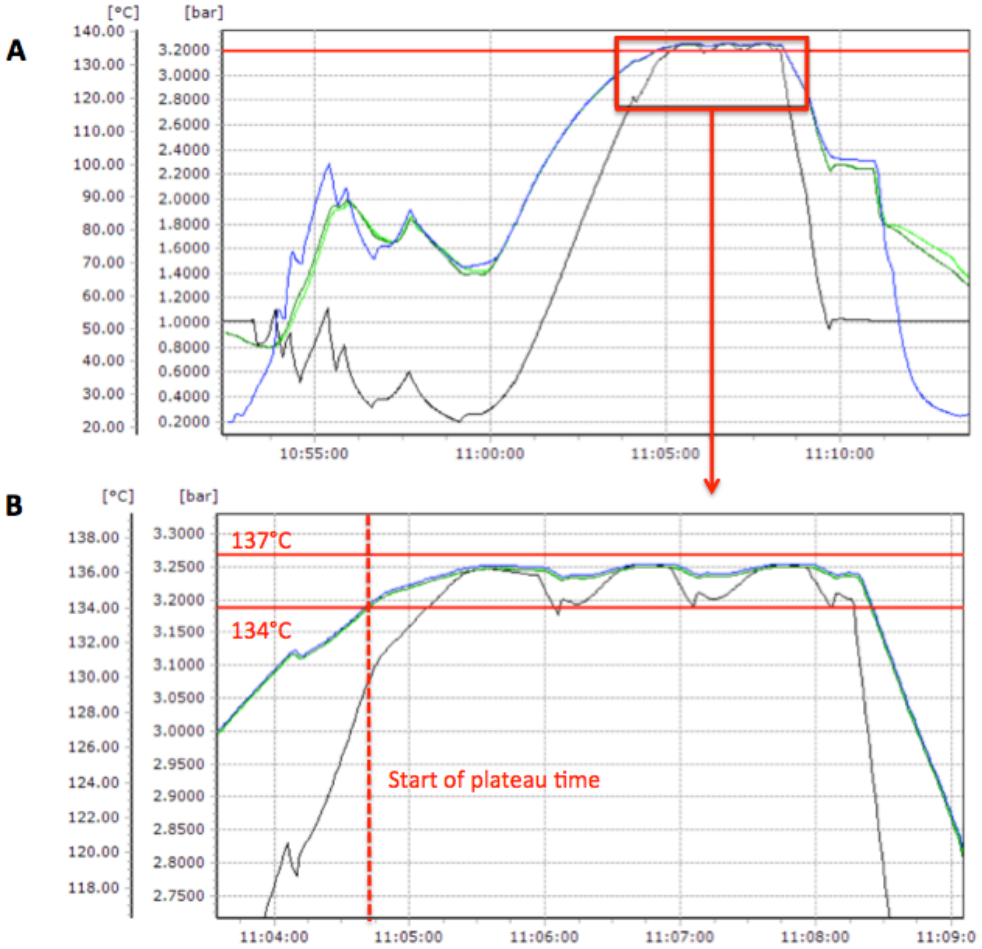

Figure 6-11 Temperature traces of B type sterilizer 2 (A full cycle, B magnification of holding time) where black=pressure, blue=free space in chamber, light green= dental air turbine 1, dark green= dental air turbine 2 (0/6 thermometric fails)

Table 6-11 Result summary B type sterilizer 2; growth in 2/54, CI fail in 0/54, growth controls showed growth (✓= pass, ✗= fail)

Sterilizer (B2) SN unknown	Cycle					
Model: W&H	00041		00042 (fast)		00043 (fast)*	
Lisa 517	CI	BI	CI	BI	CI	BI
TA-98 Head	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 CI: Spray Channels BI: Air Channel plastic	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✗
	✓	✓	✓	✓	✓	✓
S11 Back	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
WA-56 Inside	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
Helix (Browne)	✓	✓	✓	✓	✓	✗

*Faster vacuum cycle might be less effective

Table 6-12 Summary of BI, CI and thermometric fails in non-vacuum sterilizers tested in GDP

Sterilizer	BI fails	CI fails	Thermometric fails (>3 sec)**	Thermometric fails (>15 sec)**	Thermometric fails (>2°C)**
B1	0/54	0/54	0/6	0/6	0/6
B2*	1/54	0/54	0/4	0/4	0/4
Total	1/108	0/108	0/10	0/10	0/10
Total fails (%)	0.9	0	0	0	0

*x1 BI fail in helix PCD, ** refer to section 6.2

6.3.3 Humidity measurements

Humidity was measured in five different GDP for at least 1 hour and ranged from 30.75 – 90% RH, while the room temperature fluctuated between 18.5 and 26.5°C as shown in an example (Figure 6-11).

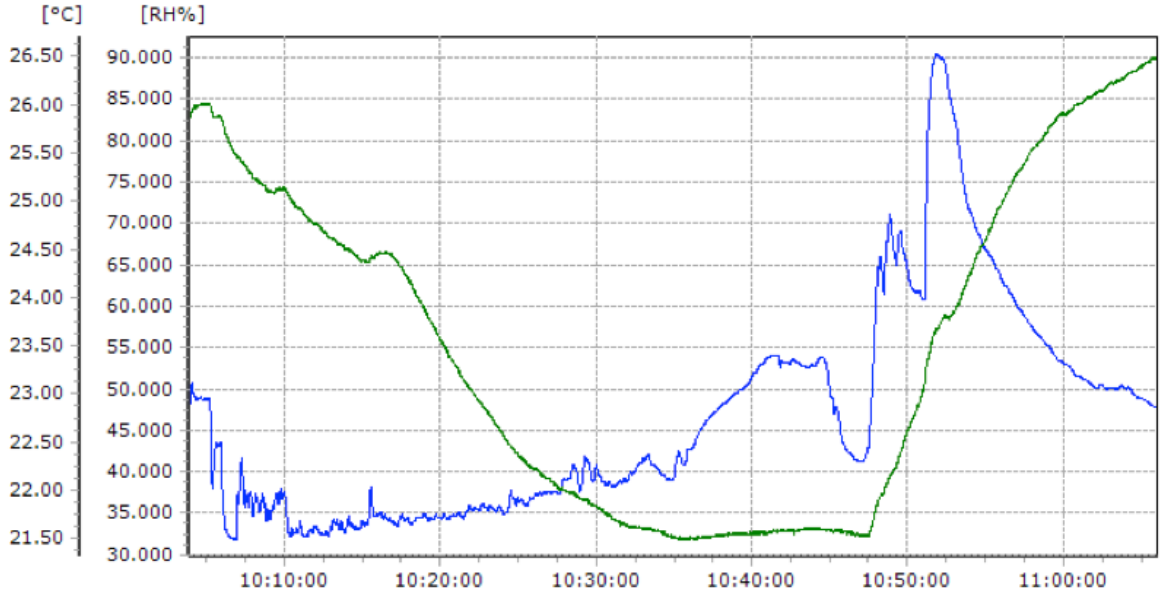

Figure 6-12 Relative Humidity (RH) in blue and temperature (°C) in green, measured in GDP 4 over the span of 1 hour

6.4 Discussion

Seven non-vacuum benchtop sterilizers were tested in total. Three cycles with nine handpieces with CI and BI each were performed in each sterilizer. A total of 378 CI and BI from inside handpieces were assessed, where 34 BI (9%) and 25 CI (7%) failed. Fails were found in all sterilizers, independently of model or cycle time, which suggests that dental handpieces cannot be sterilized in non-vacuum processes. Most fails were found in the straight surgical handpiece, which was the most contaminated type, due to its use in oral surgery (Deshpande et al., 2015, Smith, 2011). A total of 42 handpieces underwent thermometric assessment during non-vacuum sterilization, where 31 (74%) were classed as thermometric fails (for details refer to section 6.2) based on Perkins (1956) in combination with findings from previous testing in this study (Chapter 3), 30 handpieces (71%) were thermometric fails according to BS EN 13060:2014 and 28 (67%) of handpieces showed a thermometric fail according to SHTM 2010, which means that the delay inside the handpieces reaching the sterilization temperature compared to the chamber exceeded 3 sec, 15 sec or 2°C, respectively. Moreover, in non-vacuum sterilizer 3, where one of the handpieces did not achieve sterilization temperature, integrated lethality and log reduction were found to be considerably lower in the handpiece compared to the chamber (chamber F_0=102.11, log reduction=51; handpiece F_0=71.56, log reduction=36). A log reduction of 36 is more than the required 12 (SAL 10^{-6}) stated in the Pharmacopoeia (2014), but 10/54 BI fails, 9/54 CI fails and 6/6 thermometric fails were observed. Therefore, it may be assumed that residual air was present and this retarded the heating of the inside of the device. The sensing ends of the data loggers used were insulated to avoid measuring the thermal mass of the handpieces.

Two vacuum benchtop sterilizer were tested in this study. In vacuum sterilizer 1 all BI and thermometric measurements showed pass conditions. Perhaps this is not surprising since manufacturers of handpieces and benchtop sterilizers (W&H, KaVo) recommend vacuum sterilization for dental handpieces. The requirement of a vacuum sterilization process for hollow loads is also stated in the standards (285:2006+A2:2009, BS EN, 13060:2014, BS EN). However one BI fail was found in

vacuum sterilizer 2 with a “fast” cycle option, which may have been less effective than a full cycle. Additionally, a recent study involving US dental practices resulted in 25,000 BI fails over a period of 3 years (publication in print in AJIC). Most UK dental practices still work with non-vacuum sterilizers (Smith et al., 2009c). This is probably due to the fact that non-vacuum sterilization processes are much faster and less costly in terms of purchase and running costs (W&H). Lack of training and difficulties with commissioning, installation and periodic testing might also be an influencing factor in the choice of sterilizer (Smith et al., 2009a, Smith et al., 2007). Furthermore, a survey of dental practices in England showed that not all dental practices sterilized their handpieces after each patient, due to a shortage of instruments, fear of damage of instruments or unawareness of the necessity. However, media coverage resulted in a 20.6% increase of handpiece sterilization (Lloyd et al., 1995).

Some dental practices have little space for a local decontamination unit (LDU). Humidity and temperature fluctuated the most in the smallest LDU dental practice visited. This dental practice was equipped with a non-vacuum sterilizer without a wastewater tank. Humidity reached 90% RH when steam filled the room during the condensation phase of the sterilization process. This may have an impact on CI the stability in storage conditions, where large fluctuations in humidity are likely to occur.

Observations made in LDUs during dental practice visits also uncovered problems with the following: poor loading and unloading of sterilizers, lack of linear flow as described in 2005 (NHS, 2005), lack of daily, weekly, quarterly and annual testing, as well as poor or non existent record keeping. Even if a sterilizer might be considered to work according to the appropriate standards and validation criteria; it does not mean that a particular load would be sterile after the process. Narrow channels in surgical instruments can be challenging especially in terms of steam penetration and fail the sterilization process (van Doornmalen and Kopinga, 2013).

An important point in decontamination is infection control for the safety of patients and staff. The presence of cross-infection risk of HIV and Hepatitis B has been reported by Gurevich in 1996, which was proven by an incident in a

portable dental clinic in West Virginia, US in 2009 (Gurevich et al., 1996, Radcliffe et al., 2013).

Chapter 7 - Investigating the effect of cleaning on handpiece sterilization

7.1 Introduction

Dental handpieces are used in a variety of different treatments and become contaminated with protein from saliva and blood (Dreyer and Hauman, 2001, Herd et al., 2007, Chin et al., 2006) in the process. Incomplete removal of protein and microorganisms residues from handpieces can result in risk of cross-infection for patients and staff, including blood borne viruses and bacterial infections (Epstein et al., 1995, Hu et al., 2007, Lewis and Arens, 1995, Lewis and Boe, 1992, Shpuntoff and Shpuntoff, 1993, Lewis et al., 1992). The reprocessing has to include cleaning, disinfection and sterilization (Alfa et al., 2006). Cleaning and lubricating dental handpieces prior to sterilization are essential steps in the decontamination cycle (Weightman and Lines, 2004). In the European Standards, a variety of test soils for testing the cleaning of medical devices and their methods of application to such devices are described (15883-5:2005, ISO/TS). However, there is no specific test soil in the standards for dental instruments. The aim of this section was to determine the cleaning efficacy of Prototype X using Edinburgh test soil and Austrian test soil and to compare the results to those obtained with a benchtop washer-disinfector (Thermoklenz, W&H). Furthermore, the aim was to assess residual protein on dental handpieces after clinical use both before and after Prototype X or a CSSD automated washer-disinfector (Belimed) using both new and used handpieces.

The effect of pre-cleaning using Austrian test soil and Prototype X on spore inactivation in a non-vacuum sterilization cycle was investigated as well.

7.2 Material and methods

7.2.1 Cleaning efficacy of dental handpiece cleaner - In-vitro

7.2.1.1 Edinburgh test soil - OPA

10 μl of Edinburgh test soil (3.4 μg protein) were inoculated into the head and the outer sleeve of six high speed air turbines (TA-98, W&H, Austria), three slow speed motors (WA-56, W&H, Austria) and three surgical handpieces (S11, W&H, Austria). After a 30 minute drying time at room temperature the devices were put through a cleaning process in the handpiece cleaner-lubricator Prototype X. Handpieces were dismantled and the following parts were sampled after protein recovery from the device measured using the OPA method (15883-1:2009+A1:2014, BS EN ISO) as described in chapter 2, section 2.1.1:

- TA-98 (air driven high speed turbines)
    - Head (blade and cap)
    - Outer sleeve
- S11 (straight surgical handpiece)
    - Gear
    - Outer sleeve
    - Nose cone
- WA-56 (slow speed contra angled handpiece)
    - Outer sleeve
    - Head + middle gear

Positive controls consisted of leaving the Prototype X step was omitted and for negative controls the contamination step was omitted. The amounts of protein

detected were calculated in μg/ml with reference to a mucin standard curve (lower sensitivity limit 2±1 μg/ml).

7.2.1.2 Austrian and Edinburgh test soil – G-Box

Eight dental air turbines (TA-98 C LED, W&H, Austria) were manually cleaned and sterilized (Lisa, W&H, Austria). One handpiece was used as a blank control without contamination or the Prototype X cleaning process. One handpiece without contamination was chosen to represent a negative control following cleaning in the Prototype X to investigate the influence of detergent and oil on the protein detection method (G-Box, details to be found in chapter 2, section 2.1.3). Three handpieces were contaminated with Austrian test soil prepared according to ISO/TS 15883-5:2005 and three handpieces were contaminated with artificial saliva containing porcine stomach mucin (Sigma).

In each case 100 μl of contaminant were pipetted into the turbine head. 100 μl of contaminant were pipetted onto the outer sleeve of the handpiece (Figure 7-1). The handpieces were left to dry for 30 min at room temperature.

Figure 7-1 Three dental air turbines contaminated with Austrian test soil before Prototype X process

One handpiece with each contaminant was used as a positive control and was sampled in the G-Box without Prototype X cleaning and lubrication step. Three handpieces with each of two contaminants (test soil or mucin) were processed through the Prototype X before sampling in the G-Box. The efficacy of the Prototype X cleaning and lubricating device was assessed visually for the handpieces contaminated with Austrian test soil and residual protein amounts were calculated for all handpieces and turbine blades according to the mucin standard curve using Microsoft Excel. After the experiments the pipe connecting the chamber of the machine and the waste tray was wiped using a moist cotton swab.

In addition, three new turbines (TA-98, W&H, Austria) were contaminated with 100 μL of Edinburgh test soil and processed in a benchtop washer-disinfector (Thermoklenz, W&H) using the P3 cycle (intensive) before visual assessment and G-box for comparison. Experiments were performed in duplicate.

7.2.2 Cleaning efficacy of dental handpiece cleaner - In-vivo

7.2.2.1 New handpieces - OPA

For the *in-vivo* part of this chapter, W&H provided a total of 40 handpieces, 10 high-speed air turbines (TA-96), 10 high-speed motors (WA-99), 10 slow speed motors (WA-56) and 10 surgical handpieces (S11). All 40 handpieces were given tags to track and trace them and were then fed into the clinics of the Glasgow Dental Hospital (GDH), where they were used for patient treatment. Prior to reprocessing, handpieces were collected at the Dental Central Decontamination Unit (DCDU) of the GDH and transported in bags to the Oral Microbiology Laboratory for assessment. Handpieces were cleaned using the Prototype X, dismantled and parts were submerged in 1%SDS for 1 hour to facilitate protein extraction before the eluates were assessed using the OPA assay. For positive controls, handpieces were sampled directly after patient treatment without the Prototype X step. Protein levels were calculated in μg/ml by comparison to the mucin standard curve (lower sensitivity limit 1 μg/ml).

Handpiece parts sampled:

- TA-98 (air driven high speed turbines)
    - Head (blade and cap)
    - Spray channels
    - Outer sleeve
- S11 (straight surgical handpiece)
    - Gear
    - Outer sleeve + chuck lever
    - Nose cone
- WA-56 (contra angled slow speed motor) and WA-99 (contra angled high speed motor)
    - Outer sleeve
    - Head + middle gear
    - Spray channels

7.2.2.2 Used handpieces – G-Box

18 handpieces, which have been in clinical use for several years in the GDH clinics, (6 surgical handpieces (S11, W&H Austria), 6 slow speed motors (WA-56, W&H, Austria) and 6 high speed turbines (x2 synea, W&H, x1 NSK)) were collected at the DCDU after patient treatment (figure 7-2). Residual protein of all handpieces were assessed before and after the Prototype X process using the G-Box, for determining cleaning efficacy of the Prototype X on used handpieces.

Figure 7-2 Nine used handpieces from the GDH clinics (3 surgical handpieces (S11, W&H Austria), 3 slow speed motors (WA-56, W&H, Austria) and 3 high speed turbines (x2 synea, W&H, x1 NSK))

As a comparison, three used high-speed turbines (synea, W&H) were collected and assessed in the G-box after processing in an automated washer disinfector (AWD, Belimed) at DCDU.

7.2.3 Effect of cleaning on the sterilization of dental handpieces using a non-vacuum sterilization process

Two different handpiece types were included in this experiment (x3 dental air turbine (TA-98, W&H) and x3 surgical handpieces (S11, W&H)). A total of 12 handpieces were contaminated with 50 μL of Austrian test soil (15883-5, 2005 ISO/TS), inoculated into the head and air channel of x3 turbines, and the chuck lever of x3 surgical handpiece. After 30 min drying time, 3 turbines and 3 surgical handpieces were processed in the Prototype X, followed by inoculating all 12 handpieces with spore strips (Excelsior) in the contaminated locations. All dirty and clean handpieces were then sterilized in a non-vacuum benchtop sterilizer (Little Sister 3, Eschmann). Spore strips were transferred into 2 ml of TSB, as shown in figure 7-3 and incubated for 8 days at 56°C.

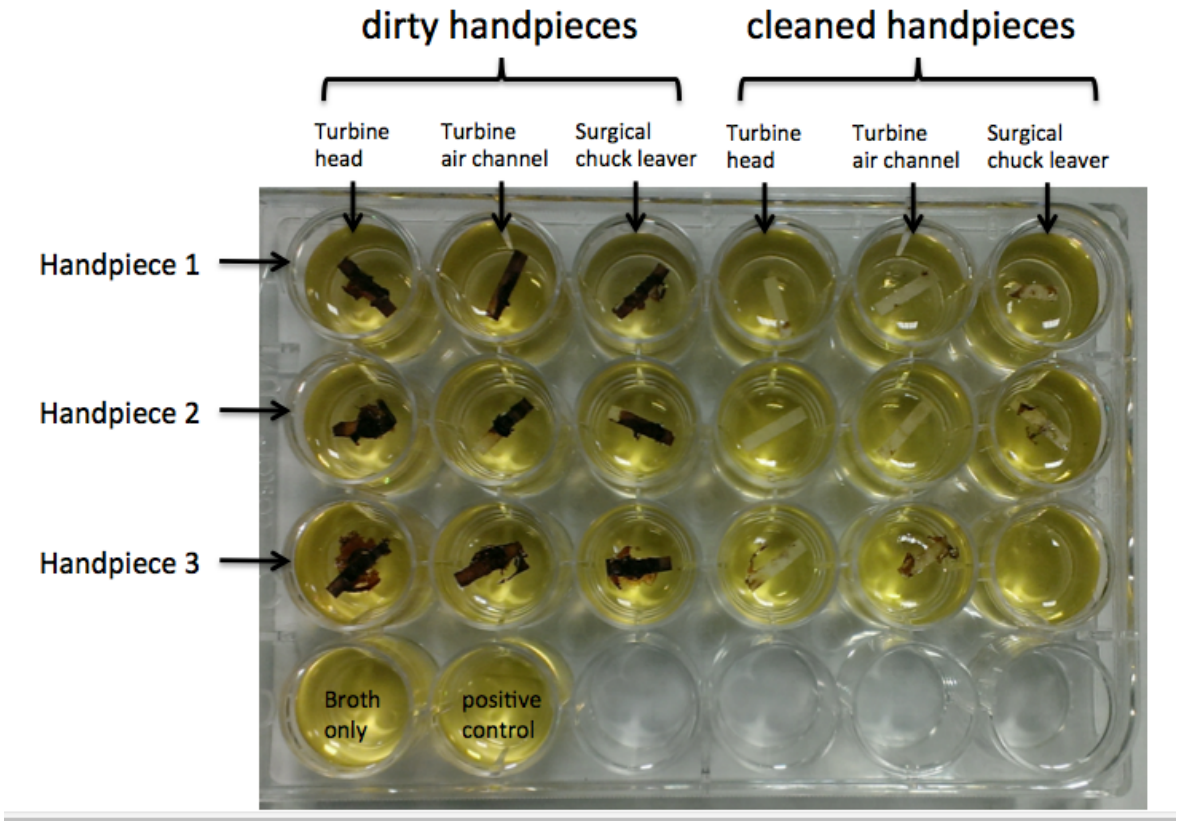

Figure 7-3 Spore strips from dental handpieces with and without cleaning in 2 ml TSB in 24 well plate (Costar)

7.3 Results

7.3.1 Cleaning efficacy of dental handpiece cleaner - In-vitro

7.3.1.1 Edinburgh test soil – OPA

Visual assessment of cleaning efficacy of Prototype X showed that the chosen amount of Edinburgh test soil was not effectively removed from handpieces externally (Figure 7-4).

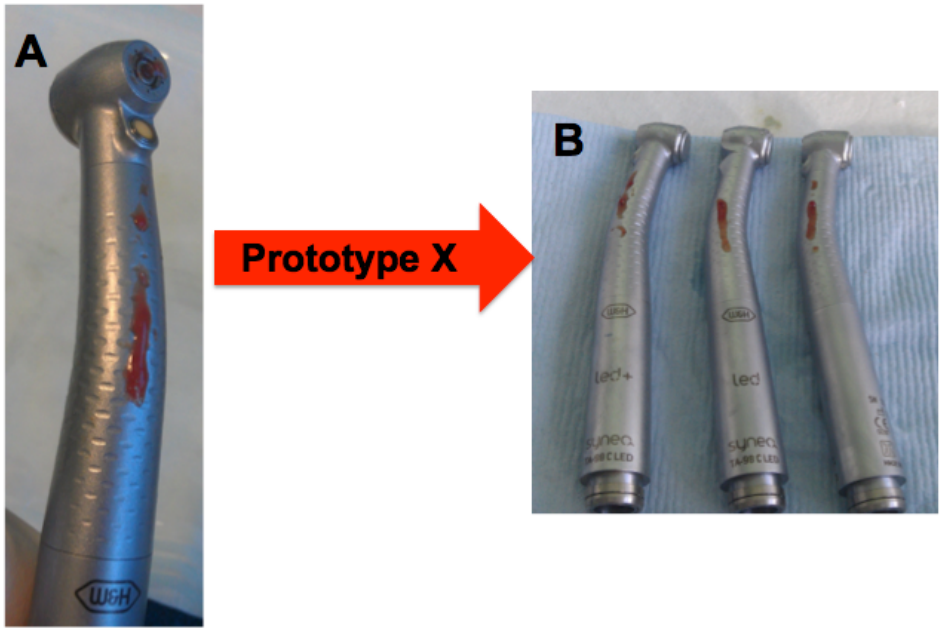

Figure 7-4 Image a shows dental air turbine (TA-98 C LED, W&H, Austria) contaminated with Edinburgh test soil; image B shows the dental air turbines after processing through Prototype X

Tables 7-1a – 7-1c show detailed results of test soil residues on different handpiece parts detected using the OPA method. Statistically significant differences in residual protein were found between soiled and cleaned head and gear of the slow speed motor ($p=0.011$) after performing a “two-sample independent t-test” in Microsoft Excel, which is a robust test, used for small sample sizes, which are normally distributed (Graham Currell, 2009). In all other tested samples, no statistical difference in residual protein was found between soiled and previously clean handpieces after the cleaning process.

Table 7-1a Residual protein detected on high-speed turbines, contaminated with Edinburgh test soil, using OPA

TA-98	n	Mean (μg)	Median (μg)	Range (μg)
Blade and cap (soiled)	3	3.6	3.0	2.1 - 5.7
Sleeve (soiled)	3	1.7	1.7	1.7 - 1.8
Blade and cap (cleaned)	6	2.1	1.7	1.5 - 4.1
Sleeve (cleaned)	6	1.7	1.7	1.7 - 1.7

Table 7-1b Residual protein detected on surgical handpieces, contaminated with Edinburgh test soil, using OPA

S11	n	Mean (μg)	Median (μg)	Range (μg)
Sleeve (soiled)	3	1.7	1.7	1.6 - 1.7
Nose cone (soiled)	3	3.6	3.9	1.8 - 5.0
Gear (soiled)	3	1.6	1.6	1.6 - 1.7
Sleeve (cleaned)	6	1.6	1.6	1.6 - 1.7
Nose cone (cleaned)	6	2.4	1.9	1.4 - 5.0
Gear (cleaned)	6	1.6	1.6	1.6 - 1.7

Table 7-1c Residual protein detected on slow-speed motors, contaminated with Edinburgh test soil, using OPA

WA56	n	Mean (μg)	Median (μg)	Range (μg)
Head and gear (soiled)*	3	3.2	3.0	2.9 - 3.6
Sleeve (soiled)	3	1.7	1.7	1.7 - 1.7
Head and gear (cleaned)*	3	2.0	1.9	1.8 - 2.3
Sleeve (cleaned)	3	1.7	1.7	1.6 - 1.7

* p=0.011

7.3.1.2 Austrian test soil – G-Box

Visual examination of handpieces contaminated with Austrian test soil showed that test soil residues were left on the outside and inside of the handpieces after processing through the Prototype X, as well as splattered across the inside of the machine (Figures 7-5 and 7-6).

Figure 7-5 Dental air turbines after Prototype X process

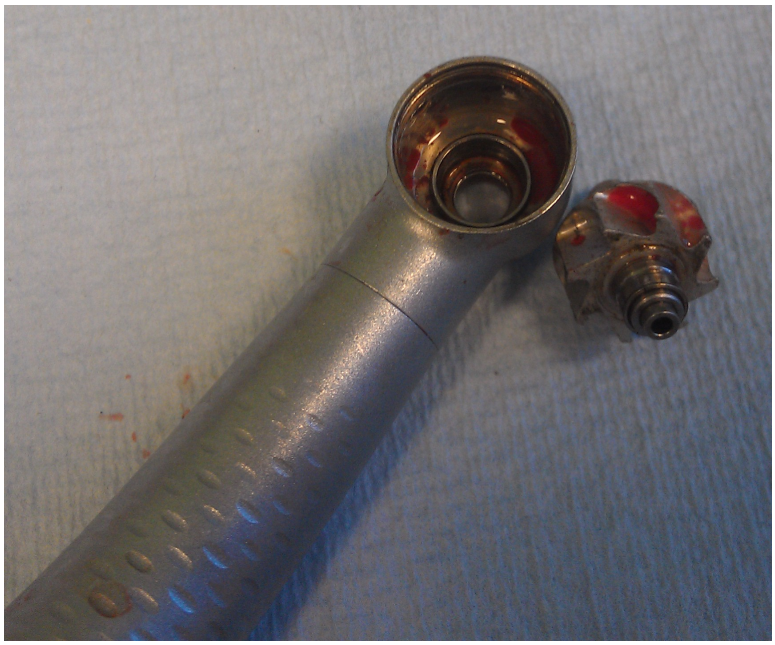

Figure 7-6 Open turbine head after Prototype X process

3D images were taken using the G-Box, to visualize protein contamination (Figures 7-7 – 7-9). The higher the peaks and the more red in colour, the more residual protein was detected.

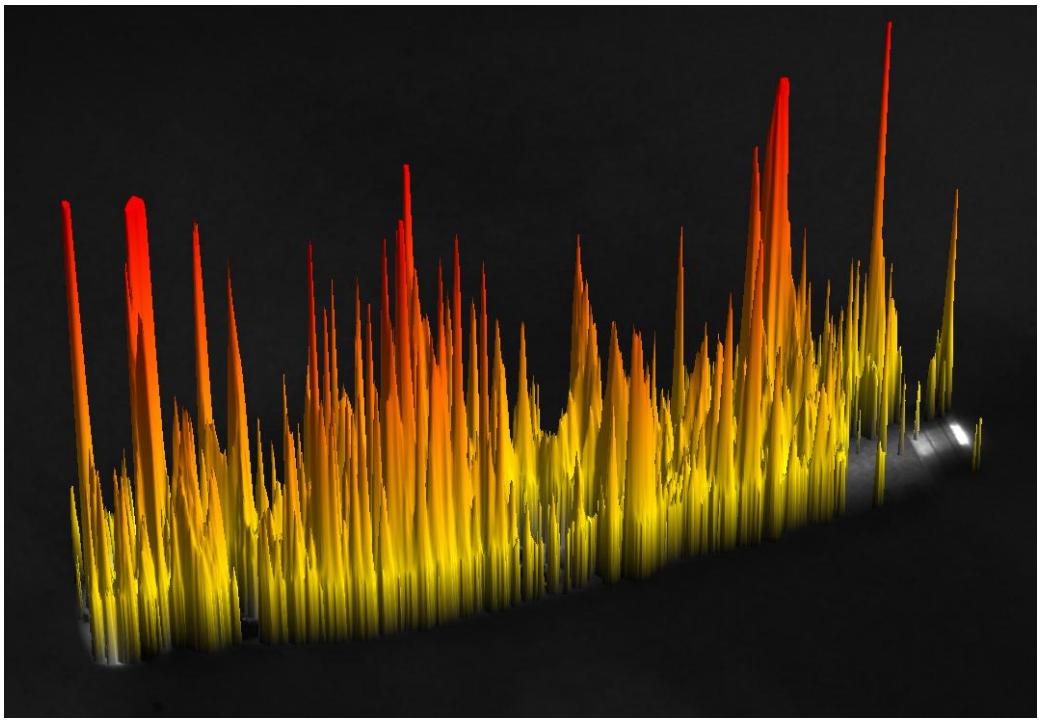

Figure 7-7 G-Box 3D image of protein on dental air turbine contaminated with Austrian test soil before Prototype X process

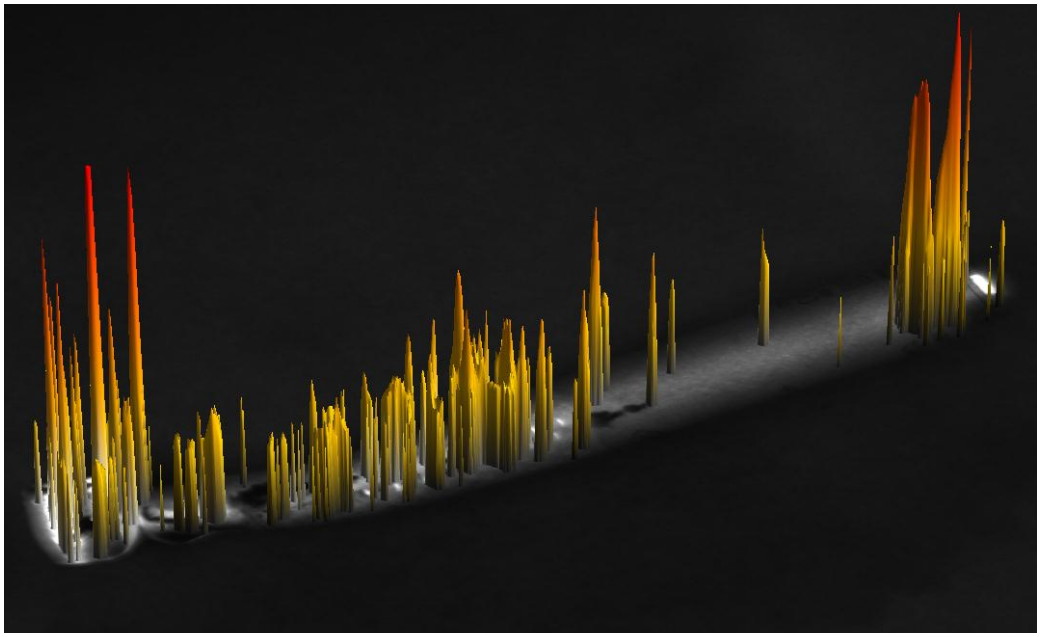

Figure 7-8 G-Box 3D image of protein contamination on dental air turbine after Prototype X process

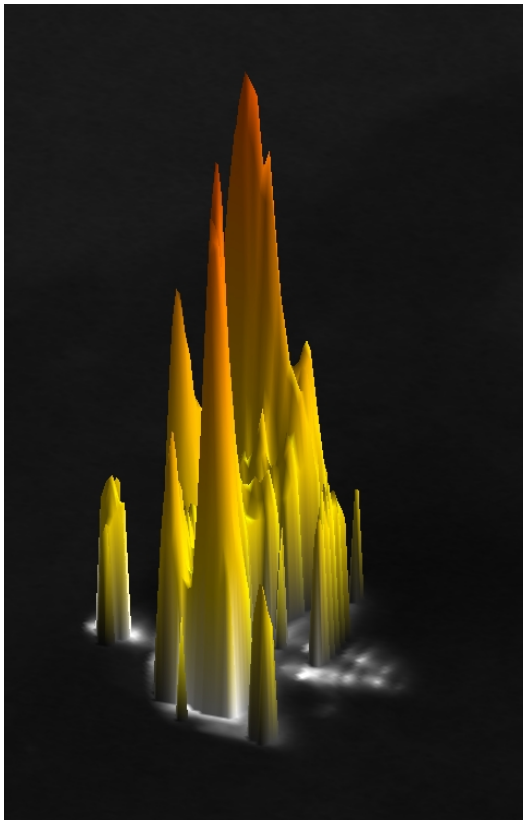

Figure 7-9 G-Box 3D image of protein contamination on dental air turbine wheel after Prototype X process

Residual protein values were calculated by comparison with a BSA standard curve (range 1.25 – 8 μg), after the blank control was subtracted, using Microsoft Excel and are displayed in table 7-2. These results clearly show that artificial saliva was removed more successfully from handpieces than Austrian test soil.

Table 7-2 Calculated values of residual proteins on all tested instruments and contaminants. One value was unreasonably high and therefore not included in the results

Residual protein	Austrian test soil (μg/instrument)	Artificial saliva (μg/instrument)
Sleeve 1	38.7	0
Sleeve 2	26	0
Turbine head 1	0.3	0
Turbine head 2	1.5	1.4

Residual contamination was observed by visual assessment after swabbing in the pipe connecting the chamber of the machine and the waste tray, as shown in figure 7-10.

Figure 7-10 Moist swab after wiping the pipe between chamber and waste tray

Figure 7-11 shows visual assessment of cleaning efficacy after using a benchtop washer-disinfector (Thermoklenz, W&H) intensive cycle (P3) and shows that no visually detectable residues of Edinburgh test soil are left on the instruments.

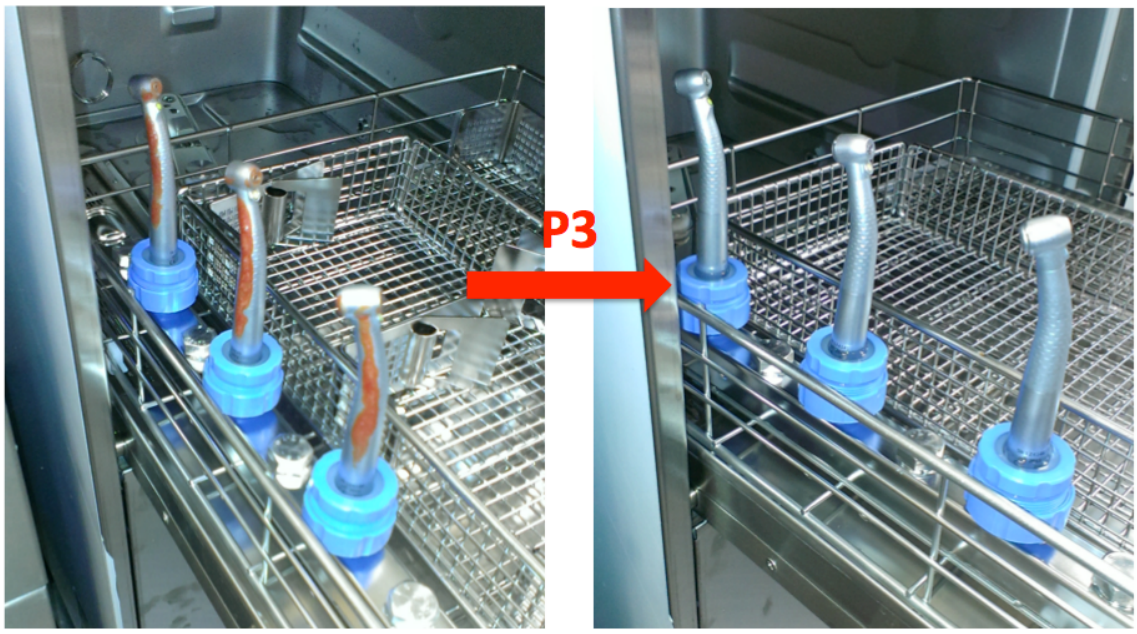

Figure 7-11 Visual assessment of residual test soil (Edinburgh test soil) after AWD

Residual protein detected on handpieces after AWD show no significant differences (Table 7-3). Figure 7-12 shows a G-box image of a handpiece, contaminated with Edinburgh test soil, after AWD reprocessing.

Table 7-3 Residual protein on high-speed turbines after AWD, assessed using G-box

Handpiece	Protein residues after Thermoklenz (µg/instrument) Run 1	Protein residues after Thermoklenz (µg/instrument) Run 2
Turbine 1	0.66	1.66
Turbine 2	1.31	1.82
Turbine 3	4.40	0.45

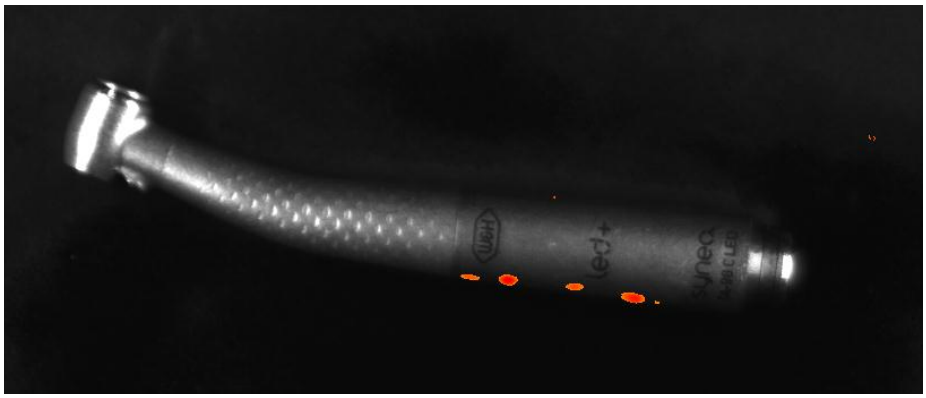

Figure 7-12 Residual protein on high-speed turbine after AWD, assessed using G-box

7.3.2 Cleaning efficacy of dental handpiece cleaner - In-vivo

7.3.2.1 New handpieces – OPA

No statistical difference was found between clinically contaminated and processed handpieces using the OPA method on new handpieces (Tables 7-4a – 7-4d).

Table 7-4a Residual protein detected on clinically contaminated high-speed turbines using OPA

TA-98	n	Mean (μg)	Median (μg)	Range (μg)
Spray (used)	14	1.5	1.5	1.5 - 1.6
Blade and cap (used)	14	1.6	1.6	1.5 - 1.7
Sleeve (used)	14	1.6	1.6	1.6 - 1.6
Spray (cleaned)	26	1.6	1.6	1.4 - 2.3
Blade and cap (cleaned)	26	1.6	1.6	1.5 - 1.7
Sleeve (cleaned)	26	1.6	1.6	1.6 - 1.6

Table 7-4b Residual protein detected on clinically contaminated surgical handpieces using OPA

S11	n	Mean (μg)	Median (μg)	Range (μg)
Sleeve (used)	4	1.6	1.6	1.6 - 1.6
Nose cone (used)	4	1.7	1.8	1.6 - 1.9
Gear (used)	4	1.6	1.6	1.6 - 1.6
Spray (used)	4	1.9	1.9	1.6 - 2.5
Sleeve (cleaned)	2	1.6	1.6	1.6 - 1.6
Nose cone (cleaned)	2	1.6	1.6	1.6 - 1.6
Gear (cleaned)	2	1.6	1.6	1.6 - 1.6
Spray (cleaned)	2	1.6	1.6	1.6 - 1.7

Table 7-4c Residual protein detected on clinically contaminated slow-speed motors using OPA

WA-56	n	Mean (μg)	Median (μg)	Range (μg)
Spray (used)	12	1.6	1.5	1.5 - 1.9
Head and gear (used)	12	1.6	1.6	1.6 - 1.6
Sleeve (used)	12	1.6	1.6	1.6 - 1.6
Spray (cleaned)	27	1.6	1.6	1.5 - 2.7
Head and gear (cleaned)	27	1.6	1.6	1.6 - 1.6
Sleeve (cleaned)	27	1.6	1.6	1.6 - 1.6

Table 7-4d Residual protein detected on clinically contaminated high-speed motors using OPA

WA-99*	n	Mean (μg)	Median (μg)	Range (μg)
Spray (used)	5	1.6	1.6	1.5 - 1.8
Head and gear (used)	5	1.6	1.6	1.6 - 1.6
Sleeve (used)	5	1.6	1.6	1.6 - 1.6

* For assessment of cleaned WA-99, too few devices were received from DCDU.

7.3.2.2 Used handpieces – G-Box

Clinically contaminated used handpieces showed a mean contamination of 763.30 μg, ranging from 114.48 – 1712.31 μg, while the mean residual protein after Prototype X process is 175.10 μg (range 12.48 – 672.96 μg). The overall cleaning efficacy is 49 – 92%. No significant difference in clinical contamination was found between the different handpiece types tested.

2D and 3D images of handpieces after patient treatment and after Prototype X process are shown in figures 7-13 and 7-14, respectively. After the use of Prototype X, contamination from the handpieces was found on the inside of the machine's chamber.

Table 7-5 Protein contamination on handpieces after clinical use and after Prototype X, using OPA

Handpiece	Contamination after treatment (μg/instrument)	Contamination after Assistina (μg/instrument)	Protein removed (%)
Surgical 1	366.39	184.16	50
Surgical 2	1332.12	672.96	49
Surgical 3	790.41	264.36	67
Surgical 4	242.63	40.79	83
Surgical 5	462.43	96.38	79
Slow 1	976.95	356.65	63
Slow 2	1712.31	167.48	90
Slow 3	773.29	417.37	46
Slow 4	1170.56	78.63	93
Slow 5	114.48	12.48	89
Slow 6	297.40	40.48	86
Turbine 1	303.27	37.65	88
Turbine 2	1690.1	136.51	92
Turbine 3	380.67	49.26	87
Turbine 4	188.15	93.75	50
Turbine 5	1411.74	152.74	89
Mean	763.30	175.10	75
Range	114.48 – 1712.31	12.48 – 672.96	49 - 92

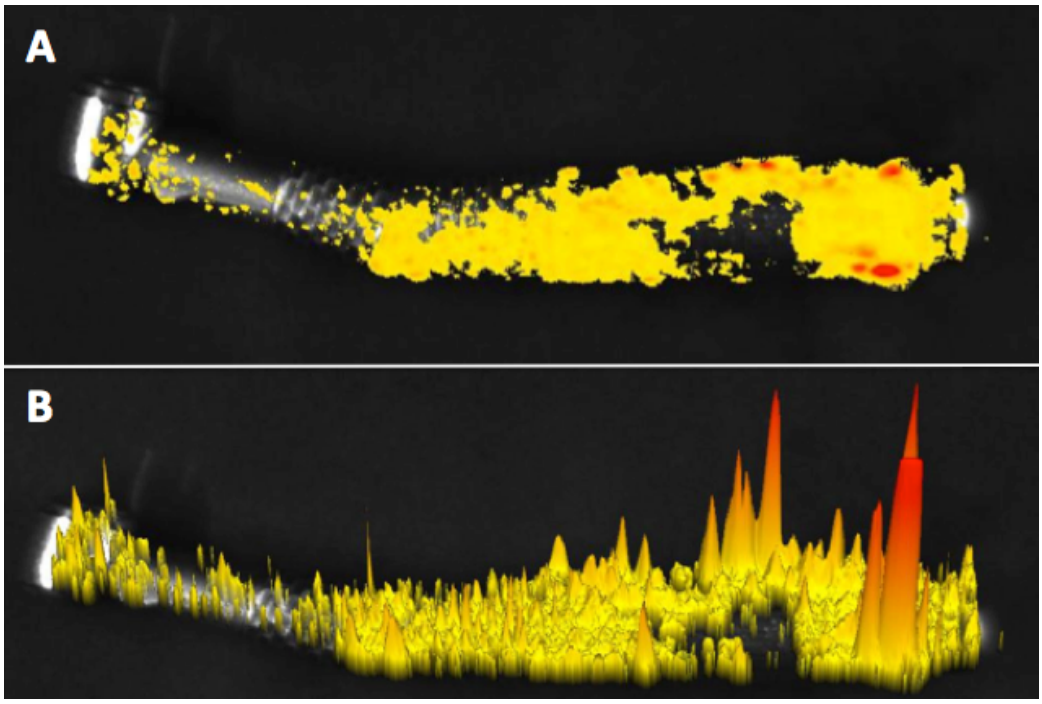

Figure 7-13 Dental high-speed turbine (W&H) after use; A shows 2D image of G-BOX; B shows 3D image of G-BOX

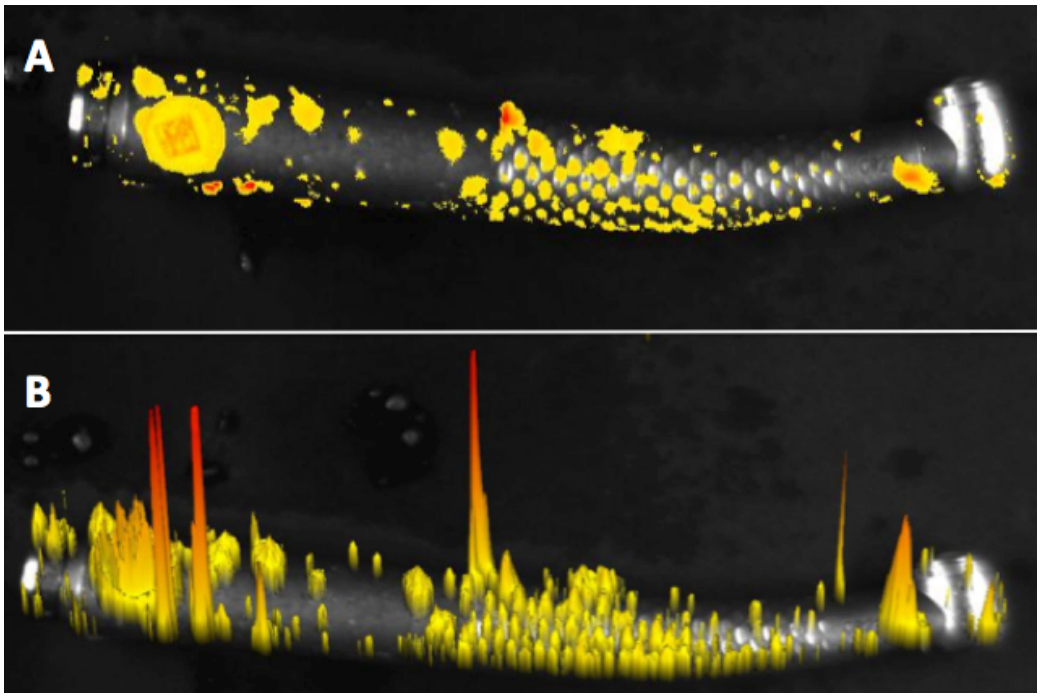

Figure 7-14 Dental high-speed turbine (W&H) after Prototype X; A shows 2D image of G-BOX; B shows 3D image of G-BOX

Three handpieces collected from DCDU after the AWD (Belimed) were assessed using the G-box and resulted in a mean residual protein value of 1.7 μg (range 0-2.8 μg). Figure 7-15 shows a 2D and 3D G-box image of a processed handpiece.

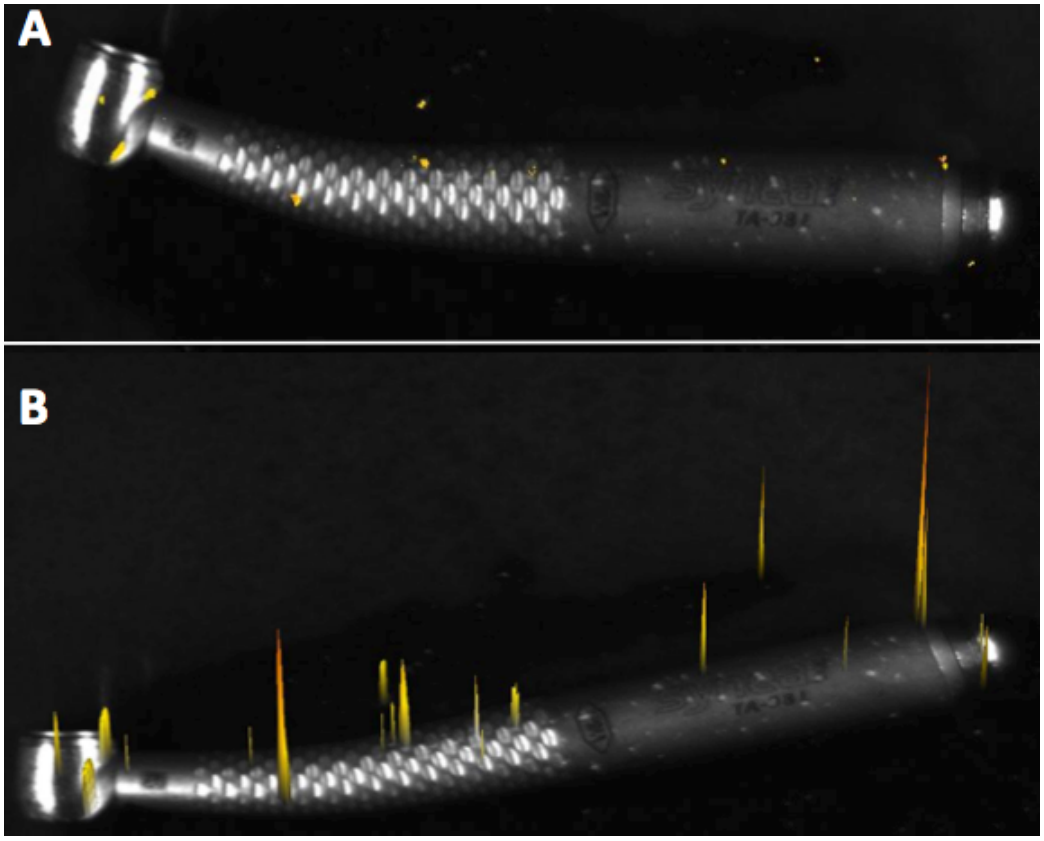

Figure 7-15 Dental high-speed turbine (W&H) after AWD; A shows 2D image of G-BOX; B shows 3D image of G-BOX

7.3.3 Effect of cleaning on the sterilization of dental handpieces using a non-vacuum sterilization process

Comparing results of BI inactivation of soiled and cleaned handpieces, one BI fail was detected in 27 samples of soiled handpieces, located in a high-speed turbine head, while three BI fails were found in 27 samples of cleaned handpieces, located in surgical handpieces (Table 7-6).

Table 7-6 BI results from inside soiled handpieces and handpieces cleaned using Prototype X (✓=pass, ✗=fail)

Sterilizer Eschmann	Cycle Number					
Model: Little Sister 3	W4249		W4250		W4251	
SN LCB8D1031	BI dirty	BI clean	BI dirty	BI clean	BI dirty	BI clean
TA-98 Head	✓	✓	✓	✓	✓	✓
	✗	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
TA-98 Air Channel	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
	✓	✓	✓	✓	✓	✓
S11 Chuck Lever	✓	✓	✓	✗	✓	✓
	✓	✓	✓	✗	✓	✗
	✓	✓	✓	✓	✓	✓

7.4 Discussion

There are several test soils listed in the standards and recommended for different types of medical devices in order to evaluate cleaning efficacy of automated washer-disinfectors (AWD) (15883-5:2005, ISO/TS), Edinburgh test soil and Austrian test soil are used for surgical instruments. There is no specific test soil for dental instruments yet listed in the standards and furthermore, there is no validated method to manually clean dental handpiece lumens. In 2007 Bagg et al. reported that the most common cleaning procedure for dental instruments is manual cleaning, in some cases with water only (Bagg et al., 2007). The challenge of cleaning lumens is well known in the field of endoscopy, where infection risk is an issue due to the limitations of validated cleaning procedures (Herve and Keevil, 2013). Handpieces contaminated with the specified quantity and location of Edinburgh test soil were not cleaned to the level specified in BS EN ISO 15883-5:2005 on the external surface, using Prototype X without pre-treatment of handpieces with disinfectant wipes; however, residual protein levels on the head and gear of slow speed motors inside the instrument were found to be significantly lower after the Prototype X process ($p=0.011$). Austrian test soil was more successfully removed using the Prototype X, however, visual residues were still detected after the cleaning step and blood spatter was found on the instruments and inside the machine. An observation of the Prototype X was that the pipe connecting the chamber of the machine and the waste tray was contaminated after use, however, there is no manufacturer's instruction concerning the decontamination of this pipe. Overall, the cleaning efficacy of the AWD tested showed no visually detectable residues of test soil. The amount of detected protein contamination on new handpieces after clinical use were very close to the limit of detection of the OPA method, therefore, no significant difference between contaminated and cleaned instruments were found. No significant difference was found between the individual handpiece types; however, it is known from the literature that surgical handpieces are the most contaminated type of handpiece (Deshpande et al., 2015, Smith, 2011). Results of the present study differ possibly because the handpieces used were new and were only used for one treatment before assessment, which may suggest biofouling of dental handpieces is cumulative over time. This notion is agreement with the publication by Smith et al., 2011.

For these reasons, handpieces taken from clinics, which have been in use for several years, were assessed before and after Prototype X. Residual protein levels after the Prototype X ranges from 12.48 – 676.96 μg. RKI guidelines state that the recommended maximum tolerable amount of protein on medical devices after cleaning is 100 μg (RKI, 2005), which was not achieved using this machine. Most recent guidelines (ACDP, 2015), however, recommend an even lower maximum tolerable level of 5 μg residual protein per instrument side.

Using a non-vacuum sterilization cycle on soiled (Austrian test soil) and cleaned handpieces using BI showed that BI fails were found in both, cleaned and soiled handpieces, which indicates that residual contamination (e.g. blood) does not appear to affect the outcome of non-vacuum sterilization. However, Further experiments will have to be performed to determine whether there is a difference in the level of contamination remaining.

Andersen et al. tested three different cleaner-lubricators on high-speed turbines, artificially contaminated with *Streptococcus salivarius* and found that only one out of three machines was able to remove bioburden with 3.9 log reduction in the colony forming units of organisms detected (Andersen et al., 1995). Simonis et al. tested the cleaning efficacy of the Turbocid handpiece cleaner-lubricator (Micro Mega) after contaminating dental handpieces with coagulated human blood containing *E. faecium*. The device did not achieve a 5 log reduction of bioburden, which is the requirement for cleaning according to the Robert-Koch Institute (A. Simonis, 2008). Another handpiece cleaner-lubricator, tested by Smith et al. failed to remove Swedish test soil successful (Smith, 2011). These results are comparable with the findings in the present study, which strongly suggests that cleaner-lubricators do not replace thorough manual cleaning or the use of an AWD, but might be a useful tool for additional flush of spray channels and lubrication of handpieces. The fact that artificial saliva was removed, using the Prototype X, while test soils were not might indicate that the applied amount of test soils was too challenging for Prototype X and do not represent realistic dental contamination. A study to determine the amount of standardized test soils that is equivalent to handpiece contamination after dental treatment would be necessary in order to develop and validate a soiling method for testing the cleaning efficacy of handpiece cleaner-lubricators,

such as Prototype X. Franz et al. also highlights the necessity for a dental specific test contamination (Franz et al., 2012). Kohek attempted to develop a specific test soil for dental instruments in his thesis. This potential ‘dental’ test soil consisting of a blood and mucin (7.5%) mixture in a ratio of 1:1, which appeared to be a promising alternative (Kohek).

In another thesis it was found that, testing the cleaning efficacy of the DAC (Sirona) handpiece cleaner-lubricator is dependent on the time between patient treatment and reprocessing. The quicker handpieces are reprocessed after treatment, the more effective the cleaning process (Radimersky, 2012). Using the OPA method to determine protein residues in a comparison of two cleaner-disinfectors for dental handpieces, the LIFEtime (Kavo) and Hygiene Center (Sirona), resulted in a significant difference of cleaning efficacy between the two machines, where LIFEtime showed better results (Schönherr, 2005).

In conclusion improvement of handpiece cleaner-lubricators is necessary in order to consistantly achieve acceptable cleaning efficacy and that these machines cannot replace manual cleaning or AWD yet. Moreover, in order to successfully determine the cleaning efficacy of cleaning devices specifically designed for dental handpieces, a standardized dental test soil should be developed and validated.

Chapter 8 – Discussion

8.1 Overview

Dental handpieces have been classed as “semi-critical” or “critical” medical devices (RKI, 2005) and are known to become contaminated during patient treatment (Smith et al., 2014, Smith, 2011). Therefore prior to reuse they must undergo decontamination, which involves cleaning, disinfection and sterilization. However, since the 1950s it has been recognized that lumens pose a challenge for steam penetration (Perkins, 1983). Therefore, vacuum sterilization is recommended for lumen containing medical devices, such as dental handpieces (13060:2014, BS EN). However, general dental practices in the UK commonly use non-vacuum benchtop sterilizers for sterilizing instruments after patient treatment (Smith et al., 2009c), including dental handpieces. The aim of this study was to investigate if benchtop steam sterilization processes commonly used in dental practice are appropriate for sterilizing dental handpieces. Critical variables affecting the outcome of steam sterilization, such as pre-cleaning and lubrication were assessed.

All results in the present thesis clearly indicate that non-vacuum sterilization in benchtop sterilizers, most commonly used in UK dental practice, are not adequate for sterilizing dental handpieces. All available methods used (biological indicators, chemical indicators and different thermometric and pressure measurements) were independent conformation and validation of all methods by showing the dame results. All our findings are in accordance with previously published scientific articles. The findings will be internationally applicable for dentistry as well as other areas in health care systems where sophisticated medical devices with narrow internal lumens are being used.

The first research question “Investigating steam penetration into dental handpieces and lumens in general (chapter 4), which was approached using thermometric measurements, chemical and biological indicators were used in different handpiece types (high-speed turbines, slow-speed motors, surgical handpieces) and process challenge devices using non-vacuum and vacuum sterilization cycles in a laboratory setting (chapter 4) and in general dental practices (chapter 6)” resulted in four main results:

1- Surgical handpieces and slow-speed motors are more difficult to sterilize than high-speed turbines, due to their mass or conductivity (chapters 4 and 5).

2- The outcome of a sterilization process differs between all types and models of handpieces (chapter 4).

3- Thermocouples placed in the load of a sterilizer facilitates steam penetration into the load and may therefore affect results (false positives) of current validation procedures for steam sterilizers (chapter 5).

4- Dental handpieces cannot be reliably sterilized in non-vacuum benchtop sterilizers, commonly used in UK dental practices (chapter 6).

The second research question “Investigating the effect of pre-cleaning dental handpieces, contaminated with different test soils from the standards or clinical contamination after patient treatment using a washer-disinfector or a handpiece cleaner-lubricator, which was assessed using the o-phtalaldehyde and G-box method (chapter 7)” showed that

1- Automated washer disinfectors (AWD) are more effective compared to dental handpiece cleaner-lubricators.

2- Surgical handpieces show the highest levels of protein contamination after the use on patients.

3- The quicker handpieces are reprocessed after treatment on patients, the more effective the cleaning process.

4- Further experiments need to be performed in order to determine the difference in the level of contamination and a standardized dental test soil should be developed and validated.

The third research question “Investigating the effect of handpiece lubricating oil on microbial inactivation by altering different parameters during a steam sterilization process using a BIER/CIER vessel in St. Paul (MN, US) (chapter 5)” resulted in two key findings:

1- Oil in thin layers might prevent spore inactivation by increasing the D value, but by blocking the channels for steam penetration.

2- The thickness of the layer of spores affects the sterilization outcome, which suggests that biofilm forming within dental handpieces, especially water spray channels, might pose an additional challenge for steam sterilization of dental handpieces.

Results for research question four “Investigating the effect of different humidity levels on chemical and biological indicators using a BIER/CIER vessel in Neuss (Germany) (chapters 3)” showed that

1- Different humidity/moisture levels affect how chemical indicators react to saturated steam, which might result in false positive results.

2- Moisture in terms of wet steam appears to be sufficient to inactivate spores. The inconsistence of results may be due to differences in spore manufacturing conditions of recovery methods.

The number of benchtop steam sterilizers and dental handpieces monitored in this study are considerably higher compared to existing scientific publications. The three independent methods (chemical indicators, biological indicators and thermometric and pressure measurements) as well as comparison of in-vitro (chapter4) and in-vivo (chapter 6) add value to the validity of findings. Moreover, extensive validation work was performed for all methods used (chapters 2, 3 and 5) in order to interpret results and increase understanding of method limitations, such as thermocouples and chemical indicators, which facilitate steam penetration into lumens rather than impair it and react to moisture rather than saturated steam, respectively. The finding that chemical indicators are likely to provide false positive results agrees with the literature (J.P.C.M. van Doornmalen, 2012, Lee et al., 1979, Rutala et al., 1996). Moreover, a weakness of this study is the small sample size of stainless steel tokens contaminated with spores (chapter 5) and surgical handpieces after patient treatment (chapter 7), due to time limitations.

8.2 Clinical implications

The three most important findings in this study are first, non-vacuum steam sterilization, even though commonly used in UK dental practice (Smith et al., 2009c), does not reliably achieve sterility of dental handpieces, second, handpiece lubrication may impair steam penetration into dental handpiece lumens and third, that handpiece cleaner-lubricators do not comply with current standards on cleaning of medical devices. Therefore, recommendations based on the findings in the present study and the interest of safety for patients and staff are not to use a handpiece cleaner-lubricator as a sole cleaning/disinfection step for dental handpieces, to refrain from relying on a non-vacuum sterilizer and to the use of vacuum sterilization of dental handpieces in order to provide a medical device that is safe to use on a patient. In general dental practice, vacuum sterilizers should replace non-vacuum sterilizers. Moreover, for better understanding of thermometric testing, it should be agreed on one definition for "thermometric fail", which could be a three second time delay, based on the findings in this work.

8.3 Future work

Using the results of this project as a foundation, it is evident that more research is required to study the effect of handpiece lubricating oil on the outcome of non-vacuum and vacuum steam sterilization cycles, in order to achieve a better understanding of the effect of handpiece lubricating oil in the heat resistance of microorganisms. Concerning PCD in dental practice, the commonly used helix does not mimic a handpiece and, as shown in this study, CI are unreliable. Therefore, the development of a dental specific PCD, simulating a dental handpiece in terms of thermal conductivity, material and dimensions, incorporating BI or electronics for faster results without incubation time, similar to the electronic Bowie Dick test (ETS, 3M), would be a good start in order to develop a PCD, which is a relevant and reliable indicator for steam sterilization of dental handpieces.

Moreover, in order to develop a standardised cleaning method for dental handpieces, the development of a dental specific test soil containing bioburden,

based on studies of instrument contamination after dental treatment, is necessary.

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