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Decontamination and Use of Endodontic Hand Files in Dental Practice in Pretoria

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DECLARATION

I, Glynn Dale Buchanan, hereby declare that this dissertation entitled, **Decontamination and Use of Endodontic Hand Files in Dental Practice in Pretoria**, which I herewith submit to the University of Pretoria in partial fulfilment of the requirements of the degree: MSc (Dent) is my own original work, and has not been submitted for any academic award or qualification at this or any other institution of higher learning.

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Signature Date

The road is long, with many a winding turn that leads us to who knows where…

– Bob Russel and Bobby Scott

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ABSTRACT

Introduction: The risk of cross-contamination validates the need to assess the adequacy of cleanliness of dental instruments. Neither the extent of single-use nor residual debris contamination of endodontic hand files following routine cleaning and sterilisation procedures in South Africa is known. **Aims:** This study aimed to determine the amount of visible debris on endodontic hand files collected from dental practices in Pretoria, South Africa, after undergoing routine decontamination procedures. The prevalence and perceptions regarding single-use of these instruments were also investigated. **Materials and Methods:** Twenty-seven dental practices voluntarily submitted 15 previously used and decontaminated endodontic hand files. A short questionnaire regarding single-use was completed by each participant. Files were examined for the presence or absence of remnant debris using a stereomicroscope. The files were scored using a novel scoring system. Statistical evaluation of the data estimated the frequency and proportions of debris in each scoring position. Cohen's Kappa statistic was used to assess examiner agreement. **Results:** In total, 401 endodontic hand files were examined. Debris was found on 94% of samples. Examiner agreement was fair to moderate over the entire dataset. No participants reported practising the single-use of endodontic hand files. Several decontamination methods were reportedly used by participants for endodontic files. **Conclusion:** Routine decontamination methods do not effectively remove debris from endodontic hand files. Single-use protocols are not practised in dental practices in Pretoria, South Africa. Great variation exists in the decontamination methods of endodontic hand files. Financial constraints are the primary reason for the re-use of endodontic files.

LIST OF ABBREVIATIONS

NiTi	-	nickel titanium
mm	-	millimetre/s
NaOCl	-	sodium hypochlorite
LED	-	light-emitting diode
n	-	number/s
µm	-	micrometre/s
m/s	-	metre/s per second
ºC	-	degrees Celsius
D_0	-	position of the tip of an endodontic file
D_{16}	-	position of the end of the cutting blade of an endodontic file
CAP	-	cold atmospheric plasma
spp	-	species pluralis
HIV	-	human immunodeficiency virus
RESCOM	-	research committee (School of Dentistry, University of Pretoria)
vCJD	-	variant Creutzfeldt-Jakob disease
min	-	minute/s
sec	-	second/s

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Endodontic hand files are instruments used during endodontic treatment to prepare the root canal system. Historically, the re-use of these instruments on multiple patients was standard practise, provided cleaning and sterilisation had taken place before re-use in subsequent clinical cases (Carrotte, 2004).

In recent years, substantial evidence has been published in the literature in support of the single-use of endodontic files, as opposed to re-using these instruments (Letters *et al*., 2005; Aasim, Mellor and Qualtrough, 2006; Walker *et al*., 2007; Morrison and Conrod, 2009; Walker *et al*., 2009).

Arguments in favour of the single-use of endodontic files include:

- Difficulty in adequately cleaning endodontic files for re-use in the clinical setting (Letters *et al*., 2005; Walker *et al*., 2009);
- The risk of transmission of viruses and/or bacteria such as; Herpes viruses, Human immunodeficiency virus (HIV), Hepatitis viruses, Mycobacterium Tuberculosis, Pseudomonas sp., Legionella sp. and several multi-resistant bacteria (Messer, Parashos and Moule, 2003; Laheij *et al*., 2012);
- The potential transmission of prion disease (Walker *et al*., 2007; Walker *et al*., 2009);
- Poor implementation of procedures assessing the cleanliness of endodontic files prior to re-use (Messer, Parashos and Moule, 2003; Marsden, 2010);
- The detrimental effects of cleaning and sterilisation processes on the structural integrity of endodontic files (Alexandrou *et al*., 2006; Sonntag and Peters, 2007; Popovic *et al*., 2014).

Literature supporting the routine re-use of endodontic files also exists (Messer, Parashos and Moule, 2003; Parashos, Gordon and Messer, 2004; Letters *et al*., 2005; Abichandani and Nadiger, 2013).

The most significant arguments in favour of re-use are:

- Laboratory trials have demonstrated that it is possible to clean 100% of the surfaces of endodontic files (Messer, Parashos and Moule, 2003; Parashos, Linsuwanont and Messer, 2004);
- The universal acceptance of re-use of other stainless steel dental instruments used in endodontics that contact the dental pulp (Messer, Parashos and Moule, 2003);
- Prion proteins have not been found in the dental pulps of individuals diagnosed with Creutzfeldt-Jakob disease (Messer, Parashos and Moule, 2003; Abichandani and Nadiger, 2013);
- The increased cost incurred in implementing single-use protocols for endodontic files may make endodontic treatment prohibitively expensive (Messer, Parashos and Moule, 2003; Parashos, Linsuwanont and Messer, 2004; Letters *et al*., 2005).

The risk of transmission of infectious diseases during dental treatment validates the need to assess the cleanliness of dental instruments before their re-use (Hartshorne, 2010). Economic constraints in a developing country such as South Africa dictate that the re-processing and re-use of endodontic files is highly likely. The presence of residual bioburden may compromise sterilisation of dental instruments (Reams, Baumgartner and Kulild, 1995). The proper implementation of infection control measures regarding dental instruments is therefore imperative.

It has been suggested that the infection control processes of dental instruments in South Africa are inadequate (Yengopal, Naidoo and Chitke, 2001; Oosthuysen, Potgieter and Blignaut, 2010; Hartshorne, 2010). The extent of the latent debris contamination of endodontic files after applying routine cleaning and sterilisation procedures, in South Africa, is unknown. It is not known whether any South African dental professionals have adopted a single-use approach regarding endodontic files. Furthermore, the reasons for or against the single-use of endodontic files have never been investigated.

South Africa does not have written guidelines recommending a standard cleaning protocol for the decontamination and reprocessing of endodontic instruments.

1.2 Literature review

1.2.1 Definitions

For the purpose of clarity, terms used to describe the cleaning and sterilisation of dental instruments within the context of this dissertation are defined as follows:

1.2.1.1 Decontamination

“Decontamination renders an area, device, item, or material safe to handle (i.e. safe in the context of being reasonably free from a risk of disease transmission). The primary objective is to reduce the level of microbial contamination so that infection transmission is eliminated.” (United States Center for Disease Control and Prevention, 2009).

1.2.1.2 Disinfection

Disinfection is a term used to describe a process that results in the elimination of most or all microorganisms from an inanimate object, with the exception of bacterial spores. Disinfection is generally a less lethal process than sterilisation (ADA Council on Scientific Affairs and ADA Council on Dental Practice, 1996; Rutala and Weber, 2008; United States Center for Disease Control and Prevention, 2009).

The level of disinfection is categorised into: high level, intermediate level or low level disinfection, based upon the ability or inability of a disinfecting agent/process to eliminate spores (Rutala and Weber, 2008; United States Center for Disease Control and Prevention, 2009).

There are several different means of achieving disinfection. According to the United States Center for Disease Control and Prevention (2009), the following factors can influence the level of disinfection:

- The number and specific nature of the microorganisms involved;
- The amount of organic debris;
- The type of instruments to be disinfected - this is especially relevant for endodontic files considering the intricate, irregular geometry;
- The condition of the instruments requiring disinfection;
- The temperature applied for disinfection.

1.2.1.3 Sterilisation

“Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms and viruses. The definition is categorical and absolute (i.e., an item is either sterile or it is not)” (Rutala and Weber, 2008; United States Center for Disease Control and Prevention, 2009). Due to the absolute nature of the definition of sterilisation, no item can be considered “partially sterile” (Rutala and Weber, 2008).

1.2.1.4 Re-sterilisation

Re-sterilisation is the repeated application of the processes required to render an object sterile, i.e. to remove all forms of microbial life from said object, prior to re-use (Morrison and Conrod, 2009; Abichandani and Nadiger, 2013). It is common practise in dentistry to re-sterilise instruments. Instruments with intricate designs may require rigorous pre-cleaning to facilitate adequate sterilisation. Some dental instruments cannot be sterilised after clinical use and are considered disposable (Morrison and Conrod, 2009; Abichandani and Nadiger, 2013).

All critical and semi-critical dental instruments, including endodontic files, should be sterilised after every clinical use (ADA, 2009). Autoclaving, dry heat sterilisation or application of chemical vapour, are effective methods of sterilising dental instruments (Morrison and Conrod, 2009). It is essential that all debris and bioburden be removed from endodontic files, mechanically and/or chemically, prior to sterilisation (Miller, 1991; Reams, Baumgartner and Kulild, 1995).

1.2.2 Cross-contamination

Any instrument should be sterile before being placed into a root canal. This prevents cross infection between patients and averts the introduction of additional microorganisms into root canals, which may compromise the outcome of endodontic treatment (Carrotte, 2004).

The oral cavity harbours microorganisms, including those native to the mouth, the naso-pharyngeal and respiratory tract (Abichandani and Nadiger, 2013). Procedures carried out in the oral cavity result in the contamination of instruments. Such procedures involve the exposure to blood, microbes, living and/or necrotic tissues and

other bodily fluids (Reams, Baumgartner and Kulild, 1995; Georgescu, Skaug and Patrascu, 2002; Laheij *et al*., 2012; Abichandani and Nadiger, 2013). For this reason it is crucial that efficient infection control practices are implemented (Georgescu, Skaug and Patrascu, 2002; Abichandani and Nadiger, 2013). Dental instruments can facilitate the transmission of diseases to dentists themselves, as well as others (Georgescu, Skaug and Patrascu, 2002; Morrison and Conrod, 2009).

The most harmful microbial contaminants to which individuals are exposed during dental procedures include:

- Hepatitis B virus (Monarca *et al.*, 2000; Georgescu, Skaug and Patrascu, 2002; Araujo and Andreana, 2002; Laheij *et al.,* 2012);
- Hepatitis C virus (Monarca *et al.*, 2000; Georgescu, Skaug and Patrascu, 2002; Araujo and Andreana, 2002; Laheij *et al.*, 2012);
- Human immunodeficiency virus (HIV) (Monarca *et al.*, 2000; Georgescu, Skaug and Patrascu, 2002; Araujo and Andreana, 2002; Mehtar *et al.,* 2007; Laheij *et al.,* 2012);
- Herpes simplex virus (Georgescu, Skaug and Patrascu, 2002; Laheij *et al.,* 2012);
- Mycobacterium tuberculosis (Smith *et al*., 1982; Georgescu, Skaug and Patrascu, 2002; Araujo and Andreana, 2002; Laheij *et al.,* 2012);
- Pseudomonas spp. (Monarca *et al.,* 2000; Georgescu, Skaug and Patrascu, 2002);
- Legionella spp. (Monarca *et al.,* 2000; Laheij *et al.,* 2012);
- Multidrug-resistant bacteria including Methicillin-Resistant Staphylococcus Aureus (MRSA) (Georgescu, Skaug and Patrascu, 2002; Laheij *et al.,* 2012).

The responsibility of minimising the risk of transmission of infectious diseases during dental treatment lies with the dentist, oral hygienist and all other auxiliary oral health professionals (Laheij *et al.,* 2012). The use of non-sterile and/or unclean dental instruments in treating patients is considered a breach of ethical duty (Hartshorne, 2010).

Healthcare workers should be vaccinated against the Hepatitis B virus due to occupational exposure to blood and other bodily fluids (Reams, Baumgartner and Kulild, 1995; Araujo and Andreana, 2002).

A study by Mehtar *et al.* (2007), demonstrated a lack of knowledge regarding adequate infection control practises in public dental clinics in at least one province of South Africa. These findings correspond with the earlier research of De Kock and van Wyk, (2001); Yengopal, Naidoo and Chitke (2001) as well as Oosthuysen, Potgieter and Blignaut (2010).

1.2.3 Prion disease

Walker *et al.* (2007) and Walker *et al.* (2009), explored the possibility of the transmission of variant Creutzfeldt-Jakob disease from one human being to another via infected endodontic files and/or instruments. Although evidence of the spread of this disease in humans via dental procedures has not been shown, an animal model has demonstrated the possibility of prion transmission in mice (Kirby *et al.,* 2012). As subclinical infection of prion disease is a possibility, endodontic files should be considered single-use only, as the routine decontamination thereof may not be effective (Walker *et al.,* 2007; Morrison and Conrod, 2009). Aasim, Mellor and Qualtrough (2006) concurred with this view, regarding endodontic files to be single-use instruments due to the potential risk of transmission of prions via contaminated endodontic instruments. In 2007, the United Kingdom (UK) Department of Health advised dentists to treat all endodontic files and reamers as single-use instruments (Walker, 2007).

Messer, Parashos and Moule (2003), opposed this viewpoint, purporting that there was no evidence of the spread of prion disease in human subjects via dental procedures. Given the low risk of transmission of prion disease via dental treatment, it was concluded that the single-use of endodontic files was not necessary.

Oosthuysen, Potgieter and Blignaut (2010), reported the lack of published data on the occurance of variant Creutzfeldt-Jakob disease or prion disease in South Africa.

1.2.4 Debridement of endodontic files

It is internationally accepted clinical practice that dental instruments contaminated with oral fluids must undergo cleaning and sterilisation processes before re-use in clinically treating a subsequent patient (Segall *et al.,* 1977; Carrotte, 2004; ADA, 2009).

The literature has described several methods of cleaning endodontic files:

- Mechanical techniques: physically wiping files with gauze or an alcohol-soaked sponge (Segall *et al.,* 1977; Murgel *et al*., 1990), ultrasonic cleaning (Aasim, Mellor and Qualtrough, 2006) and manual scrubbing with brushes (Morrison and Conrod, 2009);
- Chemical techniques: chairside sterilisation with six percent sodium hypochlorite (NaOCl) (Gnau, Goodell and Imamura, 2009) and the use of an enzymatic cleaner, in combination with ultrasonic cleaning (Aasim, Mellor and Qualtrough, 2006; Guandalini *et al.,* 2014);
- Autoclaving or steam sterilisation (Johnson *et al*., 1997; Morrison and Conrod, 2009);
- Combination cleaning: combined mechanical, chemical and/or autoclaving techniques (Parashos, Linsuwanont and Messer, 2004; Aasim, Mellor and Qualtrough, 2006);
- Washer disinfectors (Perakaki, Mellor and Qualtrough, 2007);
- Novel cleaning techniques: plasma cleaning (Whittaker *et al*., 2004; Cha and Park, 2014).

1. 2.4.1 Manual debridement

The complex design of endodontic files makes the mechanical cleaning of these instruments challenging (Ferreira *et al*., 2012). Early research has demonstrated that various manual cleaning techniques, such as physical wiping with cotton rolls or gauze, either wet or dry, as well as pushing endodontic hand files through a stretched rubberdam or foam sponge failed to render these instruments free of debris (Segall *et al.,* 1977). The same research by Segall *et al.* eliminated wire-brush cleaning as a viable decontamination method because of the generation of metal spurs and filings when using this instrument to mechanically clean endodontic instruments.

The inspection of 110 endodontic files by scanning electron microscopy aimed to assess the efficacy of three cleaning methods: manual wiping with alcohol-soaked gauze; manual wiping with an alcohol-soaked sponge and cleaning in an ultrasonic bath. This study revealed the inability of these three methods to completely clean endodontic files of debris (Murgel *et al.,* 1990).

In a position-paper, published by the Australian and New Zealand Academy of Endodontists, it was reported that routine manual cleaning procedures could be performed on endodontic files to an acceptable standard (Messer, Parashos and Moule, 2003). Furthermore, Morrison and Conrod (2009), described the manual cleaning of endodontic files and dental burs as a necessary step in removing biological debris prior to sterilisation.

More recent research has shown that manual cleaning with a nylon bristle brush can render 98.9% of endodontic hand files free of debris (Guandalini *et al.,* 2014).

1.2.4.2 Ultrasonic cleaning

Ultrasonic cleaning exposes dental instruments to vibratory pulses as sound waves carried through a fluid medium (Muqbil *et al.,* 2005; Morrison and Conrod, 2009). This process leads to the formation of bubbles with diameters of between 10 and 100µm that move from low to high-pressure zones within the fluid-medium. Subsequently these bubbles burst creating water waves with a speed up to 500m/s that impact solid surfaces. The collapse of the bubbles causes the temperature to rise to approximately 5000ºC within a fraction of a second (Jatzwauk, Schöne and Pietsch, 2001). These effects are called cavitations. Cavitations facilitate the cleaning of the surfaces of dental instruments within this environment (Jatzwauk, Schöne and Pietsch, 2001; Walker *et al,* 2007).

It has been demonstrated that cavitational activity inside a container placed within an ultrasonic bath is similar to the effect exerted on the outside of the container (Muqbil *et al.,* 2005).

Ultrasonic cleaning can reduce the number of viable organisms present in a solution (Muqbil *et al.,* 2005). Five to ten minutes of ultrasonic cleaning has been shown to be the optimum time required for cleaning endodontic files preceding steam sterilisation

(Aasim, Mellor and Qualtrough, 2006). The amount of debris-reduction from endodontic files is greater using ultrasonic cleaning as compared to the use of a washer-disinfector (Perakaki, Mellor and Qualtrough, 2007). It is, however, still difficult to clean endodontic files, even with ultrasonic cleaning. This may justify adopting a single-use protocol for endodontic files (Weightman and Lines, 2004).

The research by Van Eldik (2004a), examined the effect of cleaning procedures on stainless steel and nickel titanium (NiTi) endodontic files. This study compared the efficacy of cleaning files directly after removal from their packaging and after use on human teeth that had been contaminated in broth (cooked meat broth, Oxoid, Victoria, Australia). The study investigated three cleaning procedures: a thermal disinfector cycle, ultrasonication in a perforated container and ultrasonication in a loosely packed beaker. Neither ultrasonication within a container, nor the application of a thermal disinfector could achieve complete removal of biological debris from the endodontic files. When placed loosely in an ultrasonic bath, however, 98.33% of the files were clean of biological debris (Van Eldik *et al*., 2004a). This is an agreement with the findings of Guandalini *et al.* (2014), who found that ultrasonic cleaning with either water or detergent rendered endodontic hand files 96,2% free of debris.

The study by Aasim, Mellor and Qualtrough (2006), showed that endodontic hand files are not completely cleaned of debris by ultrasonication. The inability of conventional cleaning (such as ultrasonication) and sterilisation techniques to remove materials and medicaments such as calcium hydroxide was especially significant. It was therefore recommended that the practice of single-use of endodontic files be advocated (Aasim, Mellor and Qualtrough, 2006).

1.2.4.3 Autoclaving

Steam sterilisation by autoclave is the only method that can reliably render endodontic files sterile (ADA Council on Scientific Affairs and ADA Council on Dental Practice, 1996; Hurtt and Rossman, 1996; Morrison and Conrod, 2009).

Although autoclaves are primarily used for sterilisation and not for the cleaning of endodontic files, poor decontamination (removal of biological debris) can affect the efficacy of subsequent infection control procedures such as autoclaving (Reams,

Baumgartner and Kulild, 1995). The presence of dental materials and medicaments on endodontic files significantly decreases the efficacy of autoclaving in removing debris from these dental instrument (Aasim, Mellor and Qualtrough, 2006). This evidence demonstrates the importance of manual cleaning prior to the sterilisation of endodontic files.

Johnson *et al*. (1997), conducted an *in vitro* study that assessed 92 new, intentionally bacteria-contaminated, endodontic hand files. This study assessed the degree of contamination and efficacy of cleaning methods and sterilisation in eradicating bacteria. Despite the presence of bioburden, sterilisation of endodontic hand files by autoclave was possible (Johnson *et al.,* 1997).

More recently, it has been revealed that although it is possible to achieve sterilisation of new unused endodontic files using an autoclave, clinically used files and burs cannot be sterilised in the same way (Morrison and Conrod, 2009).

Van Eldik *et al.* (2004b), found that steam sterilisation eliminated all bacterial life from endodontic files, whether debris was still present on the files at the time of autoclaving, or not. This finding is supported by similar results from a more recent study (Souza *et al.*, 2011).

1.2.4.4 Enzymatic agents

Bagg *et al.,* (2007), found ultrasonic cleaning to be a widely accepted form of cleaning debris from endodontic files. Enzyme-based detergents may be added to the process because of their ability to disintegrate blood proteins, organic tissues and residual matter as compared to other types of detergents or water alone (Whitworth *et al*, 2007).

An experiment undertaken by Aasim, Mellor and Qualtrough in 2006, found that there was no benefit in pre-soaking endodontic files in enzymatic cleaner prior to ultrasonic cleaning. The inability of ultrasonication and sterilisation techniques to remove materials and medicaments such as calcium hydroxide was especially significant.

A study by Whitworth *et al.* (2009), assessed the efficacy of cleaning endodontic hand files via ultrasonic cleaning and washer-disinfectors with and without pre-soaking in enzymatic detergents. It was found that the most effective method to remove protein

from hand files prior to sterilisation was pre-soaking them in an alkaline detergent, Alkazyme™(Alkapharm UK ltd, England), followed by processing in a washer-disinfector. Methods of decontamination such as ultrasonic cleaning and washer disinfection could not completely remove proteins from files.

Ferreira *et al.* (2012), compared two protocols for the removal of debris from endodontic hand files. The first method entailed manual cleaning; wiping with alcohol and autoclave sterilisation. The second method entailed manual cleaning; wiping with alcohol; ultrasonication in an enzymatic solution and then autoclave sterilisation. The files that had been treated using the second method displayed a significantly higher reduction in the amount of debris as compared to the first method where no enzymatic immersion had been used. None of the files were entirely free of debris after either cleaning method had been applied (Ferreira *et al*., 2012).

Guandalini *et al.* (2014), found manual cleaning with enzymatic detergent rendered endodontic hand files 100% free of debris.

Aminozarbian *et al.* (2013), conducted a study to evaluate an effective cleaning protocol for endodontic instruments. One hundred and eighty endodontic files were intentionally contaminated by preparing root canals of extracted human teeth until debris was visible in the flutes. Cleaning protocols involved manual cleaning with sponges soaked in 0.2% chlorhexidine, manual brushing, pre-soaking in an enzymatic solution (MICRO 10 ENZYME™, Unident, Chene Bourg, Geneve, Switzerland) and ultrasonic cleaning. The only group that displayed the absence of debris used a combination of manual brushing, enzymatic soaking and ultrasonication. The conclusion was that the best method for removing debris from endodontic hand files included a combination of chemical, mechanical and ultrasonic means. This is in agreement with Parashos, Linsuwanont and Messer (2004) and Guandalini *et al.,* (2014).

Muqbil *et al.* (2005), found that the efficacy of commercial cleaning solutions used for ultrasonic cleaning decreased after repeated uses.

1.2.4.5 Washer disinfectors

There is conflicting evidence regarding the cleaning efficacy of re-processing dental instruments with a washer disinfector. Washer disinfectors have been shown to effectively clean heavily contaminated dental instruments (Miller *et al.,* 2000). Whitworth *et al.* (2009), found a combination of an enzymatic agent and washer disinfector to be more effective at removal of residual protein from endodontic files than ultrasonification. This is in agreement with Vassey *et al.* (2011), who demonstrated washer disinfectors to be significantly more effective at cleaning dental instruments than a combination of manual and ultrasonic cleaning. In contradiction to this, Perakaki, Mellor and Qualtrough (2007), found washer disinfectors to be less effective than ultrasonic cleaning at removing biological debris from endodontic files. This is in agreement with Van Eldik *et al* (2004a).

Bagg *et al.* (2007), found no dental practices included in their study of 179 participants used washer disinfectors. A suggested reason for this finding was that bench-top washer models were a relatively recent introduction on the market at the time of this research.

It has been purported that washer disinfectors could increase productivity, improve cleaning efficacy and reduce the exposure of dental staff to contaminated sharps (Miller *et al.,* 2000; Whitworth and Palmer, 2010). Vassey *et al., (*2011), found washer disinfectors improved the consistency of the levels of cleaning of dental instruments.

2.4.6 Plasma cleaning

Plasma is a partially ionized gas consisting of a collection of stripped particles. This gas is a common form of matter that can maintain its gaseous state through a wide range of temperatures. Plasma is referred to as the fourth state of matter (Hoffman, Berganza and Zhang, 2013). Everyday examples of the applications of plasma include flat panel display screens and energy-saving lamps (Cha and Park, 2014).

When applied at temperatures lower than 40ºC, plasma converts into matter referred to as either cold atmospheric plasma (CAP) (Hoffman, Berganza and Zhang, 2013) or non-thermal atmospheric plasma (Sladek *et al.,* 2004). Cold atmospheric plasma is

able to deactivate microorganisms and decontaminate irregular surfaces (Sladek *et al.,* 2004; Cha and Park, 2014).

The possible applications of CAP in dentistry and oncology have been explored (Hoffman, Berganza and Zhang, 2013).

The current applications of CAP in dentistry include:

- The treatment of dental caries (Sladek *et al.,* 2004);
- Sterilisation (Hoffman, Berganza and Zhang, 2013; Cha and Park, 2014);
- Biofilm elimination, (Hoffman, Berganza and Zhang, 2013; Cha and Park, 2014);
- Root canal disinfection (Hoffman, Berganza and Zhang, 2013; Cha and Park, 2014);
- Improved bond strength in adhesive restorations (Hoffman, Berganza and Zhang, 2013; Cha and Park, 2014);
- Dental bleaching (Hoffman, Berganza and Zhang, 2013; Cha and Park, 2014).

Whittaker and associates (2004), reported plasma cleaning to be an effective method to decontaminate endodontic files.

1.2.5 Efficacy of decontamination procedures

It has been well documented in the literature that the routine decontamination protocols used to clean endodontic files in dental practice are ineffective in rendering these instruments free of debris after clinical use (Segall *et al.,* 1977; Smith *et al.,* 2002; Letters *et al.,* 2005, Walker *et al.,* 2009). This is concerning, considering the re-use of endodontic files is common practice throughout the world including:

- Australia (Messer, Parashos and Moule, 2003);
- New Zealand (Messer, Parashos and Moule, 2003);
- United States (Segall *et al.,* 1977);
- Canada (Morrison and Conrod, 2009).

Smith *et al*. (2002), reported that 76% of the endodontic files collected from general dental practices in Scotland remained visibly contaminated with debris. Fourteen percent of the files acquired from a dental hospital in Glasgow also remained visibly

contaminated with debris after decontamination. This study concluded that the current methods used for decontaminating endodontic instruments were insufficient to completely remove biological debris. The sample size in this study was however relatively small (n = 66).

One study with a large sample base (220 endodontic files collected from 22 dental practices) reported that 98% of the files that had been used, cleaned and autoclaved: deemed ready for re-use, were still visibly contaminated by debris when examined under a dissecting light microscope (Smith *et al*., 2005).

Letters *et al.* (2005), examined the efficacy of decontamination of stainless steel and NiTi endodontic files from 25 dental practices in Scotland. It was demonstrated that 75% of files that had been put through decontamination procedures still displayed evidence of contamination. Furthermore, seven percent of these files were found to be contaminated with blood. The results of this study are highly likely to be an accurate representation of the general dental practices in this region as 25 private practices participated in the study and a total of 250 files were evaluated.

Occult blood contamination was found on 29% of the dental instruments evaluated from 24 dental clinics in one South African province (Mehtar *et al.,* 2007).

A study focused on decontamination procedures (excluding sterilisation) was conducted in Australia by Parashos, Linsuwanont and Messer in 2004. In this research a cleaning protocol, capable of 100% removal of debris from endodontic rotary NiTi files after clinical use, was demonstrated. The suggested cleaning protocol can be applied to both rotary and hand endodontic files and was designed to be workable in any general dental practice. The protocol consisted of ten vigorous strokes in a sponge pre-soaked in 0.2% chlorhexidine, pre-soaking for 30mins in an enzymatic cleaning solution, ultrasonication for 15mins in the same solution followed by a final rinse for 20secs under running tap water.

In 2007, Bagg *et al.* surveyed 179 dental surgeries in Scotland to evaluate procedures and policies relating to the cleaning of dental instruments. Forty-one percent of the dental practices surveyed, were found to only use water without any detergent during manual cleaning. Only two percent of the surgeries used a detergent that was specifically formulated for the manual cleaning of medical instruments. It was found

that the cleaning of reusable instruments was performed within a poorly controlled environment. The factors contributing to the variability of cleaning methods included the water temperature, brush-type, type of detergent used and the strength as well as the number of strokes used to clean dental instruments. This study was broad-based; representing several dental practices (n = 179). It was demonstrated that the way dental instruments, including endodontic files, were cleaned may be subjective and at times ineffective.

Lipscomb, Sihota and Keevil (2008), reported that within the context of hospital central sterile service departments (CSSD) in England the most commonly used method to determine the degree of contamination of surgical instruments was visual inspection alone. This study was undertaken to assess the effectiveness of visual inspection in detecting debris on “clean” surgical instruments compared to evaluation by episcopic differential interference microscopy, combined with a fluorescent agent. A close correlation between the level of cleanliness and instrument design was seen, especially in instruments with a simple geometry. However, when more intricate instruments were assessed by visual inspection alone, post-cleaning debris contamination was often missed. The exclusive use of visual assessment for determining the cleanliness of surgical instruments is inadequate (Lipscomb, Sihota and Keevil, 2008).

In a study undertaken in one of the South African provinces, 83.3% of participants (dental practitioners) responded that dental instruments must be visibly clean prior to re-use (Mehtar *et al.,* 2007). Bagg and colleagues (2007), however, did report that one percent of participants used magnification to inspect cleaning of endodontic files. Given that endodontic files are small instruments with an intricate design, the residual debris on files after cleaning is highly likely to be missed by visual inspection alone.

Standardisation and validation of cleaning processes are difficult to evaluate (Bagg *et al*., 2007; Walker *et al*., 2007). Dental instruments may not be effectively cleaned and sterilised to an acceptable standard within the South African context (De Kock and van Wyk, 2001; Yengopal, Naidoo and Chitke, 2001; Mehtar *et al.,* 2007; Oosthuysen, Potgieter and Blignaut, 2010; Hartshorne, 2010).

1.2.6 Cleanliness of endodontic files at the time of purchase

Newly purchased, unused endodontic files are not sterile at the time of removal from the manufacturers packaging (Van Eldik *et al.,* 2004a; Morrison and Conrod, 2009). The flutes of new endodontic files are especially prone to an accumulation of debris (Van Eldik *et al.,* 2004a). Chianello *et al.* (2008) found that 100% of new rotary endodontic files from five different manufacturers to be contaminated with debris. This is in agreement with Hanan *et al.* (2015). In contrast, Gnau and Goodell (2009), found that only six percent of new endodontic files were contaminated with debris upon removal from the manufacturer's packaging.

1.2.7 Single-use of endodontic files

Several factors must be considered when assessing the debate surrounding the single- and multiple-use protocols of endodontic files. According to the literature, the following factors have been identified as reasons why dental practitioners favour either a single- or multiple-use approach, they include:

- Cost implications of single-use protocols;
- Ethical considerations;
- Endodontic file fatigue associated with multiple-use;
- The effect of decontamination processes on endodontic files;
- Effect of multiple-use on endodontic files.

1.2.8 Cost implications of single-use protocols

On average four to seven endodontic files are used in any one root canal treatment (Messer, Parashos and Moule, 2003). Endodontic files, especially rotary NiTi files are expensive instruments and the cost of endodontic treatment is decreased when they are used multiple times (Messer, Parashos and Moule, 2003).

The single-use of endodontic files results in the cost of endodontic treatment being significantly higher (Messer, Parashos and Moule 2003). Single-use may result in endodontic treatment being less accessible to less affluent patients. Letters *et al.* (2005), agreed that the cost of endodontic procedures would likely increase due to the

implementation of single-use protocols. Regarding the single-use of both hand and rotary endodontic files, Letters *et al.* (2005), stated, “this raises important issues linked to the funding of endodontic procedures in the health service, which will need to be resolved.”

1.2.9 Ethical consideration in re-using endodontic files

The dental practitioner has moral and ethical obligations to practice infection control procedures to a high standard (Scarlett and Grant, 2015). Inadequate infection control practices may lead to the violation of ethical principles such as beneficence, non-maleficence, fairness (single-use instrument disposal) and autonomy (information on safety) (Hartshorne, 2010). A failure by a dental practitioner to ensure reasonable measures to the prevention of transmission of infectious diseases via contaminated dental instruments could constitute negligence (Hartshorne, 2010).

It has been suggested that: “The safest and most unambiguous method to ensure that there is no risk of residual infectivity on contaminated instruments and other material is to discard them after use” (Sonntag and Peters, 2007). This statement reiterates earlier literature which emphasises that to ensure maximum safety, single-use of endodontic files is prudent (Arens *et al.,* 2003).

1.2.10 Effects of multiple-use on endodontic hand files

Following multiple-use (Arens *et al.,* 2003; Neskovic *et al.,* 2010), and decontamination procedures (Sonntag and Peters, 2007), evidence of wear, surface corrosion and altered mechanical properties is seen in endodontic files. Defects on the surface of endodontic files lead to a decrease in the cutting efficiency of the cutting blades (Haikel *et al.,* 1996; Alexandrou *et al.,* 2006).

Endodontic instrument separation involves the interplay of several factors (Madarati, Watts and Qualtrough, 2008).

The most widely acknowledged factors are:

- The lack of proficiency of the operator (Arens *et al.,* 2003; Berutti *et al.,* 2009);
- The angle and radius of the curve of the root canal being prepared (Iqbal, Kohli and Kim, 2006);
- Multiple uses on several cases (Fishelberg and Pawluk*,* 2004);
- The rotary speed of the file (Fishelberg and Pawluk, 2004; Madarati *et al*., 2008).

Popovic *et al*., (2014), demonstrated the presence of defects on the cutting edge of instruments after repeated clinical use of these instruments. Even after only one use, the cutting edge of small-diameter stainless steel files demonstrated deformation. Due to deformation following single-use, this study concluded that it is advisable to only use small diameter stainless steel hand files once in narrow and/or curved canals.

1.2.11 Effects of decontamination on endodontic files

The Australian and New Zealand Academy of Endodontists reported that routine manual debridement and autoclaving procedures did not affect the functionality of either stainless steel or NiTi endodontic files (Messer, Parashos and Moule, 2003).

In contradiction, Sonntag and Peters (2007), reported that there was corrosion of the surfaces of endodontic files after prion decontamination protocols were used. The immersion in NaOCl was especially detrimental (Sonntag and Peters, 2007). Barbosa, Gomes and de Araújo (2007), however found the immersion of NiTi rotary files in NaOCl to have no influence on their resistance to file separation. This is in agreement with the findings of De Castro Martins *et al.* (2006) and Darabara *et al.* (2004).

Sodium nitrite dips have been recommended to retard corrosion prior to the autoclaving of steel dental instruments (Krell, 2009). This method has been found to be more relevant to carbon steel instruments than stainless steel instruments (Porto *et al.,* 2015).

The repeated use of endodontic files leads to surface malformations of the file's working surfaces and small diameter files are especially susceptible to separation,

indicating a need for single-use of these fragile instruments (Carrotte, 2004; Neskovic *et al*., 2010).

Ametrano *et al.* (2011), found short term contact of both NaOCl and ethylenediaminetetraacetic acid (EDTA) to cause surface alterations of ProTaper Universal NiTi rotary endodontic files using atomic force microscopy.

Spagnuolo *et al.* (2012), reported that multiple autoclave sterilisation cycles could alter the surface topography and chemical composition of conventional NiTi and titanium nitride surface-coated endodontic files.

CHAPTER 2: AIMS AND OBJECTIVES

2.1 Aims

Firstly, this study aimed to evaluate the amount of visible debris left on endodontic hand files collected from general dental practices in Pretoria, South Africa, after the application of routine cleaning and sterilising procedures. The second aim of this study was to determine the prevalence of the single-use of endodontic files and the prevailing attitudes regarding the re-use or single-use of endodontic files of dental practitioners in Pretoria, South Africa.

2.2 Objectives

The objectives of this study were:

- To determine whether dental practices in Pretoria, South Africa are efficiently cleaning endodontic hand files prior to re-use on subsequent clinical cases;
- To determine the ratio of the practise of multiple-use verses single-use protocols of endodontic hand files in dental practices in Pretoria, South Africa;
- To determine the attitudes of the individuals responsible for cleaning endodontic hand files in dental practices in Pretoria, South Africa, regarding the single-use of endodontic hand files;
- To establish a standard, recommended protocol for effectively reprocessing endodontic hand files in dental practice in South Africa.

2.3 Hypothesis

The hypothesis was that clinically used endodontic hand files would remain contaminated with debris after routine cleaning and sterilisation procedures and that single-use and standardised protocols were not in place in South African dental practice.

CHAPTER 3: MATERIALS AND METHODS

3.1 Method description

A total of 27 private dental practices in Pretoria, South Africa were included in this study. The research project commenced once 27 private dental practices had voluntarily consented to participate in the current study. Participants were selected using both random and convenience sampling. Selection was accomplished by randomly contacting dental practices in Pretoria via telephone as listed on an internet database (Medpages). Dental practitioners who were known to offer endodontic services to patients were also contacted directly, provided the practitioner had no financial affiliation with either of the researchers of the current study. An appointment was scheduled with each participant after a short telephonic conversation.

At the scheduled appointment, the owner of the practice was given a brief overview of the intended study. Those that agreed to willingly participate in the current study were then interviewed and enrolled in the study. The owner of each participating dental practice received and signed an informed consent form (Addendum A) prior to participating. A coding system was used to randomly assign a letter to each participant to guarantee the anonymity of each participant in this study. Anonymity was ensured to encourage a higher participation rate as participants would not be penalised or prejudiced in any way, even if the decontamination of the endodontic files in the associated practice was found to be lacking. The interview was administered by a single interviewer and the questionnaire (Addendum B) was actively completed with the participant. Participants included the person/s responsible for the decontamination procedures of the endodontic hand files in that specific dental practice.

The questionnaire for the present study was designed to determine:

- The applied cross-infection procedures;
- The types of endodontic files used;
- The protocols followed for endodontic file use (i.e. single- or multiple-use);
- The methods of infection control being used;
- The decontamination protocols applied to endodontic hand files.

Each participant was then requested to submit 15 endodontic hand files (Figure 3.1) of any design and/or length. One brand new endodontic hand file of a commonly used size, type and length was provided to the participant to replace each file that had been submitted for evaluation.

Figure 3.1: Samples in the collection receptacles

The inclusion criteria for the endodontic files submitted for the study were:

- The file had to have been used clinically to treat at least one patient;
- The file had to have been used clinically to prepare at least one root canal;
- The file had to have been cleaned and/or sterilised after clinical use using the routine decontamination processes followed by the participants;
- Directly following the application of the routine cleaning and/or sterilising procedures, the participant had to have placed the file into the clean collection receptacle provided by the researcher.

The exclusion criteria were:

- Rotary endodontic files;
- Barbed broaches and finger spreaders;
- Files that had separated tips.

3.2 Calibration group

Prior to analysis of the samples collected from the participants, the examiners evaluated a group consisting of fifteen unused size 015 stainless steel K-files (M•access K-FILE, Dentsply Maillefer, Ballaigues, Switzerland) (Figure 3.2) that were taken directly from the manufacturers' packaging. This exercise aimed to calibrate the examiners and to standardise scoring of samples.

Figure 3.2: New endodontic hand files (calibration group)

3.3 Microscopic analysis

The collected samples as well as calibration samples were then subjected to microscopic evaluation. The method of evaluation of debris contamination was based upon a modified version of the methodology described by Smith *et al.* (2002). The original scoring system from which the scoring system used in the current study was adapted is presented in Table 3.1 (Smith *et al*., 2002). Similar methods have been

used in subsequent research (Letters *et al,* 2005; Aasim, Mellor and Qualtrough, 2006; Souza *et al.,* 2011).

Each sample was examined for the presence of debris by two independent examiners. A novel scoring system was used to describe the presence of debris on each sample.

Debris was defined as any foreign material that adhered to the surface of a sample. Metal tags, corrosion and areas of deformation were not considered to be debris.

Table 3.1: Original scoring system described by Smith *et al.* (2002)

Extent of contamination	Score
Debris found on >75% of the instrument length	++++
Debris found on 50-75% of the instrument length	+++
Debris found on 25%-50% of the instrument length	++
Debris found on 1-25% of the instrument length	+
No debris visible at high or low power	0

The entire length of each sample was observed at ten times magnification using a stereomicroscope (Olympus, SZ-CTV, Japan) (Figure 3.3). The presence or absence of debris was confirmed and the position of any debris/contamination was then established by viewing the sample at 40 times magnification. Digital images were captured at both ten (Figure 3.4) and 40 times magnification (Figure 3.5) using a digital camera (Nikon Coolpix 950, Nikon, Japan) (Figure 3.6).

Figure 3.3: Stereomicroscope (front view)

Figure 3.4: Stereomicroscope: set at 10 x

Figure 3.5: Stereomicroscope: set at 40 x

Figure 3.6: Nikon Coolpix 950, digital camera (rear view)

3.4 Scoring

A stainless steel endodontic ruler (NSK endo-wrench, Japan) was marked and used to demarcate equal sections into which the cutting blade of each sample (endodontic file) was divided (Figure 3.7). Each demarcated section was four millimetres long, each of which would determine the four scoring positions. At 40 times magnification, it was possible to examine and photograph each “quarter” of a sample’s cutting blade in detail.

Figure 3.7: Marked stainless steel endodontic ruler and mounting device

The possible positions designated for scoring debris are defined in Table 3.2. Each "quarter" was used to represent each respective scoring position of an individual sample (Figure 3.8). During microscopic evaluation each scoring position was viewed and assigned a score of either one or zero by two independent examiners (Figure 3.9). A score of one indicated that debris was present while a score of zero indicated the absence of debris. The allocated scoring position corresponded to lie within the nearest quarter of the cutting blade of the sample – a total length of 16mm (D_0 to D_{16}).

Table 3.2: Allocated scoring positions evaluated for the presence of debris

Position	Description	Measurement
1	the most apical quarter of the blade	D_0 to D_4
2	the middle quarter nearest to the tip of the file	D_4 to D_8
3	the middle quarter closest to the handle of the file	D_8 to D_{12}
4	the quarter closest to the handle of the file	D_{12} to D_{16}

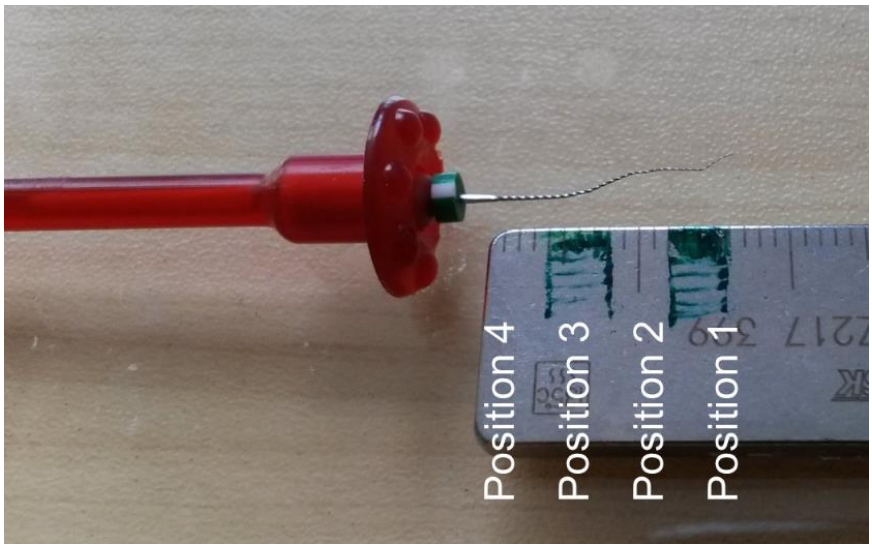
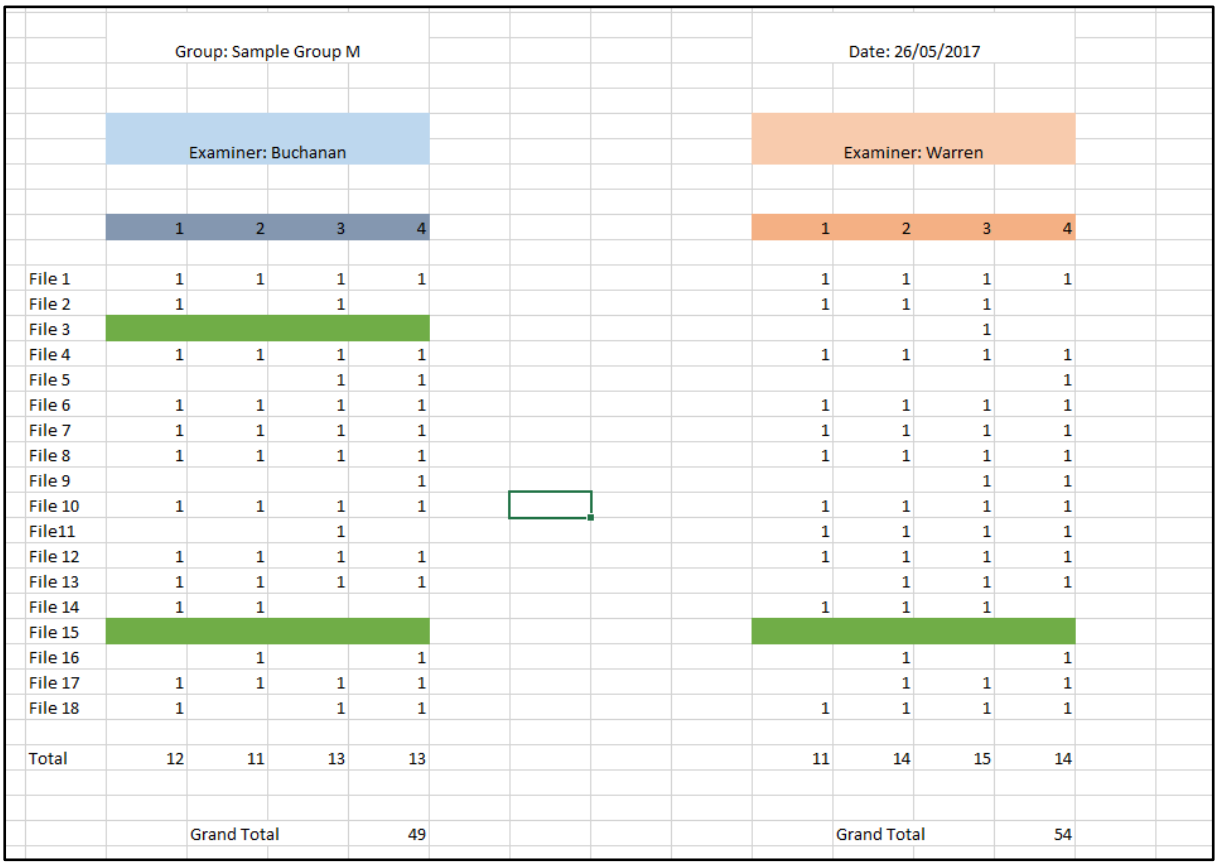


Figure 3.8: Representation of scoring positions



	Group: Sample Group M					Date: 26/05/2017			
	Examiner: Buchanan					Examiner: Warren			
	1	2	3	4		1	2	3	4
File 1	1	1	1	1		1	1	1	1
File 2	1		1			1	1	1	
File 3								1	
File 4	1	1	1	1		1	1	1	1
File 5			1	1					1
File 6	1	1	1	1		1	1	1	1
File 7	1	1	1	1		1	1	1	1
File 8	1	1	1	1		1	1	1	1
File 9				1				1	1
File 10	1	1	1	1		1	1	1	1
File11			1			1	1	1	1
File 12	1	1	1	1		1	1	1	1
File 13	1	1	1	1			1	1	1
File 14	1	1				1	1	1	
File 15									
File 16		1		1			1		1
File 17	1	1	1	1			1	1	1
File 18	1		1	1		1	1	1	1
Total	12	11	13	13		11	14	15	14
	Grand Total			49		Grand Total			54

Figure 3.9: The scoring tables of both examiners used to evaluate the samples of group M (randomly selected for illustration purposes)

3.5 Light source

The standard light source (Highlight 2000, Olympus, Japan) (Figure 3.10) accompanying the stereomicroscope failed to produce adequate, high-quality digital photographs. A custom light-emitting diode (LED) light source (Figure 3.11) was therefore specially constructed for this research project (courtesy of Dr M.Y. Gamieldien, Pretoria, South Africa).

Figure 3.10: The standard light source

Figure 3.11: The custom-built LED light source

3.6 Statistical analysis

3.6.1 Statistical considerations

The statistical evaluation of the data collected in the current study set out to estimate the frequency and distribution of debris in each scoring position of all the samples. The percentage of samples with a culminate score of zero was of interest (Smith *et al*., 2002, Smith *et al.,* 2005). The responses to the questionnaire were expressed as simple percentages.

3.6.2 Sample size

Samples were scored as either positive or negative for debris in positions 1 to 4. To attain a probability of 0.95 that each position estimation fell within 0.1 of the population proportions, a total sample of at least 400 (100 per scoring position) endodontic hand files was required (Thompson, 1987), for an expected proportion of 0.5. To achieve this number, at least 27 participants were required to submit 15 samples each which would result in a total of 405 samples.

3.6.3 Data analysis

Percentages along with confidence intervals were used to report:

- The estimates for frequency and distribution of debris (positions 1-4);
- Clean files (zero-score) and all positions (positions 1-4 together);
- The responses to the questionnaire regarding single-use.

Cohen's Kappa statistic and the Chi-Square test were used to assess the repeatability of the scoring system, measuring inter-rater agreement for each scoring position of the dataset. All samples in each scoring position were used to measure inter-rater agreement. Sample groups were further compared to the calibration group using Fisher's Exact test.

All the statistical analyses were performed on SAS (SAS Institute Inc., NC, USA), Release 9.4, running under Microsoft Windows for a personal computer. All P-values ≤ 0.05 were considered significant.

3.7 Ethical considerations

A research protocol for the present study was submitted to the Research Committee (RESCOM) of the School of Dentistry, Faculty of Health Sciences, University of Pretoria for approval. Following RESCOM approval the protocol was then submitted to the Ethics Committee of the Faculty of Health Sciences, University of the Pretoria, and ethical clearance was granted.

All parties involved in the execution of this this research project declare no financial interests in any of the materials, products, instruments or equipment used or evaluated in carrying out this research. No parties involved in this research study are financially affiliated with the consenting voluntary participants of this study.

CHAPTER 4: RESULTS

4.1 Number of samples and participants

A total number of 411 endodontic hand files were collected from 27 participants for the purposes of the present study. Seventeen of these participants provided the requested number of files. Five participants provided more than the requested 15 files and five participants provided less than the requested number of files. The calibration group consisted of 15 samples. Ten samples were excluded, and the total number of valid samples was 401.

4.2 Examiner agreement

Examiner agreement was evaluated for each scoring position over the entire dataset collected from private practice. The results of the examiner agreement are expressed in Table 4.1. The low p-values ($p < 0.0001$) obtained using the Chi-square test demonstrates substantial examiner agreement (statistically significant) over the entire dataset. The results obtained using the Kappa test also confirm examiner agreement, however when using this analysis the inter-examiner agreement is described as fair to moderate. Additionally, the Kappa scores demonstrate that the inter-rater agreement exceeds the agreement that would be expected purely by chance.

Table 4.1: Examiner agreement by scoring position over the entire dataset

	Position 1	Position 2	Position 3	Position 4
Chi-square	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
Simple Kappa	0.41	0.39	0.41	0.52
CI* (95%)	0.32 - 0.50	0.29 – 0.47	0.41 – 0.50	0.43 – 0.60
Test of H0: Kappa = 0	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
Agreement	Moderate	Fair	Moderate	Moderate
Sample size	401	401	401	401

*Confidence interval

4.3 Examiner scores

The frequency of the individual examiners' scores per scoring position, along with the correlating percentages are represented in Table 4.2.

Table 4.2: Frequency and percentages of examiner scores

Examiner 1		
Total	Frequency	Correlating percentage (%)
0	52	12.9
1	71	17.7
2	115	28.7
3	77	19.2
4	86	21.5
Examiner 2		
Total	Frequency	Correlating percentage (%)
0	42	10.5
1	56	14.0
2	111	27.7
3	76	18.9
4	116	28.9

4.4 Distribution of debris

The distribution of debris as found in the pre-designated scoring positions (as an average score of both examiners) are represented in Table 4.3.

Table 4.3: Distribution of debris by scoring position

Position	Percentage (%)	Confidence interval (%)
1	68.1	63.4 – 72.4
2	67.1	62.3 - 71.5
3	76.6	72.2 – 80.4
4	74.1	69.6 – 77.1

4.5 Percentage of clean samples

Twenty-four of the 401 examined samples from private practice were found to be completely clean of debris i.e. they scored zero in all pre-designated scoring positions by both examiners independently. This translates to 6% of the total sample size being completely debris-free. The corollary of this is that 94% of the samples displayed some debris contamination. The samples with a combined score of zero contamination are represented in Table 4.4. The calibration group contained no samples that were scored completely free of debris by both examiners.

Table 4.4: Percentage of samples with a combined score of zero.

Total	Frequency	Percent (%) Contaminated	Percent (%) uncontaminated	Cumulative Frequency
0	24	6.0	94.0	24
1	26	6.5	93.5	50
2	32	8.0	92.0	82
3	47	11.7	88.3	129
4	67	16.7	83.3	196
5	48	12.0	88.0	244
6	51	12.7	87.3	295
7	43	10.7	89.3	338
8	63	15.7	84.3	401

*A total > 0 represents samples that scored positively for debris. 1-4 represent the scoring positions assessed by the first examiner and 5-8 represent the scoring positions of the second examiner.

4.6 Number of groups with zero score

Seven out of the 27 groups contained samples that scored zero (i.e. these samples were scored as completely free of debris in all four pre-designated positions), by both examiners independently. None of the remaining 20 groups contained any samples that were deemed completely free of debris.

The seven groups, which had samples evaluated as being completely “clean” are represented in Table 4.5.

Table 4.5: The seven groups containing “clean” samples

Group	Number of samples scoring zero (total number of samples in group)	Percentage (%)
B	11(15)	73.3
L	1(16)	6.2
M	1(18)	5.6
S	3(15)	20.0
V	4(15)	26.7
W	3(15)	20.0
X	1(15)	6.7
Total	**24(401)**	**6.0**

4.7 Cleaning method by group

Five out of the seven groups that contained completely debris-free samples, reported using a common method of cleaning/decontamination of endodontic hand files, namely, a combination of manual and ultrasonic cleaning. This equates to 71.4% of the groups that displayed any completely debris-free samples making use of a combination of these two decontamination methods. The responses of all the participants to question four of the questionnaire are summarised in Table 4.6. This question was phrased: “Which decontamination methods for endodontic files is this practice using?”

Table 4.6: Groups by response to question four (see Addendum B)

Group	A Manual cleaning with brushes detergent only	A+E Manual cleaning with brushes detergent and washer disinfector	B Manual cleaning with gauze or sponge soaked in alcohol	D Both manual cleaning and ultrasonic ation	D+E Manual cleaning and ultrasonic ation plus washer disinfector	E Washer disinfector only	No answer	Total
A	0	0	0	0	0	1	0	1
AA	1	0	0	0	0	0	0	1
AB	0	0	0	1	0	0	0	1
B	0	0	0	1	0	0	0	1
C	0	0	1	0	0	0	0	1
D	0	0	0	0	1	0	0	1
E	0	0	0	0	1	0	0	1
F	1	0	0	0	0	0	0	1
G	1	0	0	0	0	0	0	1
H	1	0	0	0	0	0	0	1
I	0	0	0	1	0	0	0	1
J	0	0	1	0	0	0	0	1
K	0	0	0	1	0	0	0	1
L	1	0	0	0	0	0	0	1
M	0	0	0	1	0	0	0	1
N	1	0	0	0	0	0	0	1
O	0	0	0	0	0	1	0	1
P	0	0	0	0	0	1	0	1
Q	0	1	0	0	0	0	0	1
R	0	0	0	0	0	0	1	1
S	0	0	0	1	0	0	0	1
T	1	0	0	0	0	0	0	1
U	0	0	0	0	0	1	0	1
V	0	0	0	1	0	0	0	1
W	0	0	0	1	0	0	0	1
X	0	0	0	0	0	0	1	1
Z	1	0	0	0	0	0	0	1
Total	**8**	**1**	**2**	**8**	**2**	**4**	**2**	**27**

4.8 Comparison of groups collected from private practice

Of the 27 groups from private practice, samples from group B displayed the least amount of debris contamination (Table 4.7).

Table 4.7: Frequency of zero score for group B

Sample score	Frequency	Percentage (%)
0 (clean)	11	73.3
1 (debris present)	4	26.7
Total	**15**	**100**

When compared to other groups found to contain debris-free samples, using Fisher's Exact test, the samples from group B displayed statistically less debris (i.e. cleaner) than those of group V – the group with the second highest number of debris-free samples (Table 4.8). It is therefore extrapolated that the samples from group B were also statistically cleaner than all the remaining groups in this study.

Table 4.8: Fisher's Exact test group B to group V

Table of group B by group V			
V	B		
	1	2	Total
1	11 73.3	4 26.7	15
2	4 26.7	11 73.3	15
Total	**15**	**15**	**30**

Fisher's Exact test	
Cell (1,1) Frequency (F)	11
Left-sided Pr < = F	0.9986
Right-sided Pr > = F	0.0134
Table Probability (P)	0.0120
Two-sided Pr < = P	0.0268
Sample size	30

4.9 Comparison of all groups

The total debris contamination, taken as an average score of all scoring positions of both examiners and expressed as a percentage for each individual group is represented in Figure 4.1. In the event of examiner disagreement (i.e. one examiner scoring positive and the other scoring negative) in any given position, the decision was made to score the sample positively for the presence of debris in that position. This was done to avoid the possibility of half-scores, as a half-score was still considered to represent the presence debris and not its absence (Addendum D). As a result of this, the graphs must be interpreted with caution, as there may be a potential bias toward over-estimation of the presence of debris, however, the graphs allow for meaningful comparison of total contamination scores between all groups.

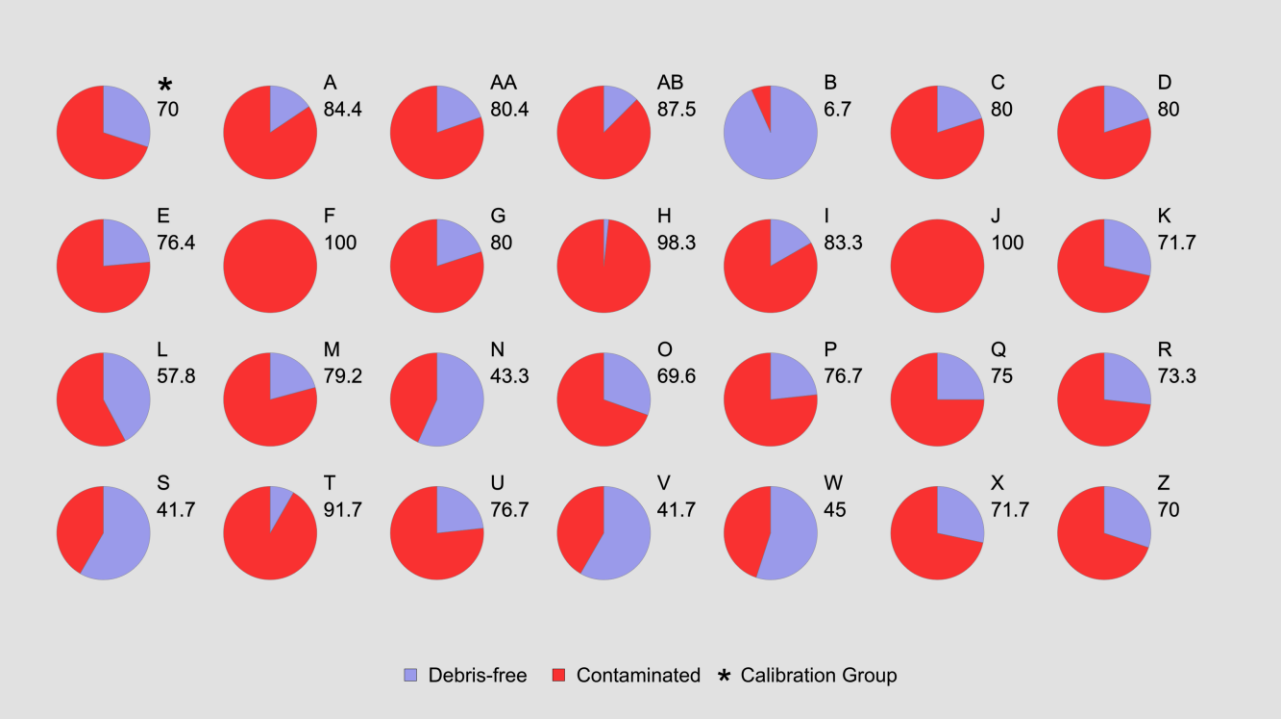


Figure 4.1: Average score of debris-free and contaminated positions per group

The red portions of the pie-charts represent the average number of contaminated scoring positions per group as a percentage (%) of the total number of possible scoring positions for that group. The blue portions represent the percentage of debris free ("clean") positions.

4.10 Comparison of participant groups to the calibration group

The total debris contamination of the calibration group was found to be 70% (Figure 4.1). The 27 groups collected from private practice were each individually compared to the calibration group using Fisher's Exact test.

The samples of five groups (viz. B, N, S, V and W), were found to have significantly less debris contamination than those of the calibration group. In contrast to this finding, the samples of four groups (viz. F, H, J and T), were found to be significantly more contaminated than those of the calibration group. The samples from the remaining 18 groups showed no statistical difference in the level of debris contamination as compared to the calibration group. The results of this analysis are demonstrated in Table 4.9.

Table 4.9: Contamination by group compared to the calibration group using Fisher's Exact test.

Group	p – value*	Statistical difference	Level of debris contamination
A	= 0.0845	Not significant	
AA	= 0.2833	Not significant	
AB	= 0.0751	Not significant	
B	< 0.0001	Significant	Less debris
C	= 0.2918	Not significant	
D	= 0.2918	Not significant	
E	= 0.4341	Not significant	
F	< 0.0001	Significant	More debris
G	= 0.2918	Not significant	
H	< 0.0001	Significant	More debris
I	= 0.1300	Not significant	
J	< 0.0001	Significant	More debris
K	= 1.0000	Not significant	
L	= 0.1922	Not significant	
M	= 0.2345	Not significant	
N	= 0.0055	Significant	Less debris
O	= 1.0000	Not significant	
P	= 0.5361	Not significant	
Q	= 0.6830	Not significant	
R	= 0.8397	Not significant	
S	= 0.0031	Significant	Less debris
T	= 0.0074	Significant	More debris
U	= 0.5361	Not significant	
V	= 0.0031	Significant	Less debris
W	= 0.0094	Significant	Less debris
X	= 1.0000	Not significant	
Z	= 1.0000	Not significant	

*p-values > 0.05 are considered significant

4.11 Results of the questionnaire

4.11.1 Type of endodontic hand files used

It was found that 48.2% of participants used stainless steel endodontic hand files. The combined use of stainless steel and nickel titanium (NiTi) endodontic files was reportedly practised by 51.9% of the participants. None of the participants reported practising the sole-use of NiTi files.

4.11.2 Number of uses

None of the participants reported the single-use of endodontic hand files. Most participants (44.4%) reported that hand files were used for five to ten separate clinical cases before being discarded. A slightly lower percentage of the participants (40.7%) stated that endodontic hand files were used for a maximum of five separate clinical cases in that dental practice. The minority of participants (11.1%) reported that endodontic files were used for more than ten clinical cases before being discarded.

4.11.3 Reasons for discarding files

Damage was the main reason for discarding an endodontic hand file as reported by 70.4% of the participants in the present study. Examples of damage included; a file that had separated, bent, deformed. Only 14.8% of the participants discarded an endodontic file after a predetermined number of uses, regardless of the condition of the file. The remaining 14.8% of participants reported taking several of the above-mentioned factors into consideration when deciding when a file must be discarded.

4.11.4 Singular decontamination methods

Regarding the application of singular decontamination methods, 29.6% of participants reported using manual cleaning with brushes and detergent as the only method used to clean endodontic hand files. The use of a gauze or sponge soaked in alcohol, unaccompanied by other adjunctive cleaning methods, was practised by 7.4% of the

participants. None of the participants in the current study made use of ultrasonic cleaning as the only cleaning method for decontaminating endodontic hand files. Decontamination of files using only a washer disinfector was reported by 14.8% of the participants. The remaining participants described using a combination of cleaning methods, as described in the following point.

4.11.5 Combined decontamination methods

Regarding the combination of decontamination methods, 29.6% of all participants reported using manual cleaning combined with ultrasonic cleaning. A combination of mechanical cleaning with brushes and the use of a washer disinfector was reportedly used by 3.7% of participants. The combined use of manual cleaning, ultrasonic cleaning and the use of a washer disinfector, was reported by 7.4% of the participants.

4.11.6 Assessment of sample cleanliness

Methods of assessment of the cleanliness of files following routine decontamination procedures were reported as either: visual inspection with the naked eye (reported by 77.8% of participants) or inspection with magnification such as magnifying loupes or an operating microscope (stated to be routinely used by 7.4% of the participants).

One participant reported that file cleanliness was confirmed by the visual examination of sterilisation pouches into which endodontic hand files were placed. As this specific response refers to a sterilisation procedure and not a decontamination procedure, this data was not taken into consideration in this study. A minority of 11.1% of participants stated that endodontic files were not inspected for cleanliness following routine cleaning, before use in subsequent clinical cases.

4.11.7 Consideration of single-use

With regards to the single-use of endodontic hand files, one-third of the respondents reported having considered using endodontic hand files once and then discarding them. The remaining two-thirds of participants indicated never having considered the single-use of endodontic hand files.

4.11.8 Reasons for multiple-use

Of the participants who had not considered single-use as an option, one-third reported that the single-use of endodontic hand files would be too expensive, negatively affecting profit margins related to endodontic treatment. Almost one-quarter of the participants (22.2%), reported considering it unnecessary to discard endodontic hand files that were still in good working condition. A combination of these factors was reported by 7.4% of participants as the reason for their not implementing the single-use of endodontic hand files. This question was not answered by more than one-third of the participants (37.0%).

A few participants (7.4%) responded that “good” decontamination of endodontic hand files was achievable with routine cleaning methods.

One participant asserted that the combination of the possibility of achieving good decontamination as well as the good working condition of files as a reason for them re-using endodontic hand files. Another participant attributed the good working condition of previously used endodontic files as the main reason for their re-use, with the caveat that a file should however be discarded after first use had it become bent or damaged in the process.

Unfortunately, invalid responses to this question regarding the reason/s for choosing multiple-use protocols discounted the views of 22.2% of the participants.

4.11.9 Reasons to consider single-use

Of the respondents who had considered single-use of endodontic hand files before, 22.2% agreed that the single-use of endodontic files would be safer for their patients. One participant responded that single-use would be safer for the personnel who worked with these instruments. Some participants (14.8%) reported that they believed they would achieve better outcomes of the endodontic treatments carried out at the practice if single-use of endodontic hand files was implemented. One participant named a combination of all the above-mentioned reasons. No answer was given for this question by 55.6% of the participants. Table 4.10 summarises the participants responses to all the questions of the questionnaire.

Table 4.10: Questionnaire responses* (see Addendum B)

Question	Response	Frequency	Percent (%)	Cumulative frequency	Cumulative percent (%)
1	A	13	48.20	13	48.20
	C	14	51.80	27	100.0
2	B	11	40.70	11	40.70
	C	12	44.40	23	85.20
	D	3	11.10	26	96.30
	No response	1	3.70	27	100.00
3	B	19	70.40	19	70.40
	B+C	4	14.80	23	85.20
	C	4	14.80	27	100.0
4	A	8	29.60	8	29.60
	A+E	1	3.70	9	33.30
	B	2	7.41	11	40.70
	D	8	29.60	19	70.40
	D+E	2	7.40	21	77.80
	E	4	14.80	25	92.60
	No response	2	7.40	27	100.00
5	A	2	7.40	2	7.40
	B	21	77.80	23	85.19
	C	3	11.10	26	96.30
	D	1	3.70	27	100.00
6i	A	9	33.30	9	33.30
	B	18	66.70	27	100.00
6ii	A	9	33.30	9	33.30
	A+B	2	7.40	11	40.70
	B	6	22.20	17	63.0
	B+C	1	3.70	18	66.70
	B+D	1	3.70	19	70.40
	C	2	7.40	21	77.80
	No response	6	22.20	27	100.00
6iii	A	6	22.20	6	22.20
	A+B+C	1	3.70	7	25.90
	B	1	3.70	8	29.60
	C	4	14.80	12	44.40
	No response	15	55.60	27	100.00

* Table 4.11 does not include the letters corresponding with possible responses where none of the participants selected that specific option.

CHAPTER 5: DISCUSSION

5.1 Efficacy of decontamination procedures

The results of the present study correlate closely with the results of previous studies on the topic (Smith *et al.,* 2002; Smith *et al.*, 2005; Letters *et al.,* 2005). In the present study, endodontic hand files were found to be 94% contaminated with debris following routine decontamination procedures. Smith *et al.* (2005), found 98 percent of the samples analysed to be visibly contaminated with debris. Letters *et al.* (2005), found that 75% of the evaluated samples still displayed visual evidence of contamination. Both these studies assessed more than 20 dental practices and included over 200 samples. Smith *et al.* (2002), also reported a high level of debris contamination (76%), however the sample size was significantly smaller (n = 66).

5.2 Spread of disease

Despite the high level of debris contamination (94%) remaining on endodontic hand files following routine cleaning procedures, it is unclear whether there is any correlation between the presence of debris and the spread of infectious diseases or not (Hartwell *et al.,* 2011). No evidence of disease transmission via debris contaminated endodontic files could be found.

5.3 Adverse endodontic treatment outcomes

In 2004, Carrotte described a link between the presence of debris on endodontic files and the possible adverse outcomes of endodontic treatment. It is known that debris can be extruded through the apex during instrumentation with endodontic hand files (Al-Omari and Drummer, 1995).

The low rate of endodontic treatment failure could however be explained by the fact that endodontic files are not simply decontaminated after use, but also sterilised. It is known that new endodontic files are contaminated with debris when taken directly from the manufacturers packaging (Chianello *et al,* 2008). Furthermore, new files are also not sterile (Van Eldik *et al,* 2004b; Morrison and Conrod, 2009). It is therefore

recommended that even new endodontic files should be both cleaned and sterilised prior to their first clinical use (Morrison and Conrod, 2009).

It is debated in the literature whether “sterile debris’ is harmful or not (Reams, Baumgartner and Kulid, 1995; Johnson *et al.,* 1997; Van Eldik *et al.,* 2004b). In fact, there is not even consensus on whether instruments containing blood, saliva and materials can be sterilised at all (Miller, 1991; Reams, Baumgartner and Kulid, 1995; Van Eldik, 2004b; Souza *et al.,* 2011). Figure 5.1 demonstrates debris on a K-file 40.

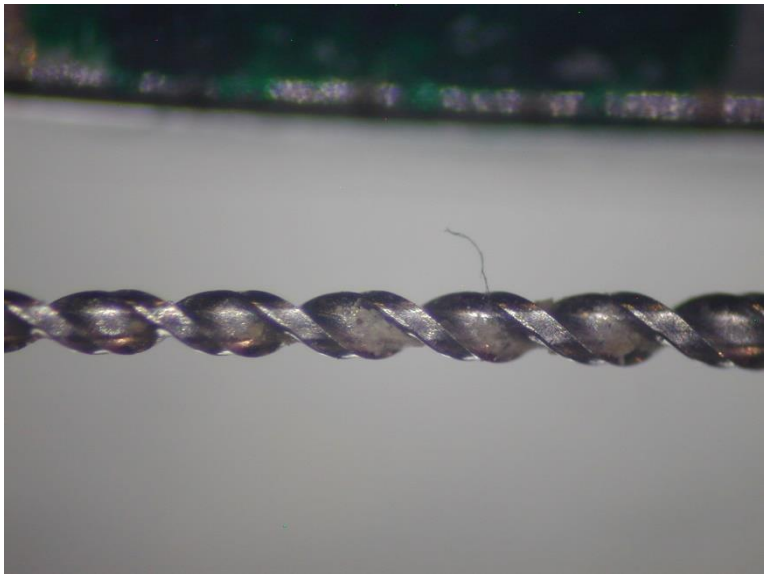


Figure 5.1: Debris on a sample (K-file) (40 x magnification)

5.4 Sterile inflammatory response

Inflammation of the dental pulp can be triggered by both mechanical and infective stimuli. Examples of such stimuli include drilling the tooth during restorative treatment and the exposure of the dental pulp to bacterial by-products of dental caries, respectively (Kim, 1990). Inflammation can also be triggered by dead cells and irritant particles, even if these particles are sterile. Sterile pro-inflammatory stimuli, such as dead cells may stimulate an inflammatory response via a common immune system pathway as compared to infective stimuli (Rock *et al.,* 2009). Sterile debris on endodontic files may be transferred between patients when files are re-used (Johnson *et al.,* 1997). Whether or not this may lead to an inflammatory response and potential

failure of endodontic treatment needs further exploration. This could be a potential explanation for the failure of otherwise clinically well-executed endodontic treatment.

5.5 Follow-up microbiological studies

Previous studies have evaluated the residual debris-contamination of endodontic files (Johnson *et al.,* 1997; Van Eldik *et al.,* 2004a; Souza *et al.,* 2011). These groups followed up with microbiological studies to determine whether remaining debris could be cultured for viable bacteria following sterilisation. All found sterilisation of endodontic files to be possible despite contamination with biological debris. A separate study did however find that files could not be sterilised after clinical use (Morrison and Conrod, 2009).

During the present study there was no intention to keep a sterile field during sample evaluation. One critique of previously undertaken studies is that it may be difficult, if not impossible, to maintain a sterile/controlled field during the handling of samples from their collection receptacles to a microscope, without cross contamination.

A potential follow-up investigation from the present study could involve microbiological evaluation the samples collected, which are known to be contaminated with debris, following the application of a standardised sterilisation protocol. These results could contribute to the existing body of evidence on this topic.

Follow-up studies have been limited to microbiological analysis alone, viz., the ability to culture bacteria on endodontic files following decontamination or sterilisation procedures. No scientific publications evaluating residual debris on endodontic files for viable viruses, fungi or parasites, could be found. Existing follow-up studies have usually been limited to laboratory studies concerned with a single-microbiological organism. For example, Johnson *et al.,* (1997) assessed only *B. Stearothermophilus.*

Siqueira and Rôças (2009), showed that over 460 unique bacterial taxa have been identified in different types of endodontic infections. Future research may be directed towards the assessment of the sterilisation of dental instruments contaminated with biofilms or multiple organisms, to possibly better reflect the clinical situation.

5.6 Small verses large diameter endodontic files

Endodontic hand files of a larger diameter seemed to be easier to decontaminate as compared to files of a smaller diameter. The reason for this may be attributed to larger diameter files being sturdier and less susceptible to bending, distortion and damage during cleaning. Neskovic *et al.* (2010), demonstrated that larger diameter endodontic hand files are more resistant to deformation and material fatigue as compared to smaller diameter files. Manual cleaning with bristle brushes may have more easily cleaned a file with a larger diameter as compared to a smaller diameter files.

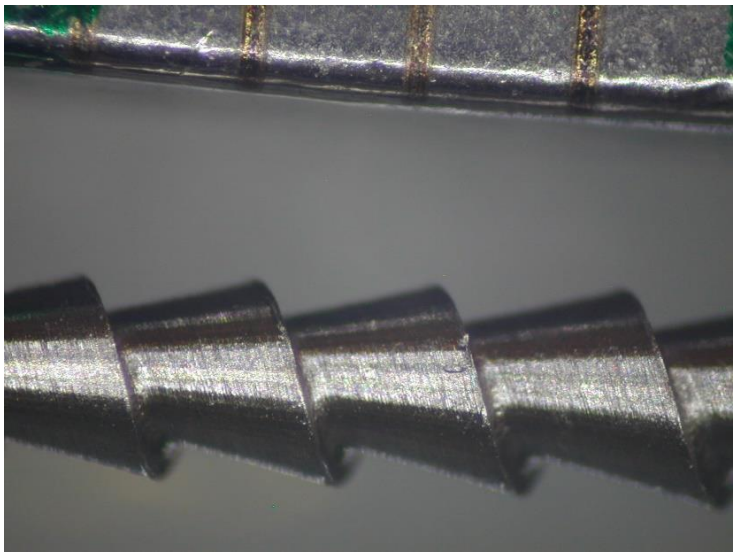


Figure 5.2: A large diameter Hedstroem file (40 x magnification)

Figure 5.3: A small diameter K-file (40 x magnification)

A study by Parashos, Linsuwanont and Messer (2004), which focussed on the decontamination of rotary endodontic files, found 100% cleaning was possible when using the cleaning protocol recommended by these researchers (Addendum C). Rotary endodontic files have a larger overall diameter and a greater surface-area of "radial land" when compared to endodontic hand files which could justify this finding.

Future research could be directed at assessing the efficacy of decontamination on small versus large diameter hand files, and/or rotary files as compared to hand files.

5.7 Examiner disagreement

Cohen's Kappa test demonstrated fair to moderate inter- and intra-examiner agreement over the entire dataset (McHugh, 2012). Analysis of the dataset revealed some samples where there appeared to be a "positional shift" of the scores allocated, by examiners. For example in Figure 5.4, the first examiner scored a sample with debris in position 1 and 3 (red), the second examiner, however, scored the debris in position 2 and 4 (blue)

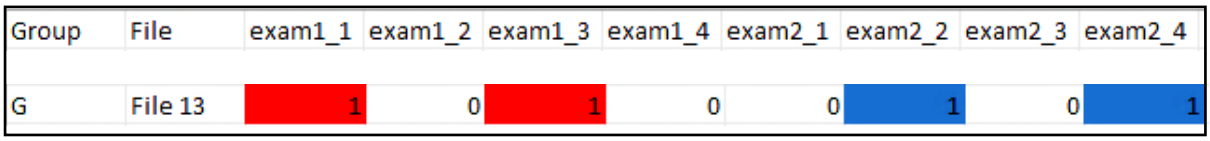


Figure 5.4: Examiner disagreement

Such scoring discrepancies could have been the result of:

- Differences in positioning of the sample by the two independent examiners;
- An inconsistent position of the modified endo ruler used to divide the samples into the four scoring positions;
- Debris overlapping the boundaries of two scoring positions (Figure 5.5).

The potential inconsistencies in the methods of mounting and examining the samples is a weakness of the methodology of the present study. Future studies should aim to circumvent this flaw.

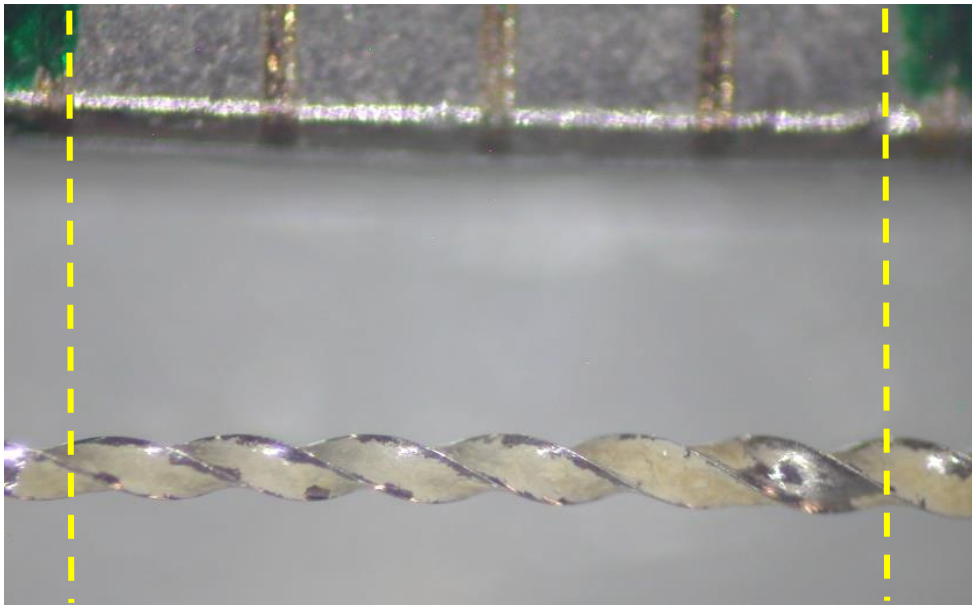


Figure 5.5: Debris on an endodontic hand file crossing three pre-designated sections (borders demarcated by dashed yellow lines) marked on the modified endodontic ruler

5.8 Length of cutting blades of endodontic hand files

The dataset included endodontic hand files from a variety of different manufacturers. The standard active blade of a hand file measured from the tip (D_0) to the end of the cutting blade (D_{16}) must be at least 16mm long (Krell, 2009). Several of the blades of the analysed samples were however longer than 16mm (Figures 5.6 and 5.8).

This phenomenon could be accounted for as follows:

- Variable designs and manufacturing methods of files from different manufacturers (Oliet and Sorin, 1973);
- The unwinding, stretching and distortion of endodontic files during the clinical preparation of root canals (Neskovic *et al.,* 2010).

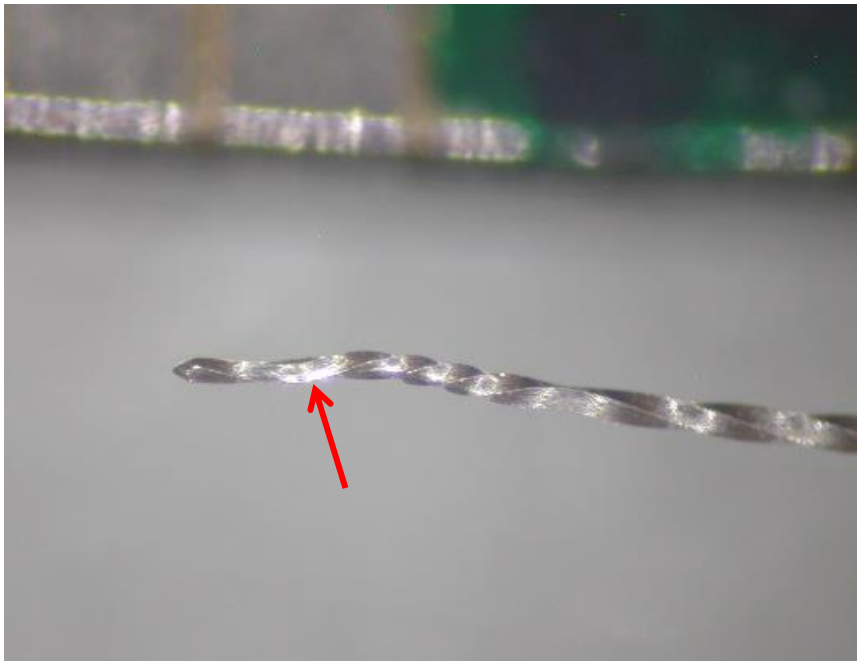


Figure 5.6: The unwound tip of one sample (indicated by the red arrow)

The opposite was also found, several samples displayed a cutting blade measuring less than 16mm due to the separation of the tips of these instruments (Figure 5.7 and 5.8). These samples were excluded from the study.

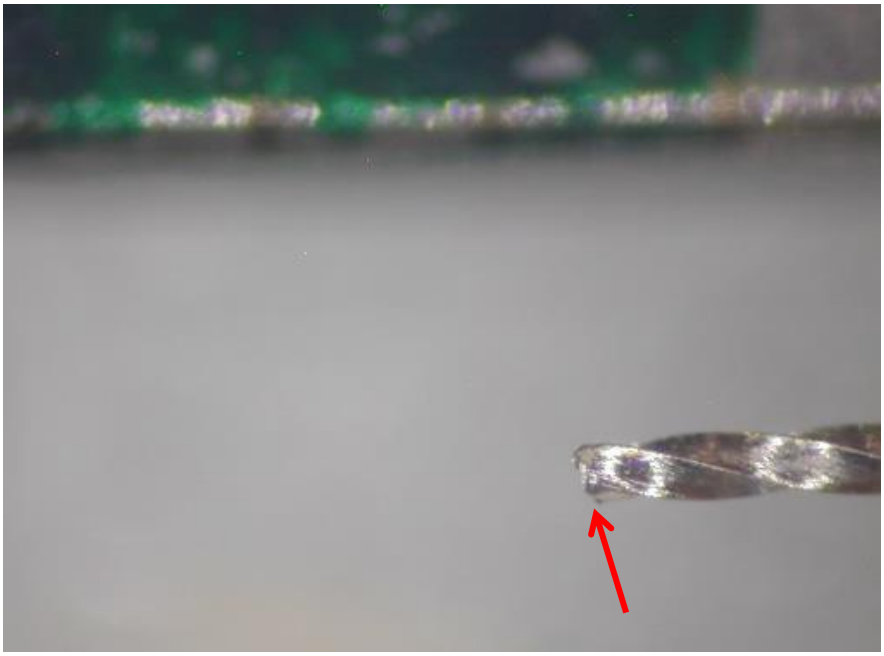


Figure 5.7: The terminal end of a sample where the tip has separated (indicated by the red arrow)

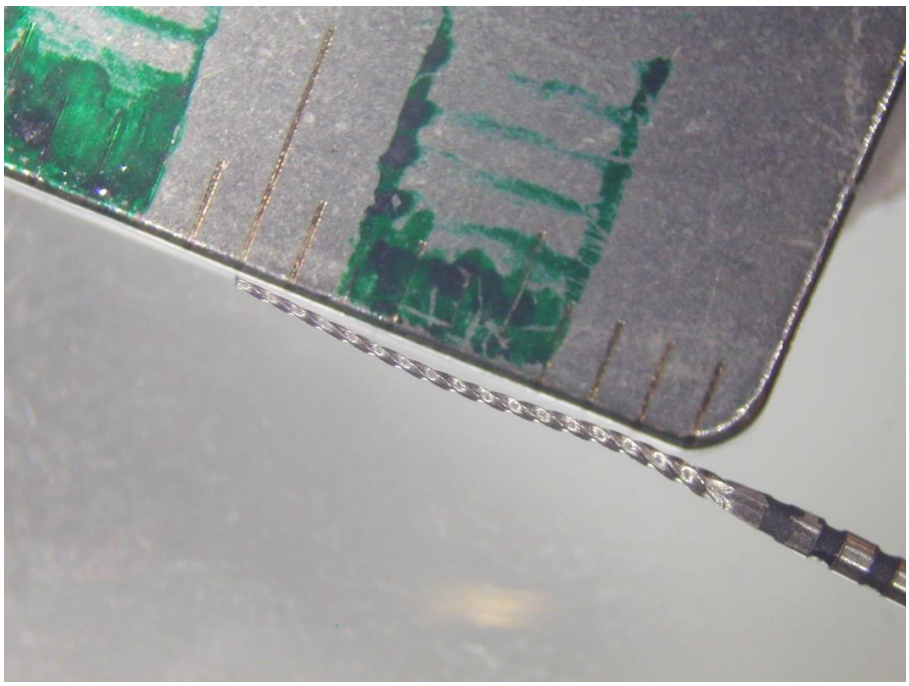


Figure 5.8: A representative sample with the tip of the cutting blade separated, no score was possible in position 1.

The modified endodontic ruler was marked with a green permanent marker to “divide” the cutting length of each sample into four-millimetre-increments, to facilitate the analysis of four sections of equal length.

The inconsistency of the length of the cutting portions of the endodontic hand files created practical implications when analysing the samples in the current study. It was found that longer files were difficult to divide equally into four sections. A decision was taken that should a sample measure longer than 16mm (Figure 5.9), any “extra” length would be included in scoring position 1. Subsequently, in such instances, scoring position 1 represented a larger proportion of the active cutting blade than the other scoring positions of that sample.

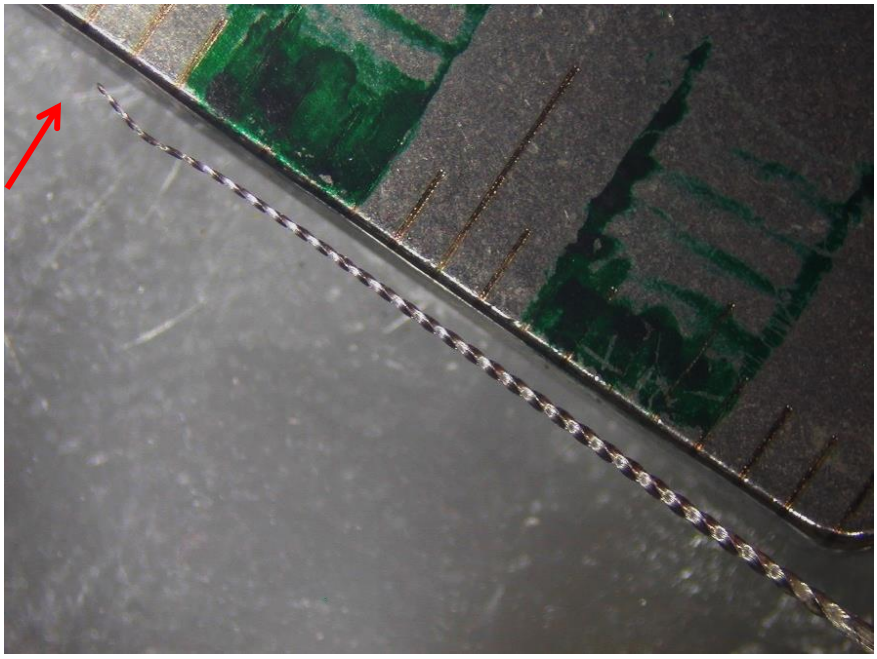


Figure 5.9: A sample measuring 1.5mm longer than expected. The additional length (indicated by the red arrow) was added to scoring position 1

It was noted that the unwound portions of samples displayed a tendency to be cleaner as compared to regular wound sections (Figure 5.10). A possible explanation for this would be that an unwound portion had a simpler geometry than the original file. An unwound file contained more flat surfaces which may have rendered decontamination processes more effective in these areas.

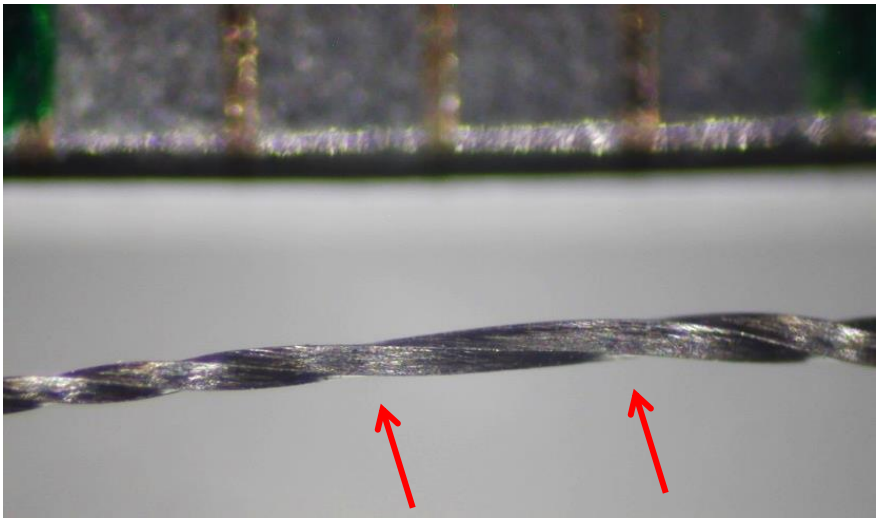


Figure 5.10: Unwound portions (indicated by the red arrows) of one sample, no debris present

5.9 Wear of endodontic instruments

Instrument wear was an incidental finding during the evaluation of the samples in current research project. Wear did make some samples more difficult to analyse. Signs of wear, such as metal tags, corrosion and areas of deformation (Figure 5.11) were sometimes difficult to distinguish from debris.

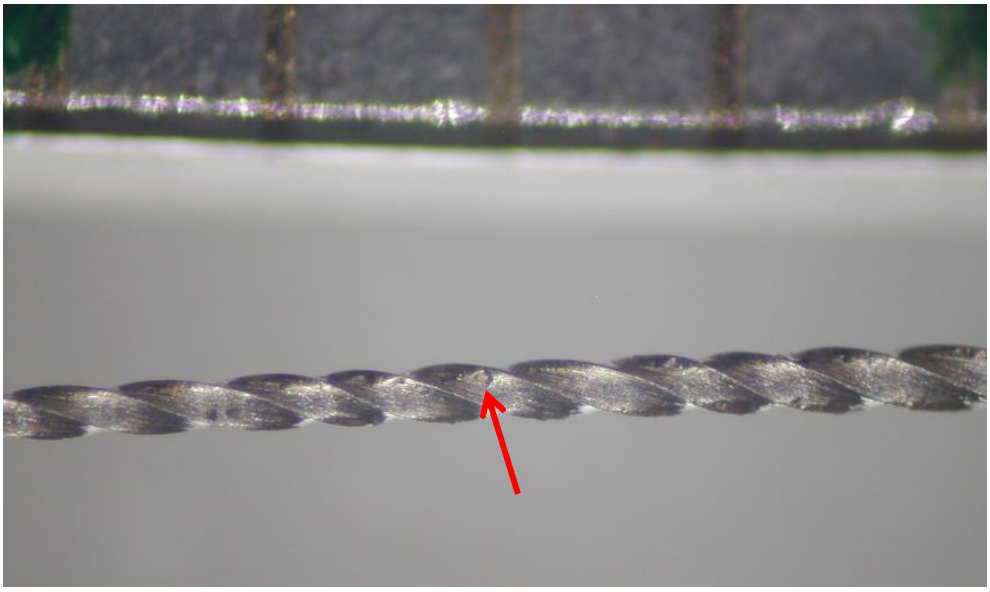


Figure 5.11: A sample with visible wear of the cutting area (indicated by red arrow)

Wear of the samples was not related to their potential to be cleaned of debris. Some well-worn instruments were found to be completely free of debris.

5.10 Debris transfer

Fifteen files were placed into the same collection receptacle, making it possible that some transfer of debris between individual files could have occurred. The rate of transfer would likely be low, as loose debris would be easily removed during the cleaning process. Such transfer, if any, would not have affected the overall results significantly. However, given the methodology used, transfer of even one small piece of debris could have influenced the score assigned to a given sample. This could place a clean sample into the contaminated category and vice versa.

5.11 Debris displacement

Debris could also have become displaced from the samples completely. This may also have changed the score that a sample received. Future studies should aim to eliminate this possibility.

5.12 Quantity of debris

The current study did not aim to assess the quantity of debris as a percentage covering the surface area of a sample (Smith *et al.,* 2002), but rather indicate the presence or absence of debris within predetermined sections of each sample. The quantity of debris on individual samples varied greatly. Some files were heavily contaminated with debris (Figure 5.12) while others were only very lightly contaminated with debris (Figure 5.13). As per the methodology of this study, the degree of contamination did not affect the score that a file received. According to Johnson *et al.* (1997) and Souza *et al.* (2011), the level of debris contamination does not affect the potential to sterilise files. Heavily contaminated files could still be sterilised in these investigations.

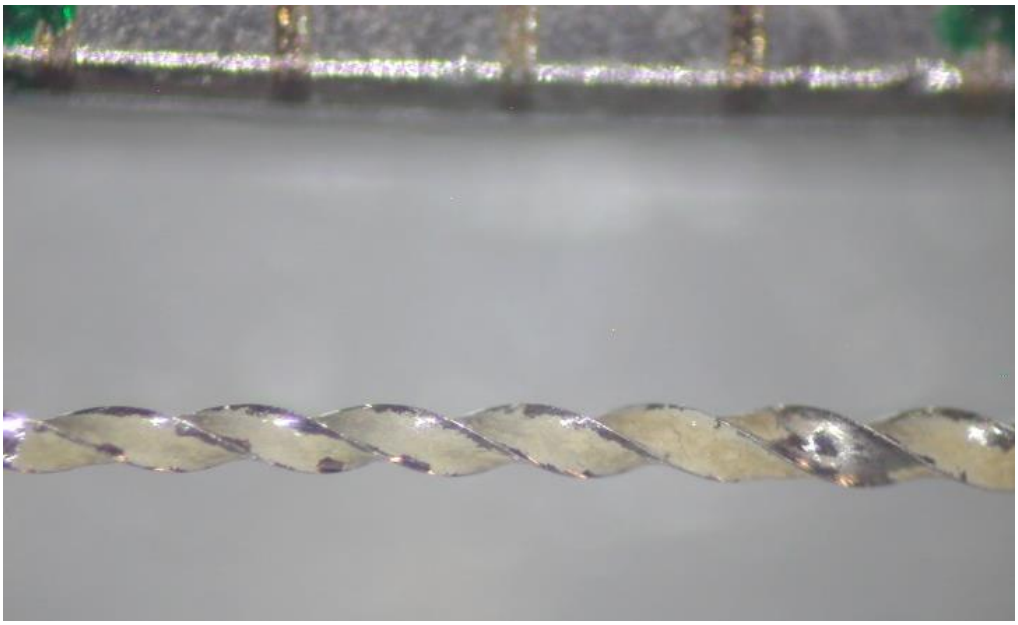


Figure 5.12: A heavily-contaminated sample

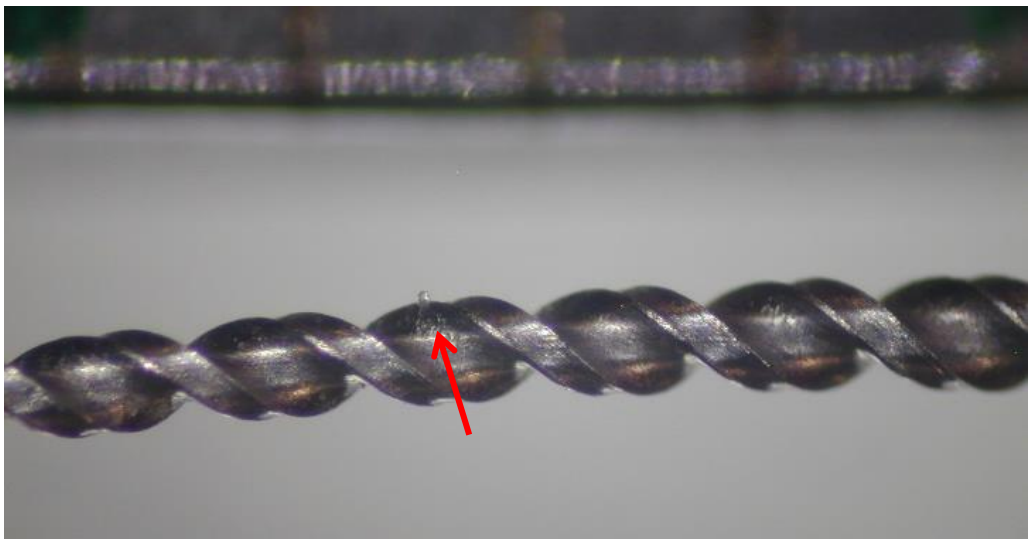


Figure 5.13: A lightly-contaminated sample (red arrow indicating debris)

5.13 Hand versus rotary endodontic files

The current study was designed to assess the level of decontamination of endodontic hand files in a predetermined area. Rotary files were not included in the criteria for this study.

Participants of the study submitted some rotary endodontic files (Figure 5.14), which presented an opportunity to gain limited insight into the current level to which rotary instruments are being decontaminated.

Endodontic hand files were chosen because these instruments have a relatively consistent design, even between different manufacturers. Comparatively, rotary files have varying metals, designs and geometries between individual files of the same brand and series as well as between different manufacturers (Parashos, Gordon and Messer, 2004). Study design intended to assess decontamination of rotary endodontic files would have to take this information into account.

The rotary files that were inadvertently acquired from participants were all found to be contaminated with debris. The sample size was however too small to draw any valid conclusions (Thompson, 1987).

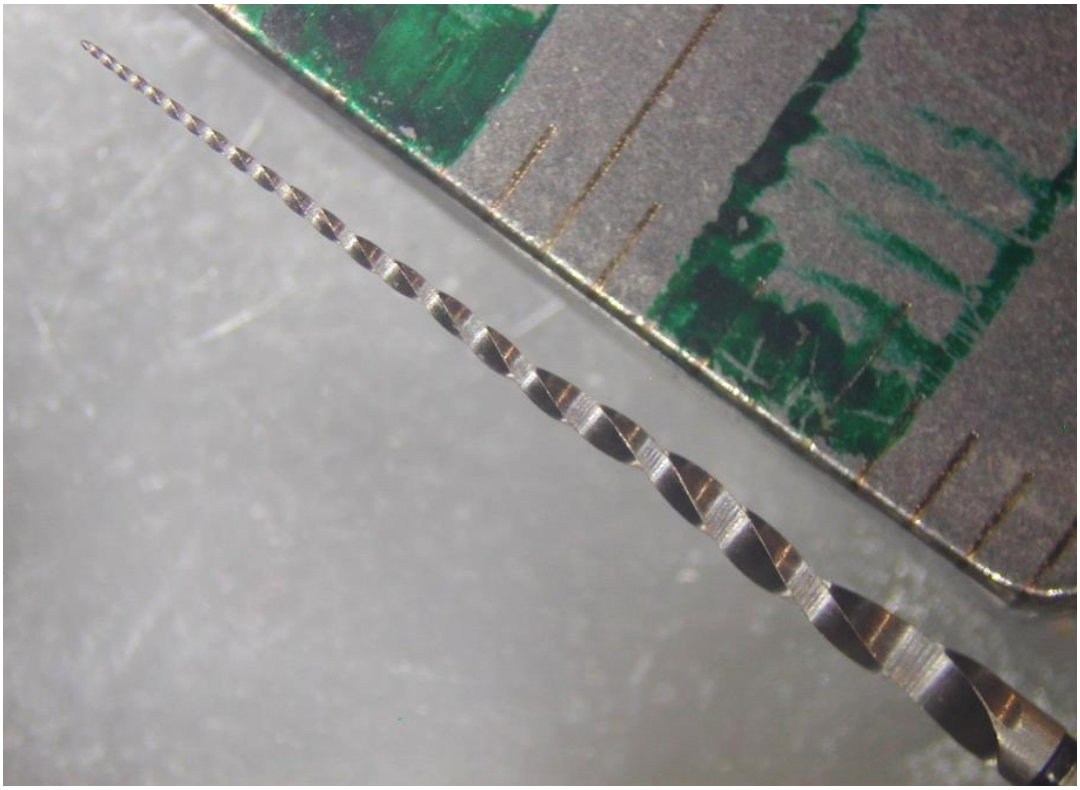


Figure 5.14: A rotary endodontic file submitted by one of the participants

5.14 Modification of the scoring system

The original scoring system (Table 5.1) used by Smith *et al.* (2002), evaluated debris contamination along the length of a file and expressed this information as the percentage of the file that was contaminated.

Table 5.1 The scoring system for measuring debris on endodontic hand files as described by Smith *et al.*, 2002.

Extent of contamination	Score
Debris found on >75% of the instrument length	++++
Debris found on 50-75% of the instrument length	+++
Debris found on 25%-50% of the instrument length	++
Debris found on 1-25% of the instrument length	+
No debris visible at high or low power	0

During the evaluation of visible debris on endodontic instruments, often an individual sample displayed debris present at both position 1 (the quarter at the tip of the file) as well as at position 4 (the quarter closest to the handle of the file). The original scoring system of Smith *et al.* (2002), makes no provision for this situation. A modified scoring system was designed and used in the present study that allowed for a better, more accurate description of the position of any debris observed. This allowed for debris-contamination to be described separately, at the apical and coronal portions of a file, as well as any possible combination of scoring positions.

5.15 Participant bias

Ideally, the samples from the participants of the current study should have been submitted on the day of the interview. This would minimise the possibility of altered participant-behaviour. Several external factors, however, prevented some participants from submitting the required number of samples until sometime after the interview.

It is possible that some participants may have changed the way they re-process endodontic files over the duration of this study, as compared to their usual behaviour. The participants' behaviour may have changed in such a way as to result in either improved or inferior levels of instrument-cleaning than usual. Such possible behaviour changes, if present, could have affected the results of this study. It is however not possible to determine the presence of such biased-behaviour in any of the participants of this study. Furthermore, it is impossible to quantify to what extent such a bias (if present) may have influenced the results of the current study.

5.16 Group comparisons

The results of the present study demonstrate a great variation between groups (i.e. individual dental practices) regarding the amount of residual debris present on endodontic files following routine decontamination procedures. This corresponds with the findings of Bagg *et al.* (2007).

Some of the groups in the current study decontaminated endodontic hand files to a better standard than that of a new file taken directly from the manufacturer's packaging. Whilst this is an encouraging finding, it must be noted that the opposite was also demonstrated in some groups. Although it has been recommended to sterilise new endodontic files prior to their initial use (Morrison and Conrod, 2009), it may be prudent to also decontaminate them to remove residual debris before clinical use.

The present study found all the samples of the calibration group, taken directly from the manufacturer's packaging, to display debris contamination. This is in agreement with previous studies (van Eldik *et al.,* 2004a; Chianello *et al.,* 2008; Hanan *et al.,* 2015) and supports the existing evidence concerning this topic.

5.17 Type and number of uses of endodontic hand files

It was found that stainless steel hand files are the instrument of choice of endodontic hand files of the dentists in private practice in Pretoria that participated in this study. This was an expected finding due to the low cost and predictability of these instruments.

Re-using endodontic hand files significantly reduces the number of instruments that must be purchased in a practice. This reduces the overheads associated with the provision of endodontic treatment and therefore increases the profit margin of providing such treatment. It was found that hand files are re-used for between five and ten cases 85.2% of the time by the participants. This finding is in agreement with Messer, Parashos and Moule (2003) and Parashos, Gordon and Messer (2004).

Most participants (70.37%) in the present study reported the main reason for discarding used endodontic hand files to be file separation, bending, deformation or damage (Figures 5.15 and 5.16). This is in agreement with the findings of Parashos, Gordon and Messer (2004).

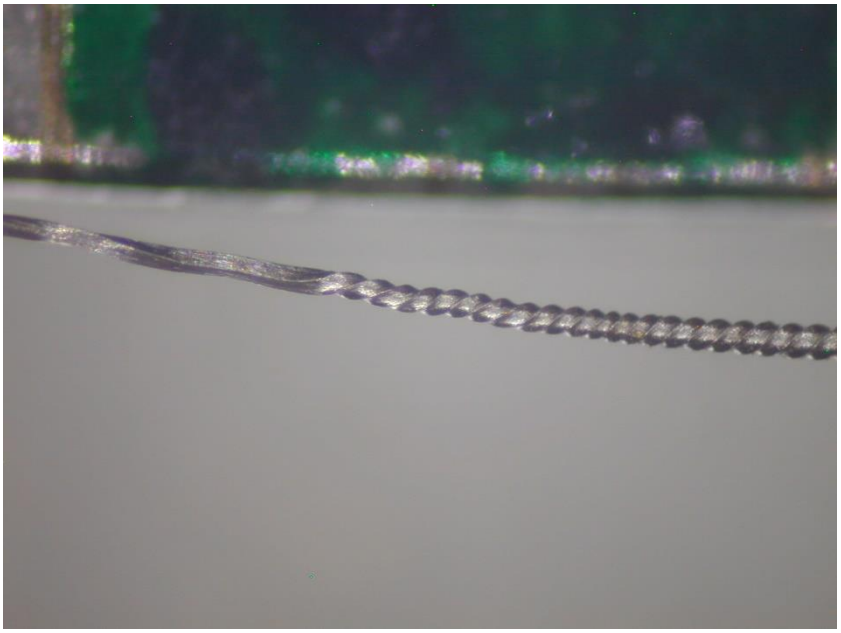


Figure 5.15: A sample displaying deformation and unwinding

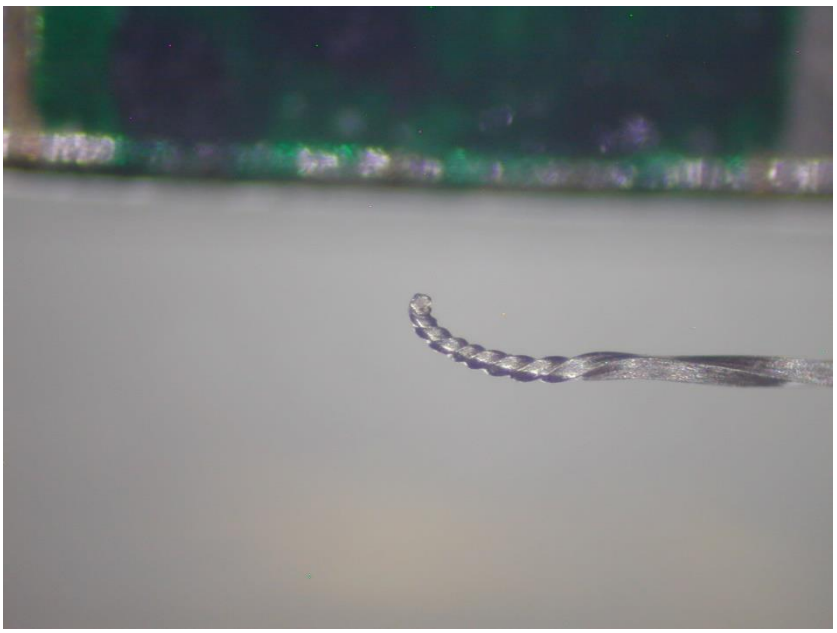


Figure 5.16: A sample displaying deformation and separation of the tip

5.18 Method of decontamination of files

In the present study, manual cleaning alone or in combination with other methods were the most common methods of decontamination employed by participants. This finding corresponds with the findings of Bagg *et al.,* (2007). However, the responses to the

survey in the current study clearly indicate that there is no uniform method or “protocol” used to decontaminate endodontic hand files for private dental practices in Pretoria.

This may indicate a possible lack in knowledge of infection control procedures of dental practitioners and auxiliary dental staff in South Africa (De Kock and van Wyk, 2001; Mehtar *et al.,* 2007; Oosthuysen, Potgieter and Blignaut, 2010; Hartshorne, 2010). Another possibility is that the important task of infection control is often delegated to a member of staff whom may lack the necessary training or experience to carry out such tasks effectively.

It has been suggested that time constraints in busy private practice may contribute to a reduced quality of instrument cleaning (Burkhart and Crawford, 1997).

Regardless of the reasons for a lack of good infection control practices, it is the ethical responsibility of all dentists and auxiliary staff involved in infection control to be up to date with the current prescribed guidelines and methods of cleaning and decontamination (Hartshorne, 2010).

There are currently no guidelines in place in South Africa regarding the best decontamination protocols for endodontic files. One of the objectives of the current study was to address this issue. As 94% of the samples collected from participants in the present study were still contaminated with debris, guidelines are clearly needed.

The results of the present study indicate that the most effective means of cleaning endodontic files was a combination of both manual cleaning and ultrasonification. This is in partial agreement with Parashos, Linsuwanont and Messer (2004), Popovic *et al.,* (2010) and Guandalini *et al.,* (2014). The aforementioned studies recommended the use of manual cleaning and pre-soaking in an enzymatic agent prior to ultrasonification. The cleaning protocol applied in the study by Parashos, Linsuwanont and Messer (2004) (Addendum C) rendered rotary endodontic files 100% clean of biological debris. No information was provided regarding hand files, although the study concluded that this cleaning protocol could be applied to all endodontic files, including endodontic hand files.

Considering the results of the present study together with the lack of additional research on the subject, it is advised that private dental practices, public dental clinics

and academic dental hospitals in South Africa should adopt the above-mentioned cleaning protocol for all endodontic files.

5.19 Quality control and assessment of cleanliness of files

The present study revealed that most endodontic hand files (77.8%) were only subjected to visual inspection for quality control following decontamination. This finding corresponds with the findings of Mehtar *et al.* (2007), who reported 83,3% of dental practitioners believed that dental instruments should be visibly clean prior to re-use. An encouraging finding from the present study was that some practices (7.4%) were using magnification to inspect files for quality control purposes after decontamination. This finding indicates the possibility that the use of magnification to verify the cleanliness of endodontic files may be gaining popularity. The study by Bagg *et al.* (2007), found only one percent of practices evaluated used magnification to inspect instruments after cleaning.

5.20 Single-use of endodontic hand files

None of the participants of this current study have adopted the policy of the single-use of endodontic instruments. One reason for this may be the amount of available research demonstrating that endodontic files can be effectively cleaned of biological debris and sterilised (Messer, Parashos and Moule, 2003; Parashos, Linsuwanont and Messer, 2004; Souza *et al.,* 2011).

One-third of the participants from the current study attributed the trend of multiple-use of endodontic hand files in South Africa to the cost of the instruments. It is a common perception that the single-use of endodontic files will significantly drive up the cost of providing endodontic treatment to patients. Financial reasons deter private dental practitioners, public and academic dental hospitals from implementing the single-use of endodontic files as policy (Messer, Parashos and Moule, 2003).

A small percentage (11.1%) of the participants in the current study stated that good decontamination of previously clinically used endodontic files, was possible. This

finding is confounding considering that all the participants in this study practise the multiple-use and reuse of endodontic files.

Just over a quarter of the participants (25.9%) in this study responded that the single-use of endodontic files would be safer for both the patients and the personnel handling these instruments. This may indicate that the concept of single-use of endodontic files is starting to gain some traction amongst dental practitioners and their staff.

5.21 Consensus

Much disparity exists in the scientific literature as to whether the single-use of endodontic instruments should be adopted or not. Evidence exists both in support of (Letters *et al*., 2005; Walker *et al*., 2009), and against (Messer, Parashos and Moule, 2003; Parashos, Linsuwanont and Messer, 2004), the idea of practising the single-use of endodontic files.

The present study demonstrates that in practice, reliably cleaning debris from endodontic hand files is difficult, which strengthens the existing scientific evidence in the literature that suggests that the single-use of these instruments is preferable.

The perception of increased overhead-costs, combined with the prevailing volatile economic climate in South Africa, dictates that the multiple-use of endodontic files will continue in most dental practices in this country. Given this situation, a cleaning protocol for endodontic files offering the best possible outcome should be implemented by all dental practitioners and institutions that provide endodontic treatment. This recommendation correlates with the conclusions from the study undertaken by Bagg *et al.* (2007). It follows that a recommended “ideal” decontamination protocol for endodontic files should be taught at an undergraduate level to dental students and all students in the auxiliary fields of dentistry.

CHAPTER 6: CONCLUSION

The following conclusions can be drawn from the present study:

- Endodontic hand files examined remained 94% contaminated with debris following routine cleaning and sterilising procedures;
- Endodontic hand files are reprocessed and re-used on multiple clinical cases in private dental practices in Pretoria, South Africa;
- The single-use of endodontic hand files is currently not practised by private dental practices in Pretoria, South Africa;
- The primary reason for the re-use of endodontic files in private dental practices in Pretoria, South Africa, is concerns surrounding the financial implications of introducing single-use protocols;
- There is a large variation in both the methods used as well as the results achieved for the decontamination of endodontic hand files in private dental practice in Pretoria, South Africa;
- Endodontic hand files can be cleaned to a higher standard than files taken directly from the manufacturer's packaging;
- The lack of written guidelines for the best practice for the decontamination and cleaning of endodontic instruments results in inconsistency in the decontamination protocols applied by dental practices in Pretoria, South Africa;
- A need exists for the development and implementation of standard guidelines for the best cleaning and decontamination of endodontic files in South Africa;
- Standard guidelines for the best cleaning and decontamination of endodontic files should be included in the academic training of dentists and dental auxiliaries in tertiary training institutions of South Africa.

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Addendum A

UNIVERSITEIT VAN PRETORIA
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CONSENT TO PARTICIPATE IN RESEARCH

TITLE: Decontamination and Use of Endodontic Hand Files in Dental Practice in Pretoria.

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STATEMENT OF RESEARCH

A person who is to participate in research must give his or her informed consent to such participation. This consent must be based on an understanding of the nature and risks of the research. This document provides information that is important for this understanding. Research projects only include subjects who choose to take part. Please take your time in making your decision as to whether to participate or not. If you have questions at any time, please ask.

WHAT IS THE PURPOSE OF THIS STUDY?

You are invited to be part of a research study about the use and decontamination/cleaning procedures currently in place in dental practice in Pretoria, South Africa, with specific regard to endodontic hand files because you are a general dental practitioner in private practice in Pretoria, South Africa.

The purpose of this research study is to assess which decontamination procedures are currently in place with regards to endodontic hand files as well as how well endodontic hand files are cleaned after clinical use, to treat patients. The single or multiple use of these instruments will also be observed. The hypothesis is that a significant percentage of endodontic files will still bear biological debris after routine cleaning and decontamination procedures and that endodontic files will be re-used for multiple cases. This will help us to determine whether current methods of cleaning/decontamination are efficient in removing

biological debris from endodontic files, or not; and if endodontic hand files are being re-used or not.

HOW MANY PEOPLE WILL PARTICIPATE?

One or two staff members from 27 participating dental practices in Pretoria will take part in this study.

HOW LONG WILL I BE INVOLVED IN THIS STUDY?

Your participation in this study will take approximately 15 minutes. You will only need to make yourself available to once to complete this survey.

WHAT WILL HAPPEN DURING THIS STUDY?

A short questionnaire will be completed by the participant to determine:

- the decontamination (part of cross infection) procedures of endodontic hand files used in the dental practice;
- the type of protocols (single of multiple use) followed in the practice;
- the methods of decontamination used to process endodontic hand files for re-use (if re-used) in the practice.

After this, the participant, if files are re-used in the dental practice, will be required to provide the researcher with 15 endodontic hand files of any size and length, each of which has been:

- used to treat at least one root canal on one patient;
- cleaned and/or decontaminated via the routine processes followed by the dental practice;
- then placed directly into the collection bottle provided.

These endodontic files will be examined by researchers in a scientific laboratory to assess the amount of debris remaining on the files. A statistician will compare the collected data to data from the following sources:

- the same type and number of endodontic files from 26 other dental practices.

WHAT ARE THE RISKS OF THE STUDY?

There are no known or foreseeable risks to participating in this study. The identities of participants and participating practices will remain anonymous.

WHAT ARE THE BENEFITS OF THIS STUDY?

You will not personally benefit from taking part in this study. The dental profession and the South African public will benefit from this study by gaining knowledge pertaining to the usage protocols in place and the current effectiveness of cleaning/decontamination of endodontic

files and can lead to the development of improved methods and protocols for infection control and cross contamination in dental practice in South Africa.

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

No cost will be incurred by the participants in this research study.

WHO IS FUNDING THIS STUDY?

The University of Pretoria and the research team are receiving no payments from other agencies, organisations, or companies to conduct this research study. The research is being funded by Dr. Glynn Buchanan in his personal capacity, some funding will be obtained from the funds in the University of Pretoria research fund account of Dr. Nichola Warren. An application will be made for a fellowship (Cornelis H. Pameijer) to obtain additional funding.

IS THIS STUDY VOLUNTARY?

Your participation in this research study is voluntary. You may choose not to participate without penalty, prejudice or loss of benefits to which you may otherwise be entitled. Your decision to participate in this research or not will not affect your current or future relations with the University of Pretoria or any other role players in this research study.

CONTACTS AND QUESTIONS?

The researchers conducting this study are Dr. Glynn Dale Buchanan and Dr. Nichola Warren. You may ask any questions you have now. If at a later time, you have any questions, concerns, or inquiries, concerning this research, please contact one of the researchers via email:
Dr. Buchanan at glynn.buchanan@up.ac.za or Dr. Warren at nichola.warren@up.ac.za

You will receive a copy of this form.

I, _____hereby confirm that this research study has been explained to me, any questions I have asked have been answered to my satisfaction, and I agree to take part in this study.

_____ _____

Signature Date

I, _____hereby confirm that I have discussed the above points with the participant before he/she commences in the completion of the aforementioned questionnaire.

_____ _____

Signature Date

Addendum B

Questionnaire

Instructions to the participant:

- Please answer the following questions honestly, your answers will be kept anonymous.
- Please circle the letter following the option that best describes the answer to each
- question (only encircle one answer please).

1. What type/s of endodontic hand files are used in this dental practice?

A. Stainless steel files only (K or Hedstroem files).
B. NiTi files only.
C. Both stainless steel and NiTi files.
D. Other: please specify: _____

2. How many separate endodontic cases (on average) will an operator treat with an endodontic hand file before discarding the file, in this practice?

A. Only once: single-use, then it is discarded.
B. One to five cases.
C. Five to ten cases.
D. More than ten cases.
E. Other: Please specify: _____

3. When will an endodontic hand file be discarded in this practice?

A. After it has been used on one patient (single-use).
B. When the file is damaged (separated, bent, deformed, damaged).
C. After a pre-determined number of uses as described in question 2.
D. Other: Please specify: _____

Instructions to the participant:

If the answer to Question 3 is A, you do not have to complete the rest of this survey.
Thank you very much for your participation.
If the answer to Question 3 is B, C or D, please complete the rest of this survey.

4. Which decontamination methods for endodontic files is this practice using?

A. Manual cleaning with brushes/ detergent only.
B. Manual cleaning with a gauze or sponge soaked in alcohol.
C. Ultrasonification only.
D. Both manual cleaning and ultrasonification.
E. Washer disinfector.
F. Other: Please specify: _____

5. Which methods, if any, are used to assess the cleanliness of endodontic files following routinely applied decontamination and sterilisation procedures in this practice?

A. Inspection using magnification (e.g. Loupes or operating microscope)
B. Visual inspection only (naked eye)
C. None
D. Other: Please specify: _____

6i. Have you ever considered routinely only using endodontic hand files once and then discarding them? (i.e. single-use of files on one patient only).

A. Yes
B. No

6ii. If No, please indicate the reason/s for choosing to use the same endodontic files on more than one patient?

A. Single-use of files is too expensive; there would be little profit to endodontic treatment if single-use protocols were in place.
B. It is not necessary to discard an endodontic file after one use as the files are still in working condition.
C. It is possible to decontaminate endodontic files between patients and re-use them.
D. Other: Please specify: _____

6iii. If Yes, please indicate the reason/s you would choose to use endodontic files on one patient only and then discard them?

A. Single-use of files would be safer for my patients.
B. Single-use of files would be safer for the staff working with these instruments.
C. Single-use would lead to better success of my endodontic treatments.
D. It is not possible to decontaminate endodontic files between patients.
E. Other: Please specify: _____

ADDENDUM C

Table: Recommended protocol for cleaning endodontic files

Step	Method
1	Ten vigorous strokes in a scouring sponge (pre-soaked in 0.2% chlorhexidine)
2	Pre-soaking for 30min in an enzymatic cleaning solution
3	Ultrasonication for 15min in an enzymatic cleaning solution
4	Rinsing for 20sec under running tap water

ADDENDUM D

Group A

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
A	File 1	1	0	0	1	1	1	0	1
A	File 2	0	1	1	1	0	0	1	1
A	File 3	1	1	1	1	1	1	1	1
A	File 4	1	1	0	1	1	1	1	1
A	File 5	1	1	1	1	1	1	1	1
A	File 6	1	1	1	1	1	1	1	1
A	File 7	1	1	1	1	1	1	1	1
A	File 8	0	1	0	1	1	1	1	1
A	File 9	1	0	1	0	0	1	1	0
A	File 10	0	0	0	1	0	0	0	1
A	File 11	1	1	0	1	1	1	1	1
A	File 12	1	1	1	1	0	0	1	1
A	File 13	1	1	0	1	0	1	0	1
A	File 14	1	1	1	1	0	0	1	1
A	File 15	1	0	1	0	0	0	1	1
A	File 16	0	0	1	1	0	0	1	1

Group	File	combined1	combined2	combined3	combined4
A	File 1	1	1	0	1
A	File 2	0	1	1	1
A	File 3	1	1	1	1
A	File 4	1	1	1	1
A	File 5	1	1	1	1
A	File 6	1	1	1	1
A	File 7	1	1	1	1
A	File 8	1	1	1	1
A	File 9	1	1	1	0
A	File 10	0	0	0	1
A	File11	1	1	1	1
A	File 12	1	1	1	1
A	File 13	1	1	0	1
A	File 14	1	1	1	1
A	File 15	1	0	1	1
A	File 16	0	0	1	1

Group AA

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
AA	File 1	1	1	1	1	1	1	1	1
AA	File 2	1	0	1	1	1	1	1	0
AA	File 3	0	0	0	0	0	0	1	1
AA	File 4	1	1	0	0	1	0	0	0
AA	File 5	1	0	0	0	1	1	1	0
AA	File 6	1	1	1	1	1	1	1	1
AA	File 7	1	1	0	0	0	1	1	0
AA	File 8	0	1	1	0	0	0	1	1
AA	File 9	0	1	0	0	0	0	1	1
AA	File 10	0	1	0	1	1	1	0	0
AA	File 11	0	1	0	1	0	0	0	0
AA	File 12	1	1	1	1	1	1	0	1
AA	File 13	1	0	1	0	1	1	1	1
AA	File 14	1	1	1	1	0	1	1	1

Group	File	combined1	combined2	combined3	combined4
AA	File 1	1	1	1	1
AA	File 2	1	1	1	1
AA	File 3	0	0	1	1
AA	File 4	1	1	0	0
AA	File 5	1	1	1	0
AA	File 6	1	1	1	1
AA	File 7	1	1	1	0
AA	File 8	0	1	1	1
AA	File 9	0	1	1	1
AA	File 10	1	1	0	1
AA	File 11	0	1	0	1
AA	File 12	1	1	1	1
AA	File 13	1	1	1	1
AA	File 14	1	1	1	1

Group AB

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
AB	File 1	1	0	0	0	0	1	1	1
AB	File 2	0	0	1	0	1	0	0	0
AB	File 3	0	1	1	1	1	1	1	1
AB	File 4	1	0	1	1	0	1	1	0
AB	File 5	1	0	1	1	0	1	1	1
AB	File 6	1	0	1	1	0	0	1	1
AB	File 7	1	1	1	1	1	1	1	1
AB	File 8	0	1	0	1	0	0	1	1

Group	File	combined1	combined2	combined3	combined4
AB	File 1	1	1	1	1
AB	File 2	1	0	1	0
AB	File 3	1	1	1	1
AB	File 4	1	1	1	1
AB	File 5	1	1	1	1
AB	File 6	1	0	1	1
AB	File 7	1	1	1	1
AB	File 8	0	1	1	1

Group B

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
B	File 1	0	0	0	0	0	0	0	0
B	File 2	0	0	0	0	0	0	0	0
B	File 3	0	0	0	0	1	0	0	0
B	File 4	0	0	0	0	0	0	0	0
B	File 5	0	0	0	0	0	0	0	0
B	File 6	0	0	0	0	0	0	0	0
B	File 7	0	0	0	0	0	0	0	0
B	File 8	0	0	0	0	0	0	1	0
B	File 9	0	0	0	0	0	0	0	0
B	File 10	0	0	0	0	0	0	1	0
B	File11	0	0	0	0	0	0	0	0
B	File 12	0	1	0	0	0	0	0	0
B	File 13	0	0	0	0	0	0	0	0
B	File 14	0	0	0	0	0	0	0	0
B	File 15	0	0	0	0	0	0	0	0

Group	File	combined1	combined2	combined3	combined4
B	File 1	0	0	0	0
B	File 2	0	0	0	0
B	File 3	1	0	0	0
B	File 4	0	0	0	0
B	File 5	0	0	0	0
B	File 6	0	0	0	0
B	File 7	0	0	0	0
B	File 8	0	0	1	0
B	File 9	0	0	0	0
B	File 10	0	0	1	0
B	File11	0	0	0	0
B	File 12	0	1	0	0
B	File 13	0	0	0	0
B	File 14	0	0	0	0
B	File 15	0	0	0	0

Group C

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
C	File 1	1	1	0	1	0	0	0	0
C	File 2	0	1	1	0	0	1	0	0
C	File 3	1	0	1	1	1	1	0	1
C	File 4	1	1	1	1	1	1	1	1
C	File 5	1	1	1	1	1	1	1	1
C	File 6	1	1	1	1	0	0	1	1
C	File 7	1	1	1	1	0	1	1	0
C	File 8	1	0	0	0	0	0	1	1
C	File 9	1	1	1	1	1	1	1	1
C	File 10	1	0	1	0	1	0	1	0
C	File 11	1	0	1	1	1	1	0	0
C	File 12	1	1	1	0	1	1	1	0
C	File 13	0	0	1	1	0	0	1	1
C	File 14	1	0	1	1	1	0	1	1
C	File 15	0	0	1	1	0	0	1	1

Group	File	combined1	combined2	combined3	combined4
C	File 1	1	1	0	1
C	File 2	0	1	1	0
C	File 3	1	1	1	1
C	File 4	1	1	1	1
C	File 5	1	1	1	1
C	File 6	1	1	1	1
C	File 7	1	1	1	1
C	File 8	1	0	1	1
C	File 9	1	1	1	1
C	File 10	1	0	1	0
C	File 11	1	1	1	1
C	File 12	1	1	1	0
C	File 13	0	0	1	1
C	File 14	1	0	1	1
C	File 15	0	0	1	1

Group D

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
D	File 1	0	1	1	0	0	0	1	1
D	File 2	0	0	1	1	1	0	0	1
D	File 3	0	1	0	1	0	0	1	0
D	File 4	0	1	0	0	0	0	1	0
D	File 5	1	1	1	1	1	1	1	1
D	File 6	0	0	0	1	0	1	1	1
D	File 7	1	1	1	1	1	1	1	1
D	File 8	1	0	1	0	0	0	1	1
D	File 9	0	0	1	1	0	0	1	1
D	File 10	0	0	1	1	0	0	1	1
D	File11	1	1	0	0	1	1	1	1
D	File 12	1	1	1	1	1	1	1	1
D	File 13	1	1	0	1	1	1	1	1
D	File 14	1	1	1	1	1	1	0	1
D	File 15	1	1	1	0	1	1	1	0

Group	File	combined1	combined2	combined3	combined4
D	File 1	0	1	1	1
D	File 2	1	0	1	1
D	File 3	0	1	1	1
D	File 4	0	1	1	0
D	File 5	1	1	1	1
D	File 6	0	1	1	1
D	File 7	1	1	1	1
D	File 8	1	0	1	1
D	File 9	0	0	1	1
D	File 10	0	0	1	1
D	File11	1	1	1	1
D	File 12	1	1	1	1
D	File 13	1	1	1	1
D	File 14	1	1	1	1
D	File 15	1	1	1	0

Group E

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
E	File 1	0	0	1	1	1	0	1	1
E	File 2	1	0	1	0	1	1	1	0
E	File 3	1	0	1	0	0	0	1	1
E	File 4	0	1	1	1	1	1	1	0
E	File 5	1	1	0	1	1	0	1	0
E	File 6	0	0	0	1	0	0	1	0
E	File 7	0	0	0	0	0	0	0	1
E	File 8	1	0	1	1	1	0	1	1
E	File 9	0	0	0	0	0	0	1	0
E	File 10	0	0	1	1	1	1	1	1
E	File 11	0	1	1	1	1	1	1	1
E	File 12	1	1	0	1	1	1	0	1
E	File 13	0	1	1	0	1	1	1	1
E	File 14	1	1	1	0	1	1	1	1
E	File 15	0	0	0	1	1	1	0	1
E	File 16	0	0	1	1	1	1	1	1
E	File 17	0	0	0	1	1	0	0	1
E	File 18	0	0	0	1	1	0	1	1

Group	File	combined1	combined2	combined3	combined4
E	File 1	1	0	1	1
E	File 2	1	1	1	0
E	File 3	1	0	1	1
E	File 4	1	1	1	1
E	File 5	1	1	1	1
E	File 6	0	0	1	1
E	File 7	0	0	0	1
E	File 8	1	0	1	1
E	File 9	0	0	1	0
E	File 10	1	1	1	1
E	File 11	1	1	1	1
E	File 12	1	1	0	1
E	File 13	1	1	1	1
E	File 14	1	1	1	1
E	File 15	1	1	0	1
E	File 16	1	1	1	1
E	File 17	1	0	0	1
E	File 18	1	0	1	1

Group F

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
F	File 1	1	1	1	1	1	1	1	1
F	File 2	1	1	1	1	1	1	1	1
F	File 3	0	1	1	1	1	1	1	1
F	File 4	1	1	1	1	1	1	1	1
F	File 5	1	1	1	1	1	1	1	1
F	File 6	1	1	1	1	1	1	1	1
F	File 7	1	0	1	0	1	1	1	1
F	File 8	1	1	1	1	1	1	1	1
F	File 9	1	1	1	1	1	1	1	1
F	File 10	1	1	1	1	1	1	1	1
F	File11	0	1	1	1	1	1	1	1
F	File 12	0	1	1	1	1	1	1	1
F	File 13	1	1	1	0	1	1	1	1
F	File 14	1	1	1	1	1	1	1	1

Group	File	combined1	combined2	combined3	combined4
F	File 1	1	1	1	1
F	File 2	1	1	1	1
F	File 3	1	1	1	1
F	File 4	1	1	1	1
F	File 5	1	1	1	1
F	File 6	1	1	1	1
F	File 7	1	1	1	1
F	File 8	1	1	1	1
F	File 9	1	1	1	1
F	File 10	1	1	1	1
F	File11	1	1	1	1
F	File 12	1	1	1	1
F	File 13	1	1	1	1
F	File 14	1	1	1	1

Group G

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
G	File 1	0	1	1	1	1	0	1	0
G	File 2	0	0	1	1	1	0	0	1
G	File 3	1	0	1	0	1	1	1	0
G	File 4	1	1	1	1	1	1	1	1
G	File 5	0	1	1	1	0	0	1	1
G	File 6	1	0	0	0	1	1	0	0
G	File 7	1	1	1	1	0	0	0	1
G	File 8	0	0	0	0	0	1	0	1
G	File 9	1	1	1	1	0	1	1	1
G	File 10	1	1	0	1	1	1	1	1
G	File 11	1	1	0	1	1	0	1	1
G	File 12	0	1	0	0	1	0	0	1
G	File 13	1	0	1	0	0	1	0	1
G	File 14	1	0	1	0	1	0	1	0
G	File 15	0	0	1	1	0	0	1	1
G	File 16	0	0	1	1	0	0	1	1

Group	File	combined1	combined2	combined3	combined4
G	File 1	1	1	1	1
G	File 2	1	0	1	1
G	File 3	1	1	1	0
G	File 4	1	1	1	1
G	File 5	0	1	1	1
G	File 6	1	1	0	0
G	File 7	1	1	1	1
G	File 8	0	1	0	1
G	File 9	1	1	1	1
G	File 10	1	1	1	1
G	File 11	1	1	1	1
G	File 12	1	1	0	1
G	File 13	1	1	1	1
G	File 14	1	0	1	0
G	File 15	0	0	1	1
G	File 16	0	0	1	1

Group H

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
H	File 1	1	1	1	1	1	1	1	1
H	File 2	1	1	0	0	1	1	1	1
H	File 3	0	1	1	1	1	1	1	1
H	File 4	1	1	1	1	1	1	1	1
H	File 5	0	0	0	0	1	1	1	1
H	File 6	1	0	0	1	1	1	1	1
H	File 7	1	1	1	1	1	1	1	1
H	File 8	1	1	1	1	1	1	1	1
H	File 9	0	1	1	1	1	1	1	1
H	File 10	1	1	1	1	1	1	1	1
H	File 11	0	1	1	1	0	1	1	1
H	File 12	1	0	1	1	1	1	1	1
H	File 13	1	0	0	1	1	1	1	1
H	File 14	1	1	1	1	1	1	1	1
H	File 15	1	1	1	1	0	1	1	1

Group	File	combined1	combined2	combined3	combined4
H	File 1	1	1	1	1
H	File 2	1	1	1	1
H	File 3	1	1	1	1
H	File 4	1	1	1	1
H	File 5	1	1	1	1
H	File 6	1	1	1	1
H	File 7	1	1	1	1
H	File 8	1	1	1	1
H	File 9	1	1	1	1
H	File 10	1	1	1	1
H	File 11	0	1	1	1
H	File 12	1	1	1	1
H	File 13	1	1	1	1
H	File 14	1	1	1	1
H	File 15	1	1	1	1

Group I

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
I	File 1	0	0	1	1	1	1	1	1
I	File 2	0	0	0	0	0	1	1	0
I	File 3	0	0	1	1	0	1	1	1
I	File 4	0	0	0	1	1	1	1	1
I	File 5	0	1	0	0	0	0	1	1
I	File 6	0	1	1	1	1	1	1	1
I	File 7	0	1	0	1	1	1	1	1
I	File 8	1	0	1	1	1	1	1	1
I	File 9	0	1	1	1	1	1	1	1
I	File 10	1	0	0	1	1	1	1	1
I	File 11	1	0	1	1	1	1	1	1
I	File 12	0	0	0	0	0	0	0	1
I	File 13	0	0	1	1	1	1	1	1
I	File 14	0	1	1	1	0	0	1	1
I	File 15	0	0	0	0	0	1	1	0

Group	File	combined1	combined2	combined3	combined4
I	File 1	1	1	1	1
I	File 2	0	1	1	0
I	File 3	0	1	1	1
I	File 4	1	1	1	1
I	File 5	0	1	1	1
I	File 6	1	1	1	1
I	File 7	1	1	1	1
I	File 8	1	1	1	1
I	File 9	1	1	1	1
I	File 10	1	1	1	1
I	File 11	1	1	1	1
I	File 12	0	0	0	1
I	File 13	1	1	1	1
I	File 14	0	1	1	1
I	File 15	0	1	1	0

Group J

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
J	File 1	1	1	1	1	1	1	1	1
J	File 2	1	1	1	1	1	1	1	1
J	File 3	1	1	0	1	1	1	1	1
J	File 4	1	1	1	1	1	1	1	1
J	File 5	1	1	1	1	1	1	1	1
J	File 6	1	1	1	1	1	1	1	1
J	File 7	1	1	1	1	1	1	1	1
J	File 8	1	1	1	1	1	1	1	1
J	File 9	1	1	1	1	1	1	1	1
J	File 10	1	1	1	1	0	1	1	1
J	File 11	1	1	1	1	1	1	1	1
J	File 12	1	0	1	1	1	1	1	1
J	File 13	1	1	1	1	1	1	1	1
J	File 14	1	1	1	1	1	1	1	1
J	File 15	1	0	1	0	1	1	1	1

Group	File	combined1	combined2	combined3	combined4
J	File 1	1	1	1	1
J	File 2	1	1	1	1
J	File 3	1	1	1	1
J	File 4	1	1	1	1
J	File 5	1	1	1	1
J	File 6	1	1	1	1
J	File 7	1	1	1	1
J	File 8	1	1	1	1
J	File 9	1	1	1	1
J	File 10	1	1	1	1
J	File 11	1	1	1	1
J	File 12	1	1	1	1
J	File 13	1	1	1	1
J	File 14	1	1	1	1
J	File 15	1	1	1	1

Group K

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
K	File 1	0	1	1	1	0	1	1	1
K	File 2	0	0	1	0	0	0	1	1
K	File 3	1	0	0	0	0	1	0	0
K	File 4	1	1	1	0	1	1	1	0
K	File 5	1	1	1	0	1	1	1	1
K	File 6	0	1	1	1	0	0	1	1
K	File 7	1	1	1	1	1	1	1	0
K	File 8	1	1	0	0	0	1	1	0
K	File 9	1	1	1	0	0	1	1	0
K	File 10	1	1	1	0	1	1	1	1
K	File 11	0	0	0	0	1	0	0	0
K	File 12	1	0	1	0	1	0	0	1
K	File 13	1	0	0	0	0	1	0	0
K	File 14	1	1	1	0	0	1	1	0
K	File 15	1	1	0	1	0	1	0	0

Group	File	combined1	combined2	combined3	combined4
K	File 1	0	1	1	1
K	File 2	0	0	1	1
K	File 3	1	1	0	0
K	File 4	1	1	1	0
K	File 5	1	1	1	1
K	File 6	0	1	1	1
K	File 7	1	1	1	1
K	File 8	1	1	1	0
K	File 9	1	1	1	0
K	File 10	1	1	1	1
K	File 11	1	0	0	0
K	File 12	1	0	1	1
K	File 13	1	1	0	0
K	File 14	1	1	1	0
K	File 15	1	1	0	1

Group L

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
L	File 1	0	0	0	0	0	0	1	1
L	File 2	0	0	0	0	0	1	0	1
L	File 3	0	1	1	1	1	1	1	1
L	File 4	1	0	0	0	0	1	0	1
L	File 5	0	0	0	0	0	0	0	0
L	File 6	0	0	0	1	0	0	0	1
L	File 7	1	0	1	0	1	0	1	0
L	File 8	0	1	0	1	0	0	0	1
L	File 9	0	0	0	0	0	0	1	1
L	File 10	0	0	0	0	0	0	0	1
L	File 11	0	0	1	1	1	1	1	1
L	File 12	0	0	0	0	0	1	1	1
L	File 13	0	0	0	1	1	1	0	1
L	File 14	0	0	0	1	1	0	0	1
L	File 15	0	0	0	1	0	1	1	1
L	File 16	0	1	1	1	0	0	1	1

Group	File	combined1	combined2	combined3	combined4
L	File 1	0	0	1	1
L	File 2	0	1	0	1
L	File 3	1	1	1	1
L	File 4	1	1	0	1
L	File 5	0	0	0	0
L	File 6	0	0	0	1
L	File 7	1	0	1	0
L	File 8	0	1	0	1
L	File 9	0	0	1	1
L	File 10	0	0	0	1
L	File 11	1	1	1	1
L	File 12	0	1	1	1
L	File 13	1	1	0	1
L	File 14	1	0	0	1
L	File 15	0	1	1	1
L	File 16	0	1	1	1

Group M

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
M	File 1	1	1	1	1	1	1	1	1
M	File 2	1	0	1	0	1	1	1	0
M	File 3	0	0	0	0	0	0	1	0
M	File 4	1	1	1	1	1	1	1	1
M	File 5	0	0	1	1	0	0	0	1
M	File 6	1	1	1	1	1	1	1	1
M	File 7	1	1	1	1	1	1	1	1
M	File 8	1	1	1	1	1	1	1	1
M	File 9	0	0	0	1	0	0	1	1
M	File 10	1	1	1	1	1	1	1	1
M	File 11	0	0	1	0	1	1	1	1
M	File 12	1	1	1	1	1	1	1	1
M	File 13	1	1	1	1	0	1	1	1
M	File 14	1	1	0	0	1	1	1	0
M	File 15	0	0	0	0	0	0	0	0
M	File 16	0	1	0	1	0	1	0	1
M	File 17	1	1	1	1	0	1	1	1
M	File 18	1	0	1	1	1	1	1	1

Group	File	combined1	combined2	combined3	combined4
M	File 1	1	1	1	1
M	File 2	1	1	1	0
M	File 3	0	0	1	0
M	File 4	1	1	1	1
M	File 5	0	0	1	1
M	File 6	1	1	1	1
M	File 7	1	1	1	1
M	File 8	1	1	1	1
M	File 9	0	0	1	1
M	File 10	1	1	1	1
M	File 11	1	1	1	1
M	File 12	1	1	1	1
M	File 13	1	1	1	1
M	File 14	1	1	1	0
M	File 15	0	0	0	0
M	File 16	0	1	0	1
M	File 17	1	1	1	1
M	File 18	1	1	1	1

Group N

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
N	File 1	1	0	0	1	0	1	0	0
N	File 2	0	0	1	1	0	0	0	1
N	File 3	1	1	0	0	1	0	0	0
N	File 4	1	0	0	0	0	1	1	0
N	File 5	0	0	0	1	0	0	0	0
N	File 6	1	1	0	0	0	0	1	0
N	File 7	0	0	1	0	0	0	0	0
N	File 8	0	0	0	1	0	0	0	0
N	File 9	0	0	0	1	0	0	0	1
N	File 10	0	1	1	0	0	0	0	0
N	File 11	0	1	0	0	0	1	0	1
N	File 12	0	0	1	0	0	0	0	0
N	File 13	0	0	1	1	0	0	1	0
N	File 14	0	0	1	0	0	0	0	0
N	File 15	0	0	0	0	0	1	0	0

Group	File	combined1	combined2	combined3	combined4
N	File 1	1	1	0	1
N	File 2	0	0	1	1
N	File 3	1	1	0	0
N	File 4	1	1	1	0
N	File 5	0	0	0	1
N	File 6	1	1	1	0
N	File 7	0	0	1	0
N	File 8	0	0	0	1
N	File 9	0	0	0	1
N	File 10	0	1	1	0
N	File 11	0	1	0	1
N	File 12	0	0	1	0
N	File 13	0	0	1	1
N	File 14	0	0	1	0
N	File 15	0	1	0	0

Group O

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
O	File 1	0	0	0	1	0	0	0	1
O	File 2	1	0	1	0	0	0	0	0
O	File 3	1	1	1	1	1	1	1	1
O	File 4	0	0	1	1	0	1	1	1
O	File 5	0	0	0	1	0	0	0	0
O	File 6	0	0	1	1	0	0	1	1
O	File 7	1	1	1	1	1	1	1	1
O	File 8	0	1	0	1	0	0	1	0
O	File 9	0	1	1	1	0	1	1	1
O	File 10	0	0	1	1	0	0	1	1
O	File 11	1	1	1	1	1	1	1	1
O	File 12	1	0	1	1	0	0	0	1
O	File 13	0	1	1	1	0	1	1	1
O	File 14	1	1	1	1	1	1	1	1

Group	File	combined1	combined2	combined3	combined4
O	File 1	0	0	0	1
O	File 2	1	0	1	0
O	File 3	1	1	1	1
O	File 4	0	1	1	1
O	File 5	0	0	0	1
O	File 6	0	0	1	1
O	File 7	1	1	1	1
O	File 8	0	1	1	1
O	File 9	0	1	1	1
O	File 10	0	0	1	1
O	File 11	1	1	1	1
O	File 12	1	0	1	1
O	File 13	0	1	1	1
O	File 14	1	1	1	1

Group P

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
P	File 1	1	1	1	1	1	1	1	1
P	File 2	1	0	0	1	0	0	1	1
P	File 3	0	1	1	1	0	0	1	1
P	File 4	1	0	0	0	1	1	0	0
P	File 5	0	1	0	1	0	1	1	1
P	File 6	1	0	1	1	0	0	0	1
P	File 7	0	0	1	0	1	1	1	0
P	File 8	1	1	0	0	1	1	1	0
P	File 9	1	1	1	0	1	1	1	0
P	File 10	1	1	1	1	1	1	1	0
P	File 11	0	0	0	1	0	0	0	0
P	File 12	0	1	1	0	1	1	1	0
P	File 13	1	1	1	1	1	1	1	1
P	File 14	1	1	1	1	1	0	1	1
P	File 15	1	0	1	1	1	0	1	1

Group	File	combined1	combined2	combined3	combined4
P	File 1	1	1	1	1
P	File 2	1	0	1	1
P	File 3	0	1	1	1
P	File 4	1	1	0	0
P	File 5	0	1	1	1
P	File 6	1	0	1	1
P	File 7	1	1	1	0
P	File 8	1	1	1	0
P	File 9	1	1	1	0
P	File 10	1	1	1	1
P	File 11	0	0	0	1
P	File 12	1	1	1	0
P	File 13	1	1	1	1
P	File 14	1	1	1	1
P	File 15	1	0	1	1

Group Q

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
Q	File 1	0	0	1	0	1	0	0	1
Q	File 2	0	0	0	1	1	0	1	1
Q	File 3	0	0	1	1	1	0	0	1
Q	File 4	0	0	0	1	0	0	0	1
Q	File 5	0	1	1	1	0	1	1	1
Q	File 6	1	0	1	1	0	1	1	0
Q	File 7	1	0	0	0	1	1	0	0
Q	File 8	1	0	1	0	1	1	0	1
Q	File 9	1	1	0	1	1	1	0	1
Q	File 10	1	1	1	1	1	0	1	1
Q	File 11	1	1	0	0	0	1	1	0
Q	File 12	1	0	1	0	1	0	1	1
Q	File 13	0	1	0	1	1	0	0	1
Q	File 14	1	1	1	1	1	0	0	0
Q	File 15	1	0	0	0	1	0	1	0

Group	File	combined1	combined2	combined3	combined4
Q	File 1	1	0	1	1
Q	File 2	1	0	1	1
Q	File 3	1	0	1	1
Q	File 4	0	0	0	1
Q	File 5	0	1	1	1
Q	File 6	1	1	1	1
Q	File 7	1	1	0	0
Q	File 8	1	1	1	1
Q	File 9	1	1	0	1
Q	File 10	1	1	1	1
Q	File 11	1	1	1	0
Q	File 12	1	0	1	1
Q	File 13	1	1	0	1
Q	File 14	1	1	1	1
Q	File 15	1	0	1	0

Group R

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
R	File 1	0	0	1	1	1	0	1	1
R	File 2	1	1	1	1	1	1	1	1
R	File 3	1	0	1	0	1	1	1	1
R	File 4	0	0	1	1	0	0	1	1
R	File 5	0	1	1	1	0	1	1	1
R	File 6	0	1	1	1	1	1	1	1
R	File 7	0	0	1	0	0	0	1	1
R	File 8	0	1	0	0	1	1	1	1
R	File 9	0	0	0	0	1	1	0	0
R	File 10	0	1	0	1	0	0	1	1
R	File 11	0	0	1	1	0	0	1	1
R	File 12	1	1	1	0	1	1	1	0
R	File 13	0	1	0	0	0	0	1	1
R	File 14	0	0	0	0	0	1	0	1
R	File 15	1	0	1	1	1	0	0	1

Group	File	combined1	combined2	combined3	combined4
R	File 1	1	0	1	1
R	File 2	1	1	1	1
R	File 3	1	1	1	1
R	File 4	0	0	1	1
R	File 5	0	1	1	1
R	File 6	1	1	1	1
R	File 7	0	0	1	1
R	File 8	1	1	1	1
R	File 9	1	1	0	0
R	File 10	0	1	1	1
R	File 11	0	0	1	1
R	File 12	1	1	1	0
R	File 13	0	1	1	1
R	File 14	0	1	0	1
R	File 15	1	0	1	1

Group S

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
S	File 1	0	0	1	1	0	0	0	0
S	File 2	0	1	0	0	0	1	0	0
S	File 3	0	1	0	1	0	0	1	0
S	File 4	1	1	0	0	0	0	0	0
S	File 5	1	1	0	0	1	0	0	0
S	File 6	1	1	0	0	0	0	1	1
S	File 7	0	0	0	1	0	0	0	1
S	File 8	1	0	0	0	1	0	0	0
S	File 9	1	1	1	0	0	1	0	0
S	File 10	0	0	0	0	0	0	0	0
S	File 11	0	1	1	0	0	0	0	0
S	File 12	0	0	0	0	0	0	0	0
S	File 13	1	0	0	1	1	0	0	1
S	File 14	1	0	0	1	1	0	0	0
S	File 15	0	0	0	0	0	0	0	0

Group	File	combined1	combined2	combined3	combined4
S	File 1	0	0	1	1
S	File 2	0	1	0	0
S	File 3	0	1	1	1
S	File 4	1	1	0	0
S	File 5	1	1	0	0
S	File 6	1	1	1	1
S	File 7	0	0	0	1
S	File 8	1	0	0	0
S	File 9	1	1	1	0
S	File 10	0	0	0	0
S	File 11	0	1	1	0
S	File 12	0	0	0	0
S	File 13	1	0	0	1
S	File 14	1	0	0	1
S	File 15	0	0	0	0

Group T

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
T	File 1	1	1	1	1	1	1	1	1
T	File 2	1	1	1	1	1	1	1	1
T	File 3	1	1	1	1	1	1	1	1
T	File 4	0	0	1	0	1	0	1	1
T	File 5	1	1	1	1	1	1	1	1
T	File 6	1	1	1	1	1	1	1	1
T	File 7	1	1	1	1	1	1	1	1
T	File 8	1	0	0	1	0	0	1	1
T	File 9	1	1	1	1	1	1	1	1
T	File 10	0	0	1	1	0	0	1	1
T	File 11	1	1	1	1	1	1	1	1
T	File 12	1	1	1	1	0	1	1	1

Group	File	combined1	combined2	combined3	combined4
T	File 1	1	1	1	1
T	File 2	1	1	1	1
T	File 3	1	1	1	1
T	File 4	1	0	1	1
T	File 5	1	1	1	1
T	File 6	1	1	1	1
T	File 7	1	1	1	1
T	File 8	1	0	1	1
T	File 9	1	1	1	1
T	File 10	0	0	1	1
T	File 11	1	1	1	1
T	File 12	1	1	1	1

Group U

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
U	File 1	0	0	1	1	0	0	1	1
U	File 2	0	1	0	1	0	1	1	1
U	File 3	1	0	0	1	1	1	1	1
U	File 4	0	1	1	1	1	1	1	1
U	File 5	0	1	0	0	0	0	1	1
U	File 6	1	0	1	0	1	0	0	1
U	File 7	0	1	0	1	1	1	1	1
U	File 8	1	0	0	0	0	0	1	0
U	File 9	0	0	1	1	1	0	1	1
U	File 10	0	0	0	1	0	0	1	1
U	File 11	1	0	1	1	1	0	1	1
U	File 12	1	1	1	1	0	1	1	1
U	File 13	0	1	0	1	0	1	1	1
U	File 14	0	1	1	1	1	1	1	1
U	File 15	0	0	1	1	0	0	1	1

Group	File	combined1	combined2	combined3	combined4
U	File 1	0	0	1	1
U	File 2	0	1	1	1
U	File 3	1	1	1	1
U	File 4	1	1	1	1
U	File 5	0	1	1	1
U	File 6	1	0	1	1
U	File 7	1	1	1	1
U	File 8	1	0	1	0
U	File 9	1	0	1	1
U	File 10	0	0	1	1
U	File 11	1	0	1	1
U	File 12	1	1	1	1
U	File 13	0	1	1	1
U	File 14	1	1	1	1
U	File 15	0	0	1	1

Group V

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
V	File 1	0	0	0	1	0	0	0	0
V	File 2	0	0	0	0	1	0	0	0
V	File 3	0	0	0	0	0	0	0	0
V	File 4	1	1	0	1	0	0	1	1
V	File 5	0	0	0	0	0	0	1	1
V	File 6	0	0	0	0	0	0	0	0
V	File 7	1	0	0	1	1	1	0	1
V	File 8	0	0	0	0	0	0	0	0
V	File 9	0	1	0	0	0	0	1	0
V	File 10	1	0	0	0	0	0	1	0
V	File11	0	0	1	1	1	1	1	1
V	File 12	0	0	0	0	0	0	0	0
V	File 13	0	0	0	0	0	0	0	1
V	File 14	0	1	0	0	0	0	1	1
V	File 15	0	0	1	0	0	0	1	1

Group	File	combined1	combined2	combined3	combined4
V	File 1	0	0	0	1
V	File 2	1	0	0	0
V	File 3	0	0	0	0
V	File 4	1	1	1	1
V	File 5	0	0	1	1
V	File 6	0	0	0	0
V	File 7	1	1	0	1
V	File 8	0	0	0	0
V	File 9	0	1	1	0
V	File 10	1	0	1	0
V	File11	1	1	1	1
V	File 12	0	0	0	0
V	File 13	0	0	0	1
V	File 14	0	1	1	1
V	File 15	0	0	1	1

Group W

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
W	File 1	1	0	0	0	1	0	1	1
W	File 2	0	0	1	0	0	1	1	0
W	File 3	1	0	0	0	1	0	1	0
W	File 4	0	1	0	0	0	1	0	1
W	File 5	0	0	0	0	0	0	0	0
W	File 6	1	0	1	0	1	0	1	0
W	File 7	0	0	0	0	0	0	0	0
W	File 8	1	0	1	1	1	0	0	1
W	File 9	0	0	0	0	1	0	0	0
W	File 10	1	0	1	0	1	1	0	0
W	File 11	0	0	0	0	0	1	0	0
W	File 12	0	0	0	0	0	0	0	0
W	File 13	1	0	1	0	1	0	1	0
W	File 14	1	1	0	0	1	1	1	0
W	File 15	1	0	0	0	1	1	1	0

Group	File	combined1	combined2	combined3	combined4
W	File 1	1	0	1	1
W	File 2	0	1	1	0
W	File 3	1	0	1	0
W	File 4	0	1	0	1
W	File 5	0	0	0	0
W	File 6	1	0	1	0
W	File 7	0	0	0	0
W	File 8	1	0	1	1
W	File 9	1	0	0	0
W	File 10	1	1	1	0
W	File 11	0	1	0	0
W	File 12	0	0	0	0
W	File 13	1	0	1	0
W	File 14	1	1	1	0
W	File 15	1	1	1	0

Group X

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
X	File 1	0	0	0	0	1	1	1	1
X	File 2	1	0	0	0	1	1	1	1
X	File 3	1	0	1	0	1	1	1	0
X	File 4	0	1	1	0	1	1	0	0
X	File 5	0	0	0	1	1	0	0	1
X	File 6	1	1	1	0	1	0	1	0
X	File 7	0	0	1	1	1	1	0	1
X	File 8	0	0	0	0	0	0	0	0
X	File 9	1	1	1	0	1	0	1	1
X	File 10	1	0	1	0	1	0	1	0
X	File 11	0	0	0	0	1	0	0	0
X	File 12	1	0	0	1	1	1	0	1
X	File 13	0	0	1	1	0	0	1	1
X	File 14	1	0	1	1	1	1	0	1
X	File 15	1	1	1	1	0	1	1	1

Group	File	combined1	combined2	combined3	combined4
X	File 1	1	1	1	1
X	File 2	1	1	1	1
X	File 3	1	1	1	0
X	File 4	1	1	1	0
X	File 5	1	0	0	1
X	File 6	1	1	1	0
X	File 7	1	1	1	1
X	File 8	0	0	0	0
X	File 9	1	1	1	1
X	File 10	1	0	1	0
X	File 11	1	0	0	0
X	File 12	1	1	0	1
X	File 13	0	0	1	1
X	File 14	1	1	1	1
X	File 15	1	1	1	1

Group Z

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
Z	File 1	0	0	0	1	0	0	0	0
Z	File 2	1	1	1	1	1	1	1	1
Z	File 3	1	1	1	1	0	0	1	1
Z	File 4	1	0	0	1	0	1	0	1
Z	File 5	1	0	0	1	1	0	0	0
Z	File 6	1	0	0	0	0	0	0	0
Z	File 7	1	0	1	0	1	0	0	0
Z	File 8	1	1	1	0	0	1	1	0
Z	File 9	1	1	0	0	1	0	0	0
Z	File 10	0	1	1	1	1	1	1	0
Z	File 11	1	1	1	1	1	0	0	1
Z	File 12	1	0	0	1	1	0	1	0
Z	File 13	1	1	0	0	0	1	0	0
Z	File 14	1	1	1	1	0	1	1	1
Z	File 15	1	0	0	1	0	1	0	0

Group	File	combined1	combined2	combined3	combined4
Z	File 1	0	0	0	1
Z	File 2	1	1	1	1
Z	File 3	1	1	1	1
Z	File 4	1	1	0	1
Z	File 5	1	0	0	1
Z	File 6	1	0	0	0
Z	File 7	1	0	1	0
Z	File 8	1	1	1	0
Z	File 9	1	1	0	0
Z	File 10	1	1	1	1
Z	File 11	1	1	1	1
Z	File 12	1	0	1	1
Z	File 13	1	1	0	0
Z	File 14	1	1	1	1
Z	File 15	1	1	0	1

Calibration group

Group	File	exam1_1	exam1_2	exam1_3	exam1_4	exam2_1	exam2_2	exam2_3	exam2_4
Calib	File 1	0	1	0	0	1	1	0	0
Calib	File 2	0	0	1	0	0	1	0	1
Calib	File 3	0	1	1	0	1	1	0	1
Calib	File 4	1	1	0	0	1	1	0	0
Calib	File 5	1	1	1	0	1	1	0	1
Calib	File 6	1	0	1	0	1	1	1	0
Calib	File 7	1	0	0	0	1	1	0	1
Calib	File 8	0	0	1	0	0	1	0	1
Calib	File 9	0	1	0	1	0	1	0	1
Calib	File 10	1	0	0	0	0	1	0	1
Calib	File11	0	0	0	0	1	0	1	1
Calib	File 12	0	0	1	0	1	0	0	0
Calib	File 13	1	0	1	0	0	1	0	0
Calib	File 14	0	1	1	1	0	0	1	1
Calib	File 15	0	0	1	1	0	0	0	1

Group	File	Combin1	Combin2	Combin3	Combin4
Calib	File 1	1	1	0	0
Calib	File 2	0	1	1	1
Calib	File 3	1	1	1	1
Calib	File 4	1	1	0	0
Calib	File 5	1	1	1	1
Calib	File 6	1	1	1	0
Calib	File 7	1	1	0	1
Calib	File 8	0	1	1	1
Calib	File 9	0	1	0	1
Calib	File 10	1	1	0	1
Calib	File11	1	0	1	1
Calib	File 12	1	0	1	0
Calib	File 13	1	1	1	0
Calib	File 14	0	1	1	1
Calib	File 15	0	0	1	1

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 28 August 2018.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

26/01/2017

Approval Certificate
New Application

Ethics Reference No.: 14/2017

Title: Decontamination and Use of Endodontic Hand Files in Dental Practice in Pretoria

Dear Dr Glynn Buchanan

The **New Application** as supported by documents specified in your cover letter dated 3/01/2017 for your research received on the 4/01/2017, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 25/01/2017.

Please note the following about your ethics approval:
- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**14/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers: MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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SUMMARY

The risk of cross-contamination validated the need to assess the adequacy of cleanliness of dental instruments following decontamination procedures. Neither the extent of single-use nor the efficacy of decontamination of endodontic hand files following routine cleaning and sterilisation procedures in South Africa was known. The first aim of this study was to determine the amount of visible debris left on endodontic hand files collected from dental practice in Pretoria, South Africa, following the application of routine decontamination procedures. Secondly, the study aimed to determine the prevalence and attitudes regarding the single-use of these instruments. Twenty-seven dental practices voluntarily took part in this study. Each participant was requested to submit 15 previously used and decontaminated endodontic hand files. A short questionnaire regarding the single-use of endodontic files was completed by participants. A coding system was used to guarantee the anonymity of the participants. Files were examined for the presence or absence of remnant debris using a stereomicroscope at ten and 40 times magnification. A novel scoring system was used to rate the position of the debris. Statistical evaluation of the data estimated the frequency and proportions of debris on the endodontic hand files, in each scoring position. Cohen's Kappa statistic was used to assess the repeatability of the scoring system. Four hundred and one endodontic hand files were collected from 27 participants. It was found that 94% of the samples were contaminated with debris. Examiner agreement was found to be fair to moderate over the entire dataset. No participants reported practising the single-use of endodontic hand files. Great variation existed in the way that endodontic hand files were decontaminated. The primary reason provided by participants for the re-use of endodontic hand files was financial concerns regarding single-use protocols.

Keywords: decontamination, single-use, endodontic hand files, debris