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EVALUATION OF THE STERILITY OF NEW RECIPROCATING ENDODONTIC FILES THROUGH MICROBIOLOGICAL CULTURE

Fabricio Victer RIBEIRO[1], Walbert A. VIEIRA[2], Marcos FROZONI[3], Iris Nogueira Bincelli SECKLER[3]

[1]Postgraduate Program in Endodontics, São Leopoldo Mandic School of Dentistry, Campinas, SP, Brazil.
[2]Departament of Dentistry, Centro Universitário das Faculdades Associadas de Ensino – UNIFAE, São João da Boa Vista, SP, Brazil.
[3]Department of Endodontic, São Leopoldo Mandic School of Dentistry, Campinas, SP, Brazil.

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Abstract

Sterility information on reciprocating endodontic instruments is often unclear on the packaging. This can make clinicians uncertain about the safe use of these instruments without prior sterilization. This study evaluated the sterility of different reciprocating file systems directly from their original packaging. Six instruments from five commercially available reciprocating systems (Reciproc® Blue, WaveOne® Gold, W File, X1 Blue MK Life, and AllPrime R Blue) were tested. Each file was aseptically removed from its packaging inside a laminar flow chamber and immediately placed into sterile glass vials containing 5 mL of Brain Heart Infusion (BHI) medium. A 0.25 mL aliquot from each vial was collected after 24, 48, and 72 hours of incubation and plated onto Petri dishes. Then, they were incubated for an additional 72 hours. To ensure methodological reliability, two instruments (one Reciproc® Blue and one AllPrime R Blue) were deliberately contaminated with *Enterococcus faecalis* and served as positive controls. The presence of colony-forming units (CFUs) was assessed at 24-hour intervals throughout the experimental period. No bacterial growth was detected in any of the test groups at any time point, while CFUs consistently appeared in the positive controls. Considering the limitations of this study, reciprocating endodontic files, in their original manufacturer-sealed packaging and without prior clinical use, seem free of microbial contamination and are safe for immediate clinical application.

Keywords: Disinfection. Endodontics. Sterilization.

Corresponding author:
Walbert A. Vieira
walbert.vieira@prof.fae.br

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1. Introduction

In dental practice, instruments are directly exposed to tissue, blood, gingival fluid, and saliva. Sterilization aims to protect patients from cross-contamination (Miller, 1993) by ensuring that instruments are free of microorganisms, including spores (Dioguardi et al. 2020).

In endodontics, all procedure stages aim to prevent the infection of the root canal system and periapical tissues by eliminating or reducing the microbial load to a level conducive to healing (Siqueira and Roças 2022; Araújo et al. 2025). In this context, the breakdown of the aseptic chain because of the handling of non-sterile instruments may be one factor that maintains viable microorganisms in the root canal after treatment (Kumar et al. 2015).

Reciprocating systems are more comfortable for patients and professionals. They reduce work time. They are also four times faster than the traditional rotary file system (Siddique and Nivedhitha, 2019; Souza et al. 2021). However, sterility information is often unclear on the package. This occurs because there is no standardization. As a result, professionals may feel uncertain about using files that have not been sterilized (Merdad and Alghmdi 2022). Longsdon et al. (2020) found that less than 35% of dentists in the United States sterilize new instruments before the first use.

Previous studies have reported mixed results on the effective sterilization of new endodontic instruments. Roth et al. (2006) evaluated new hand files from six manufacturers and found contamination in 12% of the samples. Conversely, Hortegal et al. (2022) detected contamination in all the examined hand files, including those that were supposedly sterilized by the manufacturer.

This study assessed the sterility of various reciprocating systems, namely Reciproc® Blue, WaveOne® Gold, W File, X1 Blue MK Life, and AllPrime R Blue, using microbiological culture methods. The null hypothesis proposed that none of the tested files would exhibit microbial contamination.

2. Material and Methods

The reporting of this study followed the CRIS (Checklist for Reporting *In-vitro* Studies) guidelines (Krithikadatta et al. 2014).

Sample selection

The sample size was calculated using G*Power software (version 3.1.9.4, Heinrich-Heine, University of Düsseldorf, Düsseldorf, Germany), with a 0.80 effect size, a 5% type I error, and 80% power. The minimum sample size required was five samples per experimental group. One sample was added to each group to account for potential losses, resulting in 30 samples.

This study comprised new files from two batches of different suppliers. All files were within their designated expiry dates and maintained in their original intact packages. Any files exhibiting damage or past their expiry dates were excluded from the analysis.

The experimental groups comprised six instruments from five reciprocating systems: Reciproc® Blue (VDW, Munich, Germany), WaveOne® Gold (Maillefer instruments Holding Sàrl, Ballaigues, Switzerland), W file (Shenzhen Fellhuan medical instruments, Luohu, China), X1 Blue (MK Life, Porto Alegre, RS, Brazil), and AllPrime R Blue (Shenzhen superfine technology, Guangdong, China). Each group utilized three instruments from two batches. All packaging included information on file sterility.

Microbiological analysis

One operator decontaminated the original packaging of the files with sterile gauze soaked in 70% alcohol (Rezende S/A, Duque de Caxias, RJ, Brazil) and opened it in a laminar flow chamber (Veco do Brasil).

The files were carefully extracted from the packaging using sterile tweezers (Golgran, São Caetano do Sul, SP, Brazil) and promptly placed into individual glass vials with screw caps containing 5 mL of BHI medium (Plast Labor, Curicica, RJ, Brazil). A tube shaker (VortexAp, model 56, Phoenix, Araraquara, SP, Brazil) was used to mix the contents for 60 seconds.

Aliquots of 0.25 mL of the suspension were collected at 24, 48, and 72 hours and then transferred to Petri dishes (CRAL Artigos para Laboratório Ltda, Cotia, SP, Brazil) containing BHI agar (KASVI, Curitiba, PR, Brazil). The inoculated plates were incubated in a 10% CO_2 environment at 37°C for 72 hours. A researcher evaluated the presence or absence of colony-forming units (CFUs) at 24-hour intervals.

Two additional instruments (one Reciproc® Blue and one AllPrime R Blue) were utilized as positive controls. They were immersed in 5ml vials containing BHI medium inoculated with *Enterococcus faecalis* at a concentration corresponding to level 1 on the McFarland nephelometric scale. The vials vortexed for 60 seconds to ensure adequate microbial exposure. Next, the instruments were transferred to separate vials containing sterile BHI medium and underwent the same steps as those in the experimental groups to maintain consistency in assessing antimicrobial efficacy.

3. Results

None of the experimental groups presented any bacterial growth (Figure 1). CFUs were only detected in the positive control group.

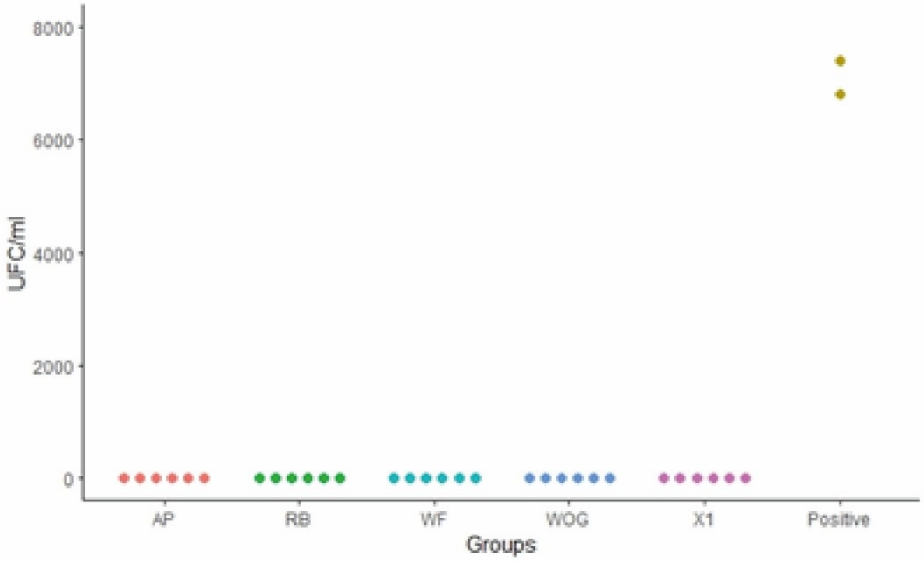

Figure 1. Bacterial growth in each experimental group. Growth was only detected in the positive control group. AP = AllPrime. RB = ReciprocBlue. WF = W File. WOG = WaveOne Gold. X1 = X1 Blue.

4. Discussion

This study assessed the sterilization efficacy of new endodontic files from different reciprocating systems. The null hypothesis was accepted, as there was no apparent microorganism growth on the tested files, indicating successful sterilization.

All tested endodontic files were sterilized via gamma radiation as specified by the manufacturer. Gamma radiation is widely recognized for sterilizing biomaterials and medical devices. It demonstrates high penetration capacity and the ability to process pre-packaged and sealed products. That makes it particularly suitable for sterilizing heat-sensitive items (Park et al. 2020). This method prevents possible structural changes in the instrument, such as loss of cutting efficiency, strength, and flexibility (Arias et al. 2020). The efficiency of this sterilization method is reflected in the microbiological evaluation of this study.

These results were similar to those of a previous study (Almehmadi and Alghamdi, 2022). However, they differed from other investigations on the contamination in new hand files (Hortegal et al. 2022; Passariello et al. 2019; Nirmala et al. 2023). One aspect that may explain these differences is that these studies analyzed files without clear documentation of their sterilization processes. Conversely, all manufacturers in the present study attested to sterilizing their files.

Another relevant characteristic is that many hand files used in previous studies were packed in box-type packaging. The reciprocating files in the present study were packed in blister-type packaging. In the study by Passariello et al. (2019), files in box-type packaging presented a higher prevalence of contamination than those in blister-type packaging.

A limitation of this study is the simple microbiological culture method used to test for bacterial contamination. Many bacterial species cannot be grown in culture media, so more sensitive detection methods are needed. Additionally, further research should assess whether new files from manufacturers that do not sterilize their products show any level of contamination.

However, this is the first study to evaluate the sterility of different file systems. It is worth noting the need for manufacturers to provide mandatory information on file sterility in an evident and standardized way. That would make it easier for professionals to use these instruments and ensure higher confidence in sterile file use.

5. Conclusions

Considering the limitations of this study, reciprocating files in their original packaging, sterilized by the manufacturer, and unused, do not present bacterial contamination and are safe to use.

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