

Analysis of the efficacy of three cleaning protocols used to remove debris during endodontic treatment: a scanning electron microscopic study

Análise da eficácia de três protocolos de limpeza usados para remover detritos durante o tratamento endodôntico: um estudo de microscopia eletrônica de varredura

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ABSTRACT

Objective: Scanning electron microscopy (SEM) was used to investigate the cleaning and debris removal by passive ultrasonic irrigation (PUI), easy clean (EC), and prophylactic canal brush (PCB) in mandibular premolars.

Material and Methods: Forty-eight single-rooted human teeth were divided into three experimental groups (PUI, EC and PCB) and a control group (CON). After access, they were prepared by irrigation with 1% sodium hypochlorite (NaOCl) up to instrument No. 40/.06 and longitudinally scored so that the specimens could be split. The split samples were taken to the SEM to detect the presence of debris and smear layer. Randomized final cleaning protocols were performed, and a second SEM image was taken for comparative analysis between groups. Kruskal-Wallis and then to the Student-Newman-Keuls tests were applied, both with $p < 0.05$. **Results:** No protocol was able to completely clean the canals. A significant difference was observed only in the cervical and middle region with PCB compared to the control group; in the other regions there was no significant difference between the techniques ($p > 0.05$). **Conclusion:** Under the conditions of the study, all techniques promoted cleaning of the examined regions of the canals, although none of the protocols completely removed debris from the canals. In the cervical third, PCB offered better results than the control.

KEYWORDS

Endodontics; Irrigation; Smear layer; Sodium hypochlorite; Ultrasound.

RESUMO

Objetivo: A microscopia eletrônica de varredura (MEV) foi usada para investigar a limpeza e a remoção de debris pela irrigação ultrassônica passiva (PUI), easy clean (EC) e escova profilática de canal (EPC) em pré-molares inferiores. **Material e Métodos:** Quarenta e oito dentes humanos de raiz única foram divididos em três grupos experimentais (PUI, EC e EPC) e um grupo controle (CON). Após o acesso, eles foram preparados por irrigação com hipoclorito de sódio (NaOCl) a 1% até o instrumento nº 40/.06 e marcados longitudinalmente para que os espécimes pudessem ser divididos. As amostras divididas foram levadas ao MEV para detectar a presença de detritos e camada de esfregaço. Foram realizados protocolos de limpeza final aleatórios e uma segunda imagem de MEV foi tirada para análise comparativa entre os grupos. Foram aplicados os testes de Kruskal-Wallis e, em seguida, o teste de Student-Newman-Keuls, ambos com $p < 0,05$. **Resultados:** Nenhum protocolo foi capaz de

limpar completamente os canais. Foi observada uma diferença significativa apenas na região cervical e média com PCB em comparação com o grupo de controle; nas outras regiões, não houve diferença significativa entre as técnicas ($p > 0,05$). **Conclusão:** Sob as condições do estudo, todas as técnicas promoveram a limpeza das regiões examinadas dos canais, embora nenhum dos protocolos tenha removido completamente os detritos dos canais. No terço cervical, a EPC ofereceu melhores resultados do que o controle.

PALAVRAS-CHAVE

Endodontia; Irrigação; Camada de esfregaço; Hipoclorito de sódio; Ultrassom.

INTRODUCTION

Successful endodontic therapy depends on the effective preparation, disinfection and obturation of the root canal system (RCS), which creates favorable conditions for the repair of the periapical tissue [1]. The RCS always has a complex anatomy, which complicates the cleaning and disinfection procedures [2]. Root canal shaping, which is performed with various manual or mechanical instruments, only works in the central area of the root canal, especially in flat canals with an oval or C-shaped cross-section [2]. Isthmuses, apical deltas, accessory canals and anatomical irregularities may contain debris, necrotic pulp remnants, microorganisms and their products that contribute to persistent periapical changes [3].

Although technological advances have significantly improved instrumentation and instrumentation techniques, a large portion of the root canal surface remains uninstrumented, regardless of the chemical mechanical preparation (CMP) technique [2,4]. Therefore, the use of irrigants during CMP is essential to remove pulp tissue, microorganisms, smear layer and debris, neutralize endotoxins and lubricate the canal walls, as well as disinfect areas that are inaccessible to instruments [5].

However, as it is not possible to carry out effective cleaning at the same time as the preparation procedures, the use of final cleaning protocols has become increasingly important [4]. In these protocols, inserts/instruments are used to clean the root canal after completion of the CMP to enhance the effect of the irrigants in removing debris and disinfection [6].

Passive ultrasonic irrigation (PUI), one of the most used protocols, can increase the efficacy of irrigants [7-9]. It is important that the ultrasonic tip oscillates freely in the root canal to promote better cleaning and avoid fractures [8], as the energy released by the insert improves the properties of the irrigant through both cavitation and acoustic flow [10,11].

The Easy Clean (EC; Bassi/Easy Equip. Odontológicos, Belo Horizonte, Brazil) is an instrument that generates movement of the irrigant, maximizes root canal cleaning and is more effective compared to PUI activation in the apical regions [12,13]. It is made of robust plastic in size #25/.04 and can be used in rotary or reciprocating systems [14,15].

The Prophylactic Canal Brush (PCB; MK Life, Medical and Dental Products Brasil, Porto Alegre, Brazil) is also available on the market. It has an active part with a diameter of 1.2 mm, including the bristles, which are made of a nylon thread. The diameter of the shaft supporting the bristles varies between 0.56 mm and 0.59 mm. The PCB is recommended for use with an electric motor, rotary kinematics and a speed between 300 and 800 RPM. It acts mechanically, dragging debris and creating a fluid movement that is limited to the neck and center region due to its size. There are few studies to prove its effectiveness. However, a previous study found that the removal of the smear layer and debris in the cervical region was more efficient than in the middle and apical region [16-18].

The aim of this study was to evaluate, under *ex vivo* conditions using scanning electron microscopy (SEM), the final cleaning of the root canal walls by using PUI, EC and PCB. The null hypothesis was that there would be no differences between the cleaning techniques tested.

MATERIAL AND METHODS

Sample calculation

Based on data from a previous study by Plotino et al. [17], an alpha error of 5%, a beta power of 80% and an N2/N1 ratio of 1 were applied and it was determined that 10 samples per group had to be included (G*Power 3.1 software; Heinrich Heine College, Düsseldorf, Germany). However, considering the possibility of samples being lost during the study, an n of 12 was accepted. Thus, 48 single-rooted human

teeth were collected by donation after being extracted for reasons unrelated to the study, following approval by the local research ethics committee (#4,983,519).

Selection and chemical-mechanical preparation of the sample

After coronal access, which was performed in a standardized manner using spherical diamond burr #1013 (KG Sorensen, Cotia, SP, Brazil) and Endo-Z drills (Dentsply/Maillefer, Ballaigues, Switzerland) driven at high speed and cooling. The teeth were standardized in size; the actual tooth length (ATL) was set at 19 mm. For this purpose, the occlusal edges were ground with a diamond disk. Chemical-mechanical preparation was performed with #25/.08 reciprocating instruments (Pro R; MK LIFE Dental Products) driven by an X-Smart Plus motor (Dentsply/Maillefer) in the "Reciproc All" program; 1% NaOCl was used as an auxiliary rinsing solution, which was introduced into the canal using a disposable syringe and a Navitip needle (29 GA) (Ultradent, South Jordan, Utah, USA). A working length of 1.0 mm below the ATL was used, and the preparation was completed with #40/.06 instruments from the same system.

SEM analysis and randomization of the samples

Longitudinal grooves were made on the buccal and lingual surfaces of the teeth. Heavy body condensation silicone (ZetaPlus Zhermack, Badia Polesine, RO, Italy) was then poured into a plastic ring into which the specimens were inserted one by one up to the amelocementary junction. The teeth were removed from the sets and their halves separated to take the first image of each third of the specimens in a variable pressure SEM (EVO MA 10, Carl Zeiss, Oberkochen, Germany). The samples were mounted on a metal base attached to a rotary stage of the microscope. A voltage of 5.0 kV and 300 μ A was used for imaging, and the images were saved in TIF format.

The images confirmed the presence of debris adhering to the walls. It was also possible to randomly score the sample using a scoring system adapted on that described by Kamel and Kataia [16]: 0 - no dentin slurry or shavings, all or almost all dentin tubules observed; 1 - small amount of dentin slurry, no shavings, more than

50% of dentin tubules observed; 2 - small amount of dentin slurry, no shavings, less than 50% of dentin tubules observed; 3 - moderate amount of shavings and dentin slurry, some dentin tubules observed; 4 - large amount of shavings and dentin slurry, no dentin tubules observed. Only samples with values between 2 and 4 in this first analysis were included in the study. Once the analysis was completed, the samples were randomly divided into an experimental group and a control group.

Application of cleaning protocols

Samples were placed back into the silicone/plastic ring set and prepared according to the final irrigation protocols established for each group:

PUI group: 1% NaOCl solution was drawn up into a plastic syringe with a 21 mm Navitip needle. The penetration of the needle was limited to 2.0 mm below the ATL. In each cycle, 5 mL of the solution was used; the excess was carefully aspirated to ensure complete filling of the canals. Ultrasonic treatment was performed using an Irrisonic tip (Figure 1^a; Helse Ultrasonics, Santa Rosa do Viterbo, Brazil) operated with Ultrawave XS ultrasound (Ultradent, South Jordan, USA) at power 1. An attempt was always made to center the insert so that it did not touch the canal walls and was limited to 2.0 mm below the ATL. The insert was used in the mesio-distal direction for 3 cycles of 20 seconds, for a total time of 60 seconds. After each cycle, a new irrigation and aspiration were performed. At the end, the same protocol was applied with a 17% EDTA solution.

EC group: After the canals were irrigated as in the previous group, agitation was performed with an EC tip (Figure 1B). This was driven by an X-Smart Plus electric motor in rotary kinematics at 1000 RPM at 2 N.cm. The tip was used with brushing movements against the mesial and distal walls of the canals, limited to 2.0 mm below the ATL, for 3 cycles of 20 seconds; each cycle was followed by a new irrigation and aspiration. At the end, the same protocol was used with 17% EDTA solution.

PCB group: Following the same pattern, the PCB (Figure 1C) was moved with the X-Smart Plus electric motor in rotary kinematics at 300 revolutions per minute at 2 N.cm. An attempt was always made to keep the PCB as central

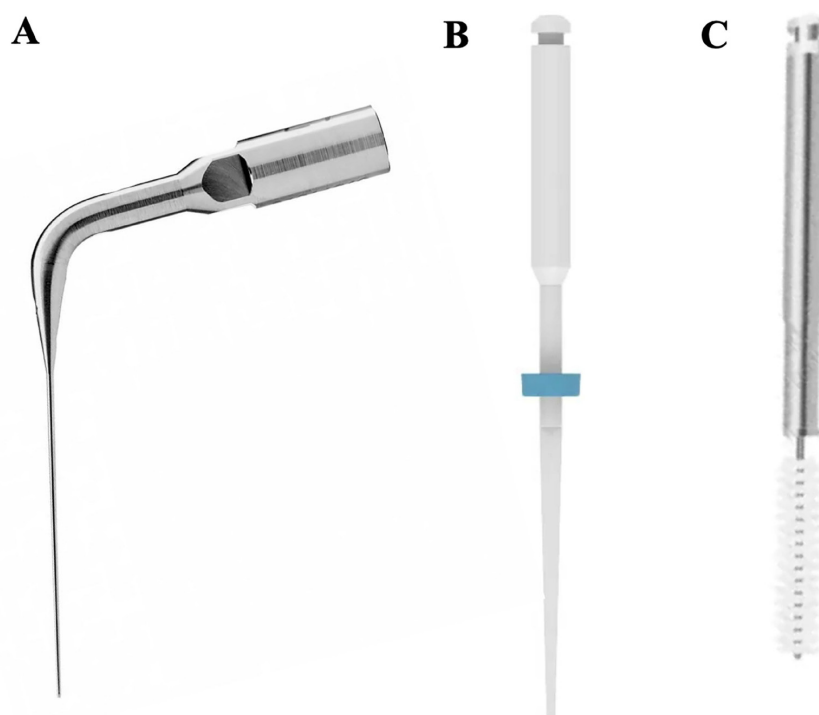


Figure 1 - Illustrative images of the equipment employed: Irrisonic (A), Easy Clean (B), and Prophylactic Canal Brush (C).

as possible, with in and out movements brushing the walls of the canal and are limited to 4.0 mm below the ATL. Similar to the previous groups, three 20-second cycles were used; each cycle was followed by a new irrigation and aspiration. At the end, the same protocol was used with a 17% EDTA solution.

CON group: The 1% NaOCl solution, packed in a 5-mL plastic syringe with a 21-mm Navitip needle, was introduced up to 2.0 mm from the ATL, injecting the solution three times and holding it in the canal for 20 seconds; the excess was carefully aspirated. At the end, the same protocol was applied with a 17% EDTA solution.

At the end, each tooth, regardless of which group it belonged to, received a total rinse of 15 mL NaOCl and 15 mL EDTA during these complementary cleanness irrigation protocol.

Analysis of the protocols

After the solution agitation/activation procedures were completed in all groups, the teeth were removed from the sets using a spatula for a new SEM image. An operator blinded to the groups took three images of each sample, one for each third, according to the localization pattern suggested by Hülsmann et al. [19].

After this step, the images were compiled and submitted for scoring by two independent, previously calibrated experts, who were again blinded to the groups being analyzed. The previously created scores were reapplied; some images were duplicated to analyze agreement between examiners.

Statistical analysis of the results

Statistical analysis of the data began with the determination of kappa values between and within investigators. After the data were validated, they were subjected to Wilcoxon test in terms to determine intra-group variation of the specimens in each third, before and after the complementary cleaning protocols. Furthermore, in terms to evaluate to individual inter-group comparisons, the Kruskal-Wallis and then to the Student-Newman-Keuls tests were applied, both with $p < 0.05$.

RESULTS

The observed kappa values confirmed the validity of the collected data with 86% and 92% for the between- and within-examiner indices, respectively. Figure 2 shows representative images of the groups. The median, minimum and maximum values offered by the groups are shown in Table I.

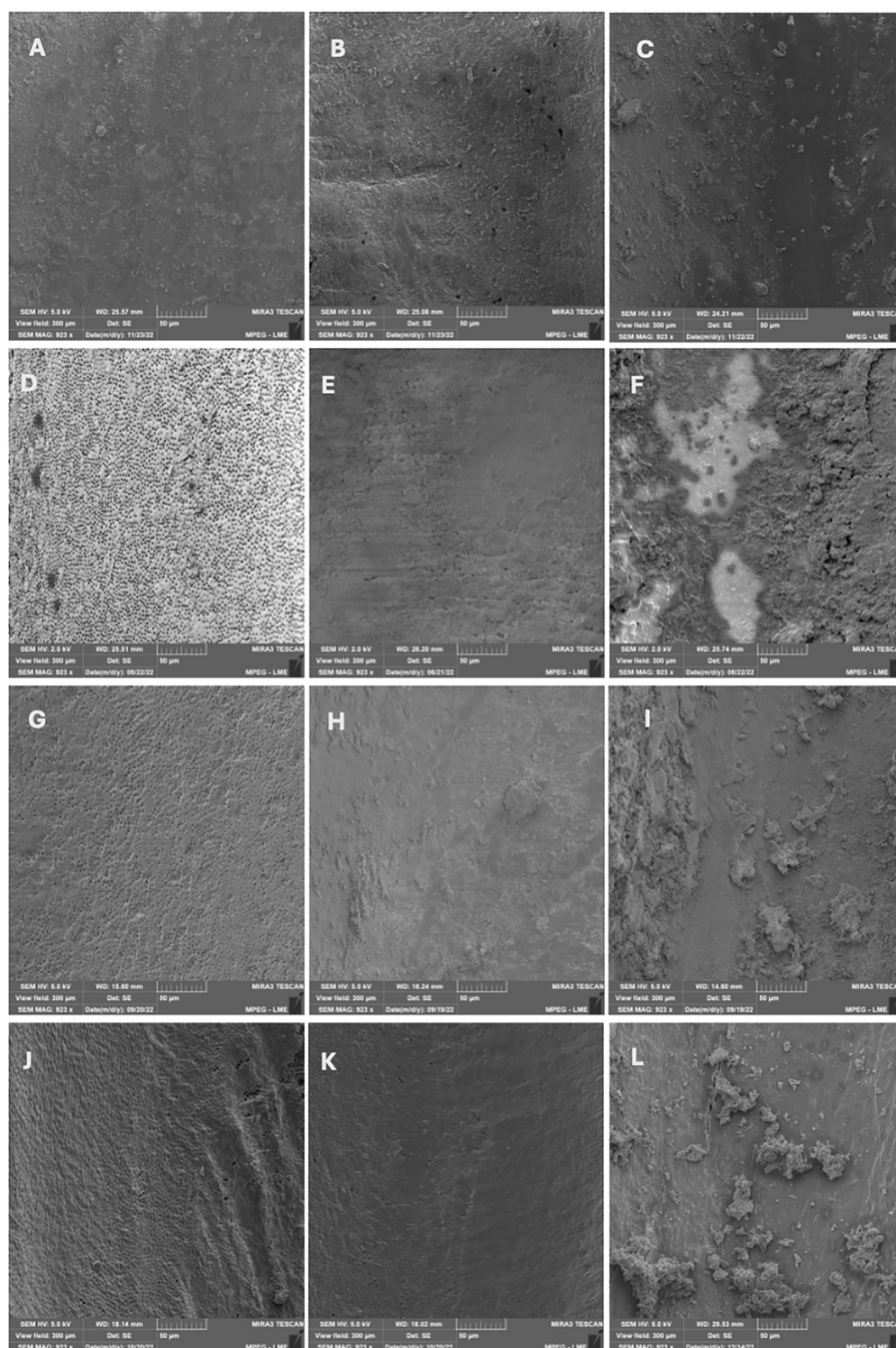


Figure 2 - Inner root surface (300µm) of the cervical (A), middle (B) and apical (C) thirds after PUI; of the cervical (D), middle (E) and apical (F) thirds after PCB; the cervical (G), middle (H) and apical (I) thirds after Easy Clean; and the cervical (J), middle (K) and apical (L) thirds after conventional irrigation (control).

This table shows the distribution of scores between the groups in the three thirds analyzed. Considering the results, none of the techniques was able to completely remove the deposits and/or fully expose the tubules. The observed values revealed no significant difference between the

experimental groups, regardless of the third analyzed ($p < 0.05$). The only difference was observed in the analysis of the cervical third when PCD and CON were compared ($p < 0.05$).

When the thirds were analyzed, there were also no variations between them when PUI or EC

Table I - Medians, minimums and maximums attributed to the groups in the three observed root canal thirds

Group	Cervical			Middle			Apical		
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.
PCB	0 ^{a,A}	0	2	0 ^{a,A}	0	2	2 ^{a,B}	0	4
PUI	1 ^{a,A}	0	3	1 ^{a,A}	0	2	2 ^{a,A}	0	3
CON	2 ^{a,A}	1	3	1 ^{a,A}	1	2	4 ^{a,B}	1	4
EC	1,5 ^{a,A}	0	2	1 ^{a,A}	0	1	2 ^{a,A}	0	3

^{a,b}Lowercase superscript letters indicate significant differences between groups, in each third, according to the Kruskal-Wallis test and Student-Newman-Keuls comparison test ($p < 0.05$). ^{A,B}Superscript capital letters indicate significant differences considering each group in different canal third, according to the Kruskal-Wallis test and Student-Newman-Keuls comparison test ($p < 0.05$).

were used ($p > 0.05$). However, less cleanliness was observed in the apical third in the PCD and CON groups ($p < 0.05$).

DISCUSSION

The chemical-mechanical preparation of root canals is of fundamental importance for the success of endodontic treatment [1,3,17]. This preparation aims to expand the inner walls of the root canal and remove vital or non-vital tissue, contaminated or not, through the chemical action of irrigating solutions and the mechanical action of endodontic instruments [20-22]. Over the years, various cleaning protocols, instruments, irrigants and methods of rinsing or activating irrigants have been developed to complement the removal of organic and inorganic debris [23,24]. Some of these methods, developed to be performed after canal shaping, offer an improvement in cleaning [22,25,26].

In this study, which was performed using the SEM, the aim was to compare the degree of cleaning of the root canal walls by using three protocols: PUI, EC and PCD. Extracted teeth were used to simulate clinical conditions. The teeth were enlarged to size #40/.06 to obtain an apical enlargement that allowed a more effective irrigation and a better effect of the agitators [1,27].

Regarding the protocols applied, no significant differences in the degree of cleaning and debris removal were found between the groups tested, regardless of the third analyzed. All agitation/activation protocols removed debris from the root walls, but not completely. Considering that only teeth with scores higher than 2 (*i.e.* specimens with a high frequency of smear layer and debris) were included, the findings confirm the change in their profile by observing scores 0 and 1. Furthermore, the

statistical analysis performed comparing the scores before and after agitation/activation demonstrated statistical significance in all groups, even in the control group, most likely due to the fact that the volume of irrigating solution was increased. The worst results were observed in the apical third in all analyzed groups. These results confirm data already found in the literature [16,28]. The lower cleanliness in the apical portion may be attributed to anatomical complexities and the smaller diameter compared to the other thirds, which could limit the physical effect of irrigation [29].

Although this was not the focus of this study, it was notable that the control group did not show a significant difference compared to the experimental groups ($p < 0.05$). This may indicate that additional cleaning protocols are not necessary. It can be concluded that apical enlargement, the caliber and length of the needle used during irrigation and the total volume applied had a positive influence on the cleaning performance of irrigation, even without agitation [5,7,12,30].

A significant difference compared to the control was only observed in the cervical third with better PCD performance ($p < 0.05$). It is hypothesized that this performance is due to a combined effect of the kinetics of the movement of the solutions and the mechanical traction (*i.e.*, contact) of the brush bristles against the canal walls, justifying a higher efficacy. As with the control, the effect of PCD was worst in the apical third. This may be due to the smaller diameter of the canal, which the brush cannot reach due to its shaft diameter of 0.56 mm [14,16].

The results found for the EC and PUI groups confirm the findings of Duque et al. [31]. The authors found no statistically significant differences between EC and PUI within the used protocol of 1.0 mm and 2.0 mm below the ATL,

respectively. However, regarding the cleaning pattern, the authors observed greater efficiency with the protocols. However, the fact that three activations of the irrigation solution were applied for 20 seconds, which were also used in this study, indicated an improvement in cleaning in the middle and apical third [31].

Thus, despite the limitation of this being an *in vitro* study that assigns scores to cleaning and debris removal, rather than quantifying them, as do several other studies in the area [6,7,12,16-19], this study suggests that when comparing protocols, PCD is more effective when the focus is on the cervical and middle region, as it does not physically affect the apical region. In addition, the fact that no differences were found between the control and final irrigation protocols tested suggests that further research could help confirm the importance of apical enlargement and irrigation volume in improving cleaning results.

CONCLUSION

Under the conditions of the study, it can be concluded that none of the protocols completely removed debris from the root canal and were less efficient in the apical third regardless of the group. Furthermore, differences between the methods applied and the control group were only found when comparing the control group and the prophylactic canal brush in the cervical third.

Author's Contributions

JVSH, BCV, MAHD: Conceptualization. JVSH, BCV, MAHD: Data Analysis. JVSH, BCV, MAHD: Statistical Analysis. JVSH, BCV, MAHD: Interpretation. JVSH, BCV, MAHD, NVG, AGL: Writing – Original Draft Preparation. JVSH, BCV, MAHD, NVG, ECPJ: Writing – Review & Editing. ECPJ, JVSH, BCV, SMLC: Visualization. SMLC: Investigation.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects

oversight committee guidelines and policies of Research Ethics Committee of the Faculdade São Leopoldo Mandic. The approval code for this study is: #4.983.519.

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