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RESEARCH ARTICLE

`REVISED` The Effect of Coronal Pre-flaring and Type of Root Canal Irrigation on Working Length Accuracy Using Electronic Apex Locators [version 3; peer review: 2 approved]

Previously titled: The effect of coronal pre-flaring and root canal irrigant on apex locators' accuracy: an in-vitro study

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Abstract

Background: Successful root canal treatment is influenced by the apical extent of root canal preparation and the eventual root canal filling. Achieving the full working length until the apical constriction, which is usually 0.5 – 1 mm shorter than the anatomical apex, is crucial. Electronic apex locators were used to detect the working length more accurately. There are six generations of electronic apex locators in the market. The selection of the appropriate irrigation with each apex locator for accurate working length determination is not fully investigated.

Methods: The actual working lengths of 120 freshly extracted human single-rooted teeth were measured and compared with their working lengths using 3rd generation (Root ZX) followed by 6th generation (Raypex 6) apex locators in dry medium, presence of 5.25% sodium hypochlorite, and 2% chlorhexidine, without coronal pre-flaring and after coronal pre-flaring using the same irrigating media. Data were

collected, tabulated, and afterward analyzed using one-way ANOVA with post-hoc to evaluate the significant difference in average working length between actual working length, Root ZX, and Raypex 6 apex locator working lengths accuracy.

Results: The significant results were shown in roots that were coronally pre-flared and their working lengths were measured in a dry medium using Raypex 6 apex locator. While using the Root ZX apex locator, the most accurate results were shown in roots that were coronally pre-flared and their working lengths were measured while using a chlorhexidine irrigating solution.

Conclusions: It is concluded that it is very important to know the specific irrigating medium to be used with each specific electronic apex locator to achieve the most accurate working length results.

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Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the Health Services gateway.

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REVISED **Amendments from Version 2**

After reviewing the comments of the reviewers, a few phrases have been added to emphasize, clarify the idea, and make the manuscript clearer and more appealing to the readers.

First, all the suggested comments from reviewers were answered and added directly to the manuscript Version3 with the supporting literature.

Second the abstract, as suggested by the reviewer that the abstract was written to be more representative of main research idea, well demonstrating the gained results.

For the methodology, further details were added for the sample preparation, steps followed, criteria of reading the working length using the Root ZX and the Raypex6 EAL to achieve the most accurate WL measurements and differences from the actual WL respectively. This will give the reader more details about the methods used in the current research. Therefore, it helps the reader to fully comprehend the methods used in the current study.

For the results, 2 Tables were added for better clarification for the gained results. A table for the frequency of deviations of apex locators from the actual WL with dry medium, NaOCl, and CHX. In addition to another table for the relative error of each apex locator with the irrigating medium used in the study. Moreover, emphasizing the method used in measuring the WL, that is the difference between the measured WL and the actual WL for each tooth.

For the discussion, a comment was added to the limitation of the study at the end of the discussion as suggested by the reviewers, to highlight the suggested modifications for the subsequent future studies. Moreover, recommendations to be followed in future research have been detailed.

For the references, references number 17, 18, 19, 21, 22, 23, 25, 26, and 27 were added to support the idea, and ended up with 48 references instead of 39.

Any further responses from the reviewers can be found at the end of the article

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Introduction

Successful root canal treatment (RCT) is influenced by the apical extent of root canal preparation and eventual root canal filling.[1] The suggested endpoint for instrumentation and obturation is apical constriction.[2] Apical constriction is defined as the minor root canal diameter. Histologically, it represents the transitional point between the pulpal and periodontal tissues at the cemento-dentinal junction (CDJ). According to anatomical research, the apical constriction is 0.5–1.0 mm from the exterior or the main foramen (anatomical apex).[3] Therefore, full WL provides a clean barrier, protecting the periodontium from bacterial invasion.[3] Moreover, underfilling or overfilling of the canals is one of the reasons for endodontic treatment failures.[4–6]

Accordingly, the determination of WL is a critical step in endodontic treatment.[3] There are many ways to determine WL: periapical X-rays (PAs), bleeding points, electronic apex locators (EALs), and the tactile sensation of the operator.[7] The most commonly used method is to combine EALs and periapical radiographs.[8] Thus, a reliable apex locator should be used to reduce patient exposure to X-rays. Six generations of EALs are used to detect WL. The most popular system is the third-generation apex locator.[9] It uses two frequencies and measures the difference between them; however, the type of moisture present may affect reading accuracy.[9,10] On the other hand, the 4th generation uses five frequencies, but they make mathematical measurements rather than measurements according to a database.[9,10] However, there are no marked differences in reading accuracy between older apex locator generations. The 5th generation uses a database of canal electrical features and compares them using a mathematical process. New technological advancements have led to the sixth generation of EAL, in which a steady algorithm was created according to the canal's moisture properties. Furthermore, working length measurements are more reproducible and accurate than those of previous generations.[9]

The use of irrigating media to determine WL has been reported in the literature. Keeping the root canal dry or moist with an irrigator while using apex locators has been questioned by many dental practitioners as to whether it increases the accuracy rate of EAL. Using 5.25% sodium hypochlorite (NaOCl) during RCT meets the prime principles of endodontics, chemomechanical cleaning, and root canal shaping. NaOCl is also used as an antibacterial agent to dissolve organic components of the root canal system.[11]

Chlorhexidine gluconate (CHX; 2%) is a broad-spectrum antiseptic cationic agent that exists in two forms: gel (CHX-G) and solution (CHX-S). However, it cannot dissolve organic materials as 5.25% NaOCl does.[11] A 2% CHX solution has been used in endodontics as an irrigating material or intracanal medicament because it has a broad spectrum of antimicrobial activity and a lower cytotoxic effect than NaOCl. While showcasing its effective clinical performance, lubricating characteristics, and gel-like rheological action, it additionally inhibits metalloproteinase, maintains chemical

stability, avoids clothing staining, lacks odor, dissolves in water, and, thanks to its cationic structure, possesses a distinct attribute referred to as substantivity (remaining antimicrobial activity).[12,13] It is well established that the chemomechanical procedure can be enhanced if followed by the use of an antibacterial intracanal medication such as chlorhexidine (CHX), especially in cases of exudation, hemorrhage, perforation, root resorption, trauma, or insufficient root development.[13]

Coronal pre-flaring of root canals has several advantages that can be reflected in the cleaning and shaping processes, including making it easier to place manual and rotary equipment in the apical region of the root canals.[14,15] The results indicated that the pre-flaring procedure provided more accurate measurements in most cases.[16]

Some research studies nowadays[17–19] recommended the contracted endodontic cavities (CECs) that were developed from the concept of minimally invasive dentistry and provide an alternative to traditional endodontic cavities (TECs). They claimed that the CECs preserve the mechanical stability of teeth and preserve more of the dentin as discussed by Alovisi et al. (2018), Weiqi et al. (2013) and Wang et al. (2023).[17–19] That's why the working length determination without coronal flaring was used in this study as it is a way some researchers recommended and supported by some of them.

Few studies have been conducted to evaluate the effect of irrigating solutions on the WL reading accuracy of various EALs in the presence or absence of coronal pre-flaring. Hence, the present study aimed to compare WL accuracy using 3rd generation (Root ZX) and 6th generation (Raypex 6) EALs in single-rooted teeth in the presence of different irrigation media: dry medium, 5.25% sodium hypochlorite (NaOCl), and 2% chlorhexidine (CHX) in root canals with or without coronal pre-flaring.

Hypothesis
No significant difference in the accuracy of WL determination will be detected by coronal pre-flaring or the type of irrigant, which varied with the different generations of EALs.

Methods
Ethical approval
The study complied with the Declaration of Helsinki, and the research protocol was approved by the Restorative Dental Science Department, Imam Abdulrahman Bin Faisal University, College of Dentistry. Ethical approval was obtained from the Institutional Review Board of Imam Abdulrahman Bin Faisal University (IRB 2022-02-171) on April 12, 2022.

Teeth were collected during disposal after receiving signed consent from dental surgery or oral procedure patients, who authorized the hospital to use their discretion in their disposal and to be used for research purposes if needed.

Sample size calculation
A power analysis was performed using the clinical sample size for this study. Means and standard deviations were obtained from previously published literature,[11] which were (0.64 ± 0.54) and (0.33 ± 0.22). The power of the sample was set at 90%, and the significance level was set at 0.05. Hence, the calculated total sample size was 109, which was increased to 120 for more precise results.

Sample selection
The study began on April 17, 2022. In total, 120 freshly extracted human single-rooted teeth of working lengths between 19 to 22 mm were used in this study. They were collected anonymously without exposing the patients' data, and were used only for this in vitro study. The samples were collected and immersed in 5.25% sodium hypochlorite (NaOCl) for 2 hours for disinfection.[3] Afterwards, the samples were stored in normal saline until further use. Root surfaces and apical regions were examined under a dental operating microscope (OMS 1950 Dental Microscope, USA) at 25× magnification. Teeth of comparable lengths and completely formed apices were included in the current study. Teeth with any possible fractures or apex immaturity were excluded from the study. The teeth were radiographed in the mesiodistal and buccolingual directions to exclude the absence of root resorption or canal curvature. Only root canals with a curvature of 0–5° were included in the study. Teeth with calcified canals, more than one canal, apical blockage, internal or external resorption, or caries were excluded from this study.

Sample preparation
The teeth were examined using periapical X-rays with a 0.08-second exposure time. Scaling of the teeth was performed using an ultrasonic scaler (Dentsply Sirona, ProUltra Piezo Ultrasonic Handpiece) and then stored in 0.9% normal saline until use. The teeth were flattened with a diamond disc of 1 mm thickness (Hi-Tech diamond disc bur) to obtain a reliable reference point based on the findings of Jakobson et al. that the rubber stopper on the file should be placed on a flat surface

to limit the possibility of errors in research with EALs, which ensured that the study findings were not influenced.[20] WL was evaluated by two evaluators, who were instructed to use the same criteria to evaluate and assess the parameters of the current study. Cohen's Kappa test was applied to ensure agreement and consistency between the two evaluators' WL evaluations. A value of 0.82 was interpreted as a high level of agreement or reliability between the two evaluators.

Working length measurements without coronal pre-flaring
Conventional access opening without coronal pre-flaring was performed. Apical patency was assessed using K-file #10 (Dentsply M-access K-file). The actual WL was measured in millimeters by two calibrated evaluators using an endodontic microscope (OMS 1950 Dental Microscope) at 25× magnification. Any tooth with an initial file larger than the #15 K-file (Dentsply M-Access K-File) was excluded. The file was placed beyond the apical constriction and retrieved until it was flushed through the apical foramen. 0.5 mm was subtracted from the total, and the final measurement was considered as the actual WL.[2,8] A double rubber stopper was used.[3] Using freshly mixed alginate, each tooth was mounted at the cemento-enamel junction (CEJ) level using freshly mixed alginate.

Working length determination
The WL in millimeters for each tooth was measured using the 3rd generation EAL Root ZX (J. Morita Corp., Kyoto, Japan), followed by the 6th generation EAL Raypex 6 (VDW, Munich, Germany). Apex locators were used while the teeth were dry, using 5.25% NaOCl and 2% CHX. Irrigation was done with 23G with apical vented needle (Ultrdent Products, South Jordan, UT, USA). The needle was introduced until resistance was felt and then reduced 1 mm from the needle penetration depth to prevent obliteration of the root canal lumen as illustrated by Guerreiro-Tanomaru et al. (2013).[21] 3 ml of 5.25% NaOCl and 2% CHX were used alternatively in the presence and absence of coronal pre-flaring as used by Spoorthy et al. (2013).[22] Irrigation was for 2 minutes as suggested by Fernandes et al. (2021).[23] Between each irrigating solution and the next, distilled water was used to neutralize each irrigating effect before using the next one. The canals were dried using paper points. All measurements were taken by two calibrated examiners. A K-file size #15 (Dentsply M-access K-File) was inserted inside the canal, a lip clip was placed inside a fresh alginate mix, and a file holder was placed on the file.

The file was fixed to the apex locators: third generation EAL Root ZX (J. Morita Corp., Kyoto, Japan) and sixth generation EAL Raypex 6 (VDW, Munich, Germany) for 5 seconds before recording measurements. The measurements were recorded when the file reached the mid-green area on the EALs' screen.[24] For Root ZX, working length determination was established at the point when the screen displays the line just in the middle of apex and 1 mark which corresponds to 0.5 mm short of radiologic apex. For Raypex 6, the third green line, just before the yellow lines, which corresponds to 0.5 mm short of radiographic apex was determined for working length. The measures were recorded as electronic measurement (EM) if they were stable for at least 5 seconds as described by Aydin et al. (2015).[25]

Moreover, When the file tip reaches or is close to the apical constriction, the apex locator's screen display typically indicates this by showing the file position within the mid-green area. Moreover, both Root ZX and Raypex 6 EALs also have an audio indicator that emits a sound, such as a continuous or intermittent beep, when the file approaches or reaches the apical constriction. The change in sound indicates that the file is close to the desired working length, assisting the clinician in achieving precise measurements. Furthermore, both Root ZX and Raypex 6 feature a digital display that shows the distance between the file tip and the apical constriction in millimeters as reported by Higa et al. (2009).[26] Besides, Calibration of EALs before each use, according to the manufacturer's instructions was performed to ensure precision of WL measurements as recommended by Çinar et al. (2020).[27]

Working length measurements with coronal pre-flaring
All teeth were collected, and coronal pre-flaring of the root canals was performed. An access opening was prepared for each tooth. The penetration depth of the gate-glidden drills was as follows: #3 to the canal orifice, #2 to the coronal third, and maximum to the coronal half of the canal to avoid perforations and achieve straight-line access.[28] Measuring the WL in millimeters of all teeth was repeated after coronal pre-flaring with both Root ZX (3rd generation) and Raypex 6 (6th generation) apex locators using dry medium, 5.25% NaOCl, and 2% CHX irrigating solutions, as shown in Figure 1.

In an attempt to eliminate any variable that may take place among each group, each tooth was subjected with its same anatomical variation, diameter, length, dentin thickness to all the study variables. This way provided more precise calculations of differences from the actual working length for all the used samples.

Statistical analysis
The data were represented in the differences between the actual WL and the WL measured by each EAL. It was recorded and tabulated in an Excel spreadsheet and then transferred to SPSS version 24 (IBM, Inc., Chicago, IL, USA). The
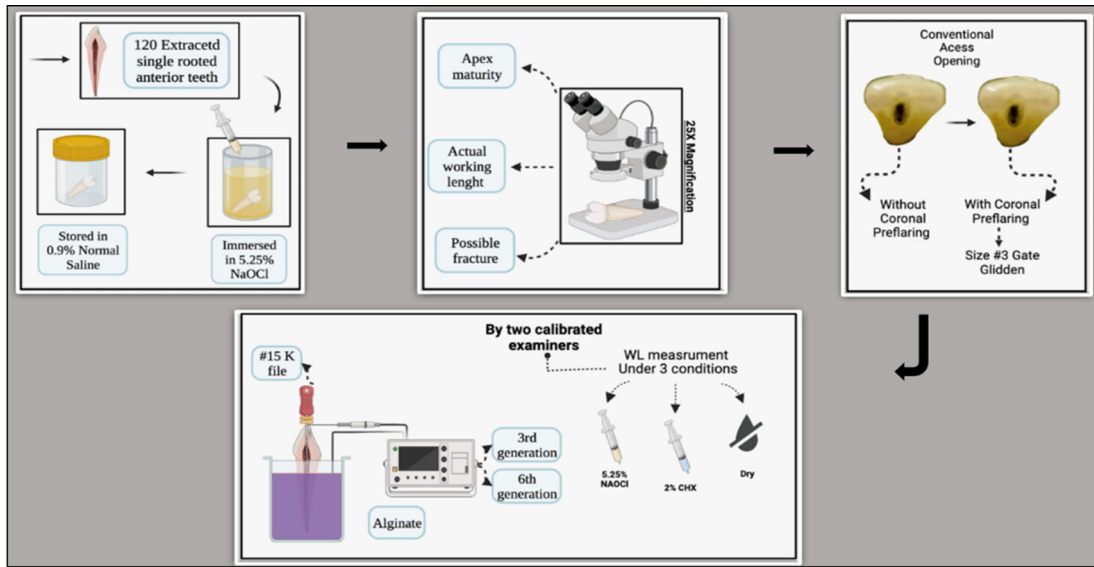
Figure 1. Schematic drawing showing the following steps for the study: tooth selection, tooth storage, measuring the actual working length under the microscope, access opening preparation without coronal pre-flaring followed by access opening preparation with coronal pre-flaring, teeth mounted in alginate, holding the file in the double stopper technique, and measuring the working length (WL) in millimeters using different irrigation media (dry medium, 5.25% NaOCl, 2% CHX) with 3rd and 6th generation EALs.

difference or deviation from the zero (actual WL) either increased by (+) or decreased by (-) were measured. The means and standard deviations were calculated and presented in tables as descriptive statistics. Comparisons between the irrigant solutions for Root ZX and Raypex 6 EAL were performed using an independent sample t-test and ANOVA. Where ANOVA was significant, multiple comparisons were made using Tukey's post hoc test. A comparison of WL differences between the 3rd (Root ZX) and 6th generation (Raypex 6) apex locators in teeth without coronal pre-flaring and with coronal pre-flaring in all media was also performed using an independent sample t-test. Statistical significance was set at p < 0.05.

Results

Table 1 compares the mean WL differences of Root ZX and Raypex 6 EALs between the irrigating solutions and within the irrigating solutions without coronal pre-flaring. Mean length measurement differences for Root ZX in CHX were significantly the (0) that represents the actual WL (0.087 ± 0.445) than the other two irrigants; the least close measurement from the WL was with NaOCl (0.252 ± 0.553), and the difference was statistically significant (p = 0.025). However, with Raypex 6, the dry medium should have the closest readings to WL, and the overall mean difference among the irrigants used was insignificant. Within each irrigating solution, the mean differences of Raypex 6 was close to (0) representing the actual WL and statistically significant. Moreover, the dry medium showed the most accurate WL differences with the Raypex 6, the NaOCl demonstrated the most accurate WL differences with the Raypex 6, and the CHX displayed the most accurate WL differences with Root ZX in all non-pre-flaring conditions. Figures 2 and 3 present the pre-flaring WL differences for Root ZX and Raypex 6 for all irrigating solutions. The median lengths for dry medium and NaOCl were almost the same, whereas the median length for CHX significantly decreased and was closer to (0) that represents the actual WL; asterisks indicate outliers.

Table 1. Comparison of mean working length differences (WL) of Root ZX and Raypex 6 EALs in the presence of Dry medium, 5.25% NaOCl, and 2% CHX media without coronal pre-flaring.

	Without pre-flaring			p-values of ANOVA
	Dry	**NaOCl**	**CHX**	
Root ZX (3rd generation)	0.146 ± 0.399	0.252 ± 0.553[a]	0.087 ± 0.445[a]	F = 3.7, p = 0.025*
Raypex 6 (6th generation)	-0.074 ± 0.486	-0.121 ± 0.506	-0.161 ± 0.534	F = 0.861, p = 0.42
p-values of t-test	T = 3.8, p = 0.001*	T = 5.4, p = 0.001*	T = 3.85, p = 0.001*	

*Statistically significant at 0.05.
[a]Significant differences between solutions horizontally.
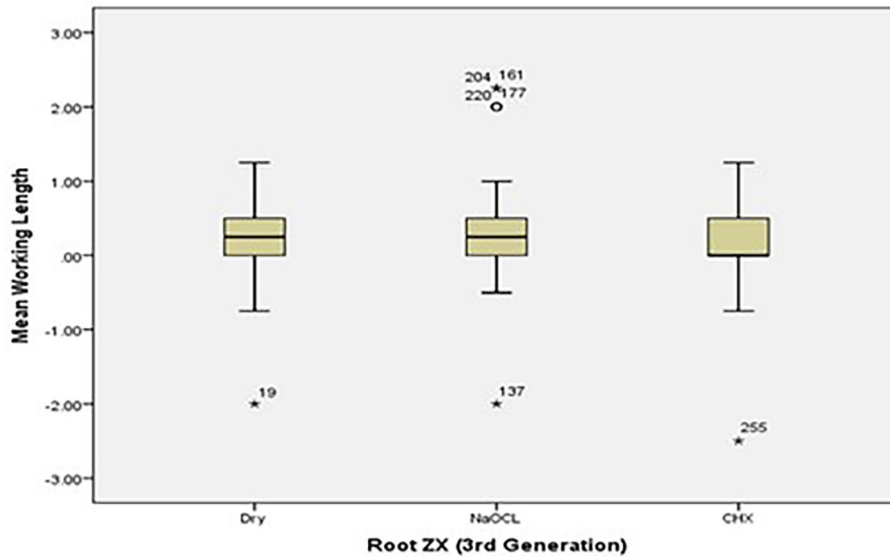
Figure 2. Working length differences for Root ZX for all irrigating solutions without canal pre-flaring.
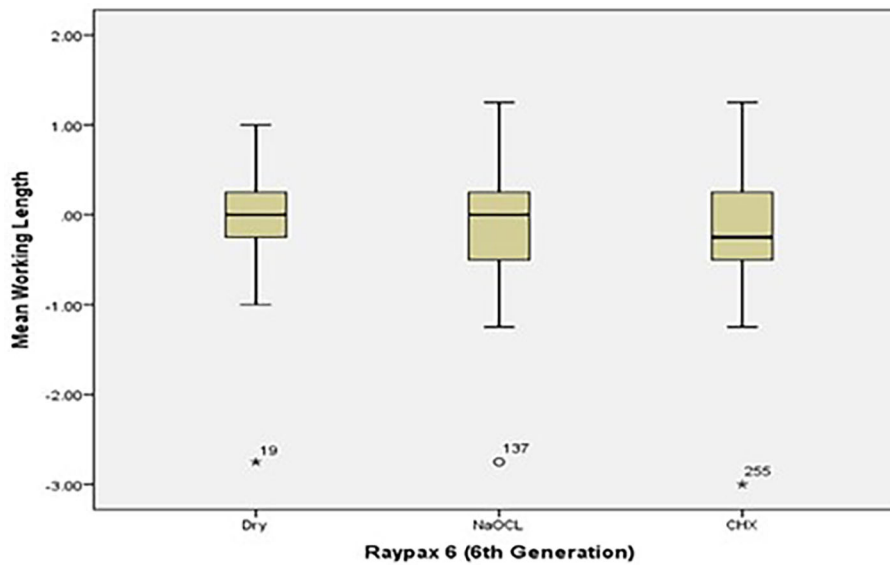
Figure 3. Working length differences for Raypex 6 for all irrigating solutions without coronal pre-flaring.

Similarly, Table 2 presents the mean WL difference among the solutions for both apex locators in the presence of coronal pre-flaring. The mean WL for the Root ZX locator differed significantly among the solutions (p = - 0.038). The closest mean to the (0) representing the actual WL was recorded with CHX (0.068 ± 0.586), whereas the mean that differed the most from the (0) representing actual WL was recorded in a dry medium (0.269 ± 0.621). Likewise, the most accurate mean WL difference for the Raypax 6 locator was recorded in a dry medium (-0.464 ± 0.641), the least accurate WL difference was recorded with NaOCl (-0.174 ± 0.584), and the difference between the (0) representing actual WL and the mean was statistically significant. Both apex locators differed significantly between irrigation solution groups.

Moreover, the dry medium displayed the most accurate WL with Raypex 6, followed by NaOCl achieved the most accurate WL with Root ZX, and CHX showed the most accurate WL with Root ZX in all coronal pre-flaring conditions (Table 2). The box plots in Figures 4 and 5 show the median WL spread of the measurements. Figure 4 shows that the median WL in NaOCl and CHX media was almost equal, but significantly different in the dry medium. Figure 5 illustrates a similar pattern for dry medium and CHX when using the Raypex 6 apex locator, where the asterisks in each box plot refer to outliers.

Table 2. Comparison of mean WL differences of Root ZX and Raypex 6 EALs in the presence of Dry medium, 5.25 % NaOCl, and 2% CHX media with coronal pre-flaring.

|	With pre-flaring			p-values of ANOVA
	Dry	**NaOCl**	**CHX**	
Root ZX (3rd generation)	0.269 $\pm$ 0.621[a]	0.206 $\pm$ 0.64	0.068 $\pm$ 0.586[a]	F = 3.28, p = 0.038*
Raypex 6 (6th generation)	-0.174 $\pm$ 0.584[a]	-0.464 $\pm$ 0.641[a]	-0.28 $\pm$ 0.74	F = 5.87, p = 0.003*
p-values	T = 5.4, p = 0.001*	T = 8.02, p = 0.001*	T = 3.99, p = 0.001*	

*Statistically significant at 0.05.
[a]Significant differences between solutions horizontally.
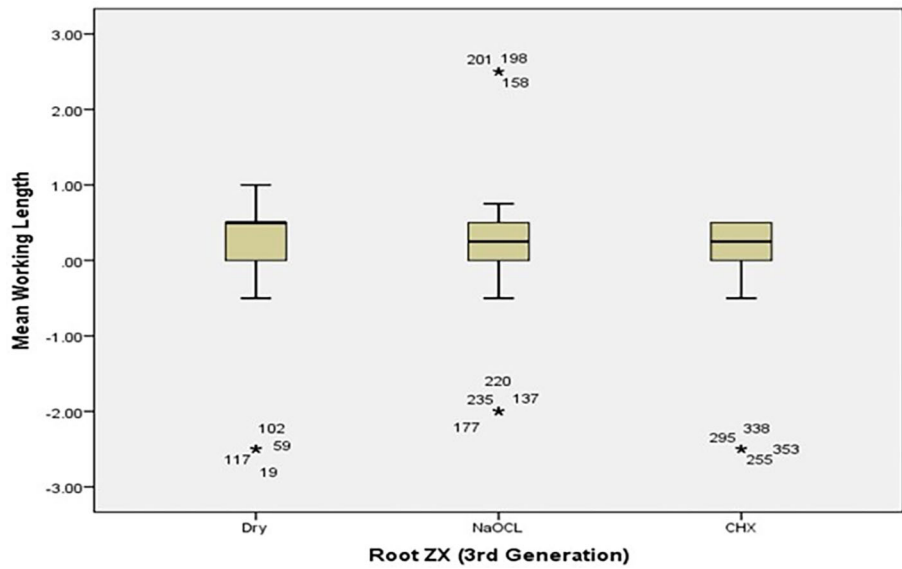
Figure 4. Working length differences for Root ZX for all irrigating solutions with coronal pre-flaring.
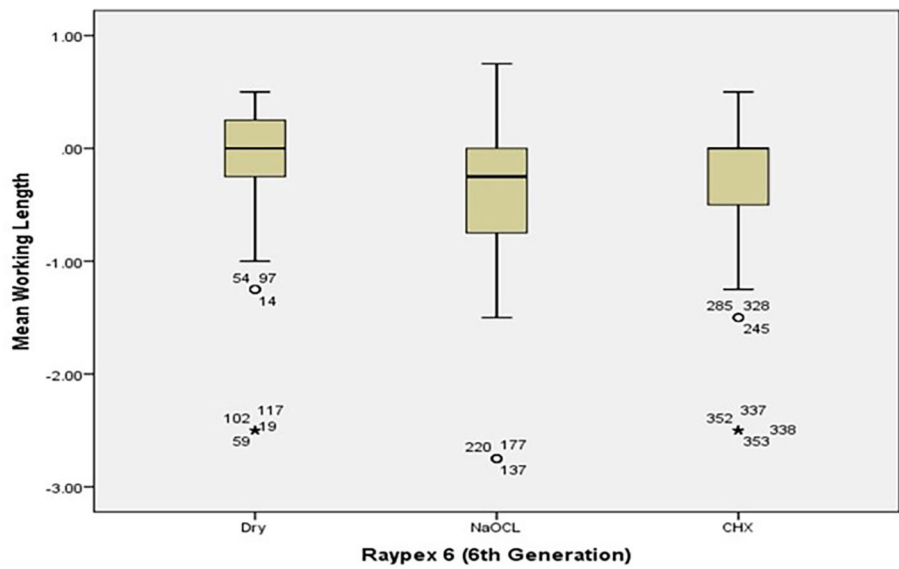
Figure 5. Working length differences for Raypex 6 for all irrigating solutions with coronal pre-flaring.

Table 3. Comparison of WL differences of roots irrigated with different irrigating media under all flaring conditions (with or without coronal pre-flaring).

Groups	Dry		NaOCl		CHX		Overall	
	Root ZX	Raypex 6	Root ZX	Raypex 6	Root ZX	Raypex 6	Root ZX	Raypex 6
Without pre-flaring	0.146 ± 0.39	-0.074 ± 0.48	0.252 ± 0.55	-0.121 ± 0.50	0.087 ± 0.44	-0.161 ± 0.53	0.161 ± 0.37	-0.118 ± 0.44
With pre-flaring	0.269 ± 0.62	-0.174 ± 0.58	0.206 ± 0.64	-0.464 ± 0.64	0.068 ± 0.58	-0.28 ± 0.74	0.181 ± 0.54	-0.30 ± 0.57
p-value	T=-1.807, p = 0.072	T=-1.407, p = 0.156	T = 0.598, p = 0.550	T = 4.5, p = 0.0001*	T = 0.281, p = 0.779	T = 1.41, p = 0.159	T = 3.14, p = 0.734	T = 2.86, p = 0.005*

*Statistically significant at 0.05.

Table 4. Frequency of deviations from actual working length for both electronic apex locators with each irrigation medium in the absence and presence of coronal pre-flaring.

Irrigation system	Apex locator	Without pre-flaring				With pre-flaring			
		WL difference deviation in ± 0.5		WL difference deviation >1		WL difference deviation in ± 0.5		WL difference deviation >1	
		Number	Frequency	Number	Frequency	Number	Frequency	Number	Frequency
Dry medium	Root ZX	111	94.1	2.5	2.5	99	83.9	4	3.4
	Raypex 6	102	86.4	10.2	10.2	104	88.1	14	11.9
5.25% NaOCl	Root ZX	104	88.1	2.5	2.5	102	86.4	7	5.9
	Raypex 6	100	84.7	12.7	12.7	84	71.2	32	27.1
2% CHX	Root ZX	107	90.7	4.2	4.2	114	96.6	4	3.4
	Raypex 6	96	81.4	15.3	15.3	101	85.6	17	14.4

Table 5. The relative error for each electronic apex locator for their accuracy of measurement of WL.

Irrigation solution	Apex locator	Relative error	
		Without coronal pre-flaring	**With coronal pre-flaring**
Dry medium	Root ZX	1.57%	2.48%
	Raypex 6	1.58%	1.74%
5.25% NaOCl	Root ZX	1.97%	2.21%
	Raypex 6	1.76%	2.61%
2% CHX	Root ZX	1.54%	1.93%
	Raypex 6	2.03%	2.28%

The WL difference for both apex locators was then compared between the presence and absence of coronal pre-flaring, as presented in Table 3. In both cases (with and without coronal pre-flaring), the mean length did not differ significantly for either apex locator in dry medium (p = -0.072), but the closest mean WL difference to (0) representing the actual WL was recorded in irrigation groups without coronal pre-flaring (0.146 ± 0.39, -0.074 ± 0.48, respectively). Similarly, in the NaOCl solution, the most accurate mean WL difference was recorded without coronal pre-flaring media for the Raypex 6 apex locator (0.121 ± 0.50), and the difference was statistically significant (p = -0.001). The closest mean WL difference to (0) representing the actual WL in CHX for Root ZX was found in pre-flaring media (0.068 ± 0.58), whereas for Raypax 6, it was the closest in the same media (0.28 ± 0.74), but the difference was not statistically significant. When compared regardless of the irrigation solution, the most accurate mean WL difference to (0) representing the actual WL was recorded in the absence of coronal pre-flaring media groups for both apex locators (0.161 ± 0.37, -0.118 ± 0.44, respectively), and the difference was only statistically significant for the Raypex 6 apex locator (p = -0.005).

The frequencies presented in Table 4 were calculated for the differences between the differences and 0 (the actual WL) within the range of ±0.5 and greater than 1. In the absence of coronal pre-flaring the Root ZX (3rd generation) showed more accuracy as most of the values were within the range of ±0.5 in each irrigation system, whereas Raypex 6 (6th generation) showed less accuracy than Root ZX while having majority of the values greater than 1 which is showing the far distance from (0) representing the actual WL. A similar trend was observed in irrigation media used in the presence of coronal pre-flaring except in dry medium where Raypex 6 apex locator showed more accuracy with the most values near to ±0.5.

In Table 5 Root ZX was found to have the most accurate measurement tool for WL differences in Dry medium (1.57%) and CHX (1.54%) irrigating solution respectively. Whereas, with 5.25% NaOCl, Raypex6 showed high accuracy (1.76%) in teeth without coronal pre-flaring. However, in presence of coronal pre-flaring, Root ZX showed high accuracy in NaOCl (2.21%) and CHX (1.93%) compared to Raypex 6.

Discussion

The establishment of an accurate WL is a crucial step during root canal treatment, particularly in cases of anatomical limitations. It is useful when used with radiographs to ensure proper determination of the canal WL.[29] The coronal pre-flaring of root canals provides many advantages during meticulous cleaning and shaping procedures, such as facilitating the insertion of manual and/or rotary files into the apical third of the root canals by removing cervical dentin interferences.[30] In addition, coronal flaring improved the flow of the irrigating solution within the root canal, minimizing the risk of bacterial invasion into the periapical tissue and reducing the risk of canal debris and irrigant extrusion during the root canal preparation procedure.[31,32]

In recent years, a growing body of evidence has suggested a correlation between the type of root canal irrigating solution used and the success of coronal pre-flaring efficacy. Moreover, the use of root canal irrigants is a major contributor to the success of endodontic treatment. Some studies have shown a correlation between the irrigant and the root canal sealer used to ensure a proper hermetic seal,[33] while others have discussed the effect of the proper irrigant on the accuracy of WL determination.[34]

The current investigation evaluated the influence of coronal pre-flaring on the accuracy of actual working length determination, and the results showed that the canals without coronal preflaring at the coronal end provided more consistent and accurate outcomes than canals that were pre-flared (Table 3). These results are consistent with those of a

previous study conducted by João Marcelo da Silva Teixeira et al. (2012), who concluded that the use of Gates Glidden burs for cervical pre-flaring did not significantly influence the accuracy of apical placement of the apex locator when determining the actual WL because of insufficient removal of coronal dentin when compared with the rotary system for preparing coronal pre-flaring.[35] However, this contradicts a previous study that showed that pre-flaring improved the efficiency of EALs in mandibular and anterior root canals.[16]

Fernandes et al. (2021)[23] showed that the 5.25% NaOCl showed a significantly greater reduction in E. faecalis than 2.5% NaOCl. This coincided with the results of Elnaghy et al. (2017), who found that the use of 5.25% sodium hypochlorite (NaOCl) as an irrigant without coronal pre-flaring was associated with greater success rates than a 2% chlorhexidine (CHX) solution. This may be due to the good electrical conductivity of NaOCl, which contributes to the accurate detection of Raypex 6 at the apical constriction. Moreover, this may be due to the advanced technology of Raypex 6, which can accurately operate under different canal conditions, including the presence of debris and/or obstructions in the canal.[9] Furthermore, researchers also noted that sodium hypochlorite irrigants were more effective than other irrigants that may be used to prevent blockage and ledge formation in the root canal.[36] Therefore, even without pre-flaring, Raypex 6 may provide accurate measurements.

The results showed that the readings when using Raypex 6 (6th generation EAL) were significantly closer to the actual WL than when using Root ZX (3rd generation EAL) for the groups with and without coronal pre-flaring. This coincides with the results of Pegum Unsal Peker et al., who concluded that Raypex 6 is not influenced by irrigation solutions because of its multifrequency technology, which shows precise WL results.[9,37] The 6th generation apex locator is believed to be less sensitive to external factors that increase measuring reliability,[38] such as the number and taper of the files used in coronal pre-flaring, which may influence the enlargement of the coronal portion of the canal.[39] The sixth generation EALs have been proven to have a preliminary determination of canal moistness, and based on the constant determined moisture, the sixth-generation EALs adapt the measuring method for either a dry or wet root canal environment.[40]

It is important to remember that the accuracy of an EAL can vary depending on the type of irrigant used. Hence, it is important to use the most appropriate irrigant for the situation at hand. The current study showed the best results with the Raypex 6 apex locator in the dry medium under all conditions of pre-flaring (without pre-flaring and with pre-flaring) ($T = 3.8$, $p = 0.001$) and ($T = 5.4$, $p = 0.001$), respectively. This may be due to the ability to detect changes in canal resistance, as it is easier to obtain accurate measurements in dry root canals.[41] Moreover, our findings agree with those of a study conducted by Koçak et al. for WL measurements in dry conditions, which showed more accurate readings than wet canals.[42] However, our findings contradict those of a previous study by Nayif et al. (2011), who stated that when saline was used as an irrigant, readings were closer to the actual length, whereas those conducted in dry root canals were shorter than the actual WL.[43]

In accordance with our study, the Root ZX apex locator achieved significant results with CHX in all conditions of pre-flaring (without pre-flaring and with pre-flaring) ($T = 3.85$, $p = 0.001$) and ($T = 3.99$, $p = 0.001$), respectively. This may be because of the different electrical conductivities of the irrigants, which are defined as the intrinsic ability of the irrigant to conduct electric currents.[11] Moreover, single-rooted teeth with a single canal orifice were used, which may have contributed to the lack of difference between the groups with and without coronal pre-flaring. In contrast, multirooted teeth with more canal orifices have a higher potential for more anatomical variations and may have differences when coronal pre-flaring is performed prior to WL determination.[16]

Moreover, electro-conductivity was enhanced in the current study by using alginate as an embedding material to determine electronic WL. The alginate model provides reliable and reproducible results as it has favorable characteristics that mimic the clinical situation by ensuring the required electric circuit for proper measurement of the EAL. This is because they mimic the electrical resistance of the human periodontal ligament.[44] Despite the consistency of alginate, it can remain a gel that allows ions to circulate and promotes adequate electro-conductivity. Hence, it is recommended that alginate be used as an embedding material in laboratory applications.[44,45]

Lucena-Martin et al. reported that electronic WL measurements should be concluded within 2 hours after mixing the alginate to minimize moisture loss.[46] Lipski et al. reported that the most accurate readings were obtained within 30 minutes after mixing alginate to enhance the electrical conductivity of the irrigants and EAL.[47] Consequently, alginate was used for only the first 30 minutes of mixing in the current study to ensure accuracy.

The null hypothesis was partially rejected because of the differences in the results obtained from the different irrigation solutions used with each EAL. However, there was no significant difference between groups with and without coronal flaring. This was within the limitations of the current study on using single-root teeth with an initial file size of 15, while

using one type of irrigant at a time to be used as a benchmark for future studies. The study's limitations included using curved canal teeth with various degrees of curvature and larger apical foramen sizes, which may have affected the results. In the current research one irrigant was used to prevent the results bias and ensure each irrigant electroconductivity as a benchmark. However, the results may be affected while using a multi-irrigation sequence protocol in the WL reading accuracy. Future results were indicated to evaluate the EAL accuracy after various irrigation protocols.

Moreover, agitation of the irrigated solution may affect the electrical conductivity and, hence, the reading accuracy of the EAL. Irrigation volume, concentration, temperature, and application method may also influence reading accuracy. In addition, applying the current study in vivo in the presence of patient's body fluids may affect the results. Furthermore, measuring the specificity and the sensitivity of the variables will be more valuable to collect more accurate results of clinical significance in future studies.

Conclusions

In conclusion, the study results suggest that adherence to the endodontic principles of conventional access opening, coronal pre-flaring, and patency are the cornerstones for achieving the most accurate and reproducible WL measurements in Root ZX and Raypex 6 EALs. It was concluded that irrigant-type selection plays a major role in the accuracy of EAL readings. Generally, using the 6th generation EAL (Raypex 6) is the most accurate choice for measuring WL. However, it provides the most accurate WL measurements when used in a dry medium. Regarding the 3rd generation EAL (Root ZX), it is better to use it with 2% CHX to achieve the most accurate WL of the root canal. Hence, it is important to know the specific irrigating medium used with each specific EAL to achieve the most accurate WL results.

Recommendations

The dry medium with 6th generation EAL (Raypex 6) and 2% CHX with the 3rd generation EAL (Root ZX) showed the most accurate WL measurements. Therefore, it is very important to know the specific irrigating medium used with each specific EAL to achieve the most accurate WL results during the root canal treatment.

Data availability

Underlying data

Figshare: The Effect of Coronal Pre-flaring and Root Canal Irrigant on Apex Locator Accuracy: An in vitro Study (https://doi.org/10.6084/m9.figshare.22492354.v4.[48])

This project contained the following underlying data:

- Apex locators results.xlsx

- Microscope images for cases 1–6

Due to the size of the original microscopy images, they were not uploaded to a public repository. Readers and reviewers can request additional images from the corresponding author (srhussein@iau.edu.sa).

Extended data

Figshare: The Effect of Coronal Pre-flaring and Root Canal Irrigant on Apex Locator Accuracy: An in vitro Study (https://doi.org/10.6084/m9.figshare.22492354.v4.[48])

This project contains the following extended data:

- Manuscript tables and figures

- Additional images

Acknowledgments

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Open Peer Review

Current Peer Review Status: ✓ ✓

Version 3

Reviewer Report 13 November 2023

https://doi.org/10.5256/f1000research.157772.r217941

✓ **Amira Salem** (iD)

[1] Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, Otago, New Zealand
[2] Blis Technologies Limited, Dunedin, New Zealand

The authors have addressed all of the reviewer's concerns and clarified it in the discussion and recommendation.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endodontics, microbiology, molecular biology, biotechnology, bioengineering, product development, drug delivery

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 27 October 2023

https://doi.org/10.5256/f1000research.157772.r217942

✓ **Rania El Backly** (iD)

Endodontics, Conservative Dentistry Department and Tissue Engineering Laboratories, Faculty of Dentistry, Alexandria University, Alexandria, Alexandria Governorate, Egypt

I believe the authors have made substantial revisions and I can endorse the manuscript for indexing.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 27 September 2023

https://doi.org/10.5256/f1000research.153312.r203786

?   **Rania El Backly** (iD)
     ¹ Endodontics, Conservative Dentistry Department and Tissue Engineering Laboratories, Faculty of Dentistry, Alexandria University, Alexandria, Alexandria Governorate, Egypt
     ² Endodontics, Conservative Dentistry Department and Tissue Engineering Laboratories, Faculty of Dentistry, Alexandria University, Alexandria, Alexandria Governorate, Egypt

In general:

The authors have attempted to address the previous comments however, some changes still need to be made for the manuscript to be suitable for publication. All the responses to the comments should be clearly highlighted in the main manuscript file AND mentioned in the responses letter especially the page no. and line no. where these corrections were made.

New comments are bolded.

Introduction:
- The rationale of the study is unclear. Coronal pre-flaring is a routine part of endodontic instrumentation and the irrigants used in this study are routinely used in everyday clinical practice in association with apex locators, so why are the authors assuming that these variables will affect the overall accuracy? This must be clearly shown.

The authors responded to this point in the response letter but did not incorporate these reasons in the manuscript itself. This must be added to the introduction section along with the appropriate references.

Methods:
- The settings used for each apex locator must be clearly defined. "mid-green area on the screen" is not precise.

For this comment, the responses of the authors are highly appreciated however, these details should be mentioned in the manuscript for accurate reproducibility of the work

along with adding the references.

  ○ How was irrigation done? The volume used? The type of needle and gauge used? And depth of insertion of the needle? How long was the duration of irrigation?

Again, the authors responses should be incorporated as modifications in the main text. All these details should be added for the reader to see as well.

  ○ Why did the authors not use EDTA? Additionally, the sequence of using EDTA and NaOCl is the most common combination of irrigating solutions used especially since working length is often verified in the middle of instrumentation procedures under the influence of mixtures of these irrigants together. It is well-known in endodontics that combinations of irrigants are often used so testing combinations of irrigants would have given higher validation of the results of this study.

While the response of the authors to this comment is not convincing since they could have used different groups with and without EDTA, this justification or rationalization of why they did NOT use EDTA should be mentioned in the discussion section.

  ○ There is a major concern that the same samples were used for all the different variables. Other studies separated the entire sample into groups to avoid the influence of all of the intermediate procedures on the measurements taken last. How do the authors justify this? It would have been better to divide the sample into sub-groups for each tested variable especially since each sample represents its own control and this division of the samples would not have influenced the results, on the contrary would have enhanced the precision of the results.

The same as above, this explanation should be added to the discussion section.

Results:

  ○ The tables and figures demonstrate that the values measured are the working length values in mm however, this is impossible since the values are even in the negative? So what do these values represent? Do they represent the values registered on the apex locator screens according to the pre-determined settings? I.e. the number registered on the screen when the device indicates that this is the CORRECT working length? If so these values are quite misleading. Also, how is it possible that the ACTUAL working length is 0.087+-0.445? The authors should record the entire working length in mm from the coronal reference point to the apical constriction minus 0.5 mm as they mentioned in the methodology.

Again, the authors must clearly show or describe that the values are the DIFFERENCE in readings and that they were recorded as either + or -.

  ○ The frequency of the difference between the actual working length and that measured in each condition by the two apex locators should be shown as this is the true measure that would truly demonstrate which technique was of higher accuracy in determining the WL.

  ○ Check:

  Ramezani, M.; Bolbolian, M.; Aliakbari, M.; Alizadeh, A.; Tofangchiha, M.; Faegh, S.M.; Patini, R.; D'Amato, G. Accuracy of Three Types of Apex Locators versus Digital Periapical Radiography for Working Length Determination in Maxillary Premolars: An In Vitro Study. *Clin. Pract.* **2022**, *12*, 1043-1053.

  Kaufman AY, Keila S, Yoshpe M. Accuracy of a new apex locator: an in vitro study. Int Endod J. 2002 Feb;35(2):186-92.

The authors respond with a table but it was NOT included in the revised manuscript. Please

add this table.
- The choice of 5.25% NaOCl should be justified. It would have been more practical to use 2.5% as this is the most commonly used concentration clinically.

Kindly add justification in the discussion section.
- It is recommended that diagnostic accuracy of the apex locators in presence of the different variables be recorded in terms of specificity and sensitivity as compared to the actual working length measured. This information would be a better guide for the clinician and as such authors can make a recommendation regarding the most "accurate" apex locator to use and in association with which conditions.

I appreciate the authors' attempts by including this table which again should be added to the main revised text however, this is not what is meant by specificity and sensitivity. While I understand that this may be laborious to measure now, it should again be mentioned in the discussion section that diagnostic accuracy studies usually require these two measures to make a valuable deduction of clinical significance.
- The results section should include a table for the demographic data of the sample including mean and SD of the types of teeth included, lengths of the teeth, number of canals (the authors mention single rooted but not the number of canals nor canal configurations), age of patients if present, gender....etc.

Perhaps age and gender might not be available (and this should be mentioned in the text for the reasons the authors mentioned about anonymity of samples) however, the type of teeth, lengths, number of canals....etc should all be easy to demonstrate and should be included.

Discussion:
- The discussion is well-written but the authors should show the clinical significance of the results as previously commented placed in the context of the multiple variables that are present in the clinical situation. The authors showed based on the results of their study that the type of irrigating solution has an influence on measuring WL using these two apex locators? So what do the authors recommend? A particular sequence to use? A specific irrigant to use only during WL determination? In order to give value to results from laboratory studies their clinical significance must be shown.

The response to this comment again should be included in the discussion section.
- There should also be a section on the limitations of the study such as the limitation of performing the study on single rooted teeth, the influence of curvatures, the influence of combining different irrigants, influence of embedding medium, volume of irrigant and other limitations that may influence the extrapolation of the results in a clinical sense. Especially since most of these points were not mentioned in the discussion and it is advisable to do so.

Thank you for adding a limitations section

References

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2. Kaufman AY, Keila S, Yoshpe M: Accuracy of a new apex locator: an in vitro study.*Int Endod J*. 2002; **35** (2): 186-92 PubMed Abstract | Publisher Full Text

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 Oct 2023

Shimaa Rifaat

Reviewer (2) comments and authors' responses:

In general:

The authors have attempted to address the previous comments however, some changes still need to be made for the manuscript to be suitable for publication. All the responses to the comments should be clearly highlighted in the main manuscript file AND mentioned in the responses letter especially the page no. and line no. where these corrections were made.

New comments are bolded.

Introduction:
   ○ The rationale of the study is unclear. Coronal pre-flaring is a routine part of endodontic instrumentation and the irrigants used in this study are routinely used in everyday clinical practice in association with apex locators, so why are the authors assuming that these variables will affect the overall accuracy? This must be clearly shown.

In updated Version 3, Page: 5, Line: 48-54

The authors responded to this point in the response letter but did not incorporate these reasons in the manuscript itself. This must be added to the introduction section along with the appropriate references.

Methods:
   ○ The settings used for each apex locator must be clearly defined. "mid-green area on the screen" is not precise.

In updated Version 3, Page: 7, line: 134-148

For this comment, the responses of the authors are highly appreciated however, these details should be mentioned in the manuscript for accurate reproducibility of the work along with adding the references.
   ○ How was irrigation done? The volume used? The type of needle and gauge used? And depth of insertion of the needle? How long was the duration of irrigation?

In updated Version 3, Page: 6, Line: 120-125

Again, the authors responses should be incorporated as modifications in the main text. All these details should be added for the reader to see as well.

○ Why did the authors not use EDTA? Additionally, the sequence of using EDTA and NaOCl is the most common combination of irrigating solutions used especially since working length is often verified in the middle of instrumentation procedures under the influence of mixtures of these irrigants together. It is well-known in endodontics that combinations of irrigants are often used so testing combinations of irrigants would have given higher validation of the results of this study.

While the response of the authors to this comment is not convincing since they could have used different groups with and without EDTA, this justification or rationalization of why they did NOT use EDTA should be mentioned in the discussion section.

Thank you for your comment. At the first steps of WL determination, we use NaOCl for removal of organic debris inside the canal without doing any cleaning and shaping with no formation of smear layer inside the canal to use any chelating agent. Hence, At the step of WL determination usually EDTA wasn't yet introduced. Furthermore, as per the literature discussing the accuracy of WL using EAL most of the studies that showed accurate WL was with NaOCl and CHX.

In updated Version 3, Page: 14-15, Line: 372-376
○ There is a major concern that the same samples were used for all the different variables. Other studies separated the entire sample into groups to avoid the influence of all of the intermediate procedures on the measurements taken last. How do the authors justify this? It would have been better to divide the sample into sub-groups for each tested variable especially since each sample represents its own control and this division of the samples would not have influenced the results, on the contrary would have enhanced the precision of the results.

The same as above, this explanation should be added to the discussion section.
In updated Version 3, Page: 7-8, Line: 167-170

Results:
○ The tables and figures demonstrate that the values measured are the working length values in mm however, this is impossible since the values are even in the negative? So what do these values represent? Do they represent the values registered on the apex locator screens according to the pre-determined settings? I.e. the number registered on the screen when the device indicates that this is the CORRECT working length? If so these values are quite misleading. Also, how is it possible that the ACTUAL working length is 0.087+-0.445? The authors should record the entire working length in mm from the coronal reference point to the apical constriction minus 0.5 mm as they mentioned in the methodology.

Again, the authors must clearly show or describe that the values are the DIFFERENCE in readings and that they were recorded as either + or -.

Add it in the discussion section (corrected in the statistical analysis, results, and the tables labels)

In updated Version 3, Page: 8-11, Line: 173-257
○ The frequency of the difference between the actual working length and that measured in each condition by the two apex locators should be shown as this is the

true measure that would truly demonstrate which technique was of higher accuracy in determining the WL.

○ Check:

Ramezani, M.; Bolbolian, M.; Aliakbari, M.; Alizadeh, A.; Tofangchiha, M.; Faegh, S.M.; Patini, R.; D'Amato, G. Accuracy of Three Types of Apex Locators versus Digital Periapical Radiography for Working Length Determination in Maxillary Premolars: An In Vitro Study. *Clin. Pract.* **2022**, *12*, 1043-1053.

Kaufman AY, Keila S, Yoshpe M. Accuracy of a new apex locator: an in vitro study. Int Endod J. 2002 Feb;35(2):186-92.

The authors respond with a table but it was NOT included in the revised manuscript. Please add this table.

In updated Version 3, Page: 12, Line: 261-280

○ The choice of 5.25% NaOCl should be justified. It would have been more practical to use 2.5% as this is the most commonly used concentration clinically.

Kindly add justification in the discussion section.

In updated Version 3, Page: 13, Line: 308-309

○ It is recommended that diagnostic accuracy of the apex locators in presence of the different variables be recorded in terms of specificity and sensitivity as compared to the actual working length measured. This information would be a better guide for the clinician and as such authors can make a recommendation regarding the most "accurate" apex locator to use and in association with which conditions.

I appreciate the authors' attempts by including this table which again should be added to the main revised text however, this is not what is meant by specificity and sensitivity. While I understand that this may be laborious to measure now, it should again be mentioned in the discussion section that diagnostic accuracy studies usually require these two measures to make a valuable deduction of clinical significance.

In updated Version 3, Page: 15, Line: 382-383

○ The results section should include a table for the demographic data of the sample including mean and SD of the types of teeth included, lengths of the teeth, number of canals (the authors mention single rooted but not the number of canals nor canal configurations), age of patients if present, gender....etc.

Perhaps age and gender might not be available (and this should be mentioned in the text for the reasons the authors mentioned about anonymity of samples) however, the type of teeth, lengths, number of canals....etc should all be easy to demonstrate and should be included.

All the data were collected and tabulated and uploaded on the link bellow:

Rifaat S: The Effect of Coronal Pre-flaring and Root Canal Irrigant on Apex Locators Accuracy: In-Vitro Study. [Dataset]. *figshare.* **2023. 10.6084/m9.figshare.22492354.v4**

In updated Version 3, Page: 5, Line: 82

Discussion:
- ○ The discussion is well-written but the authors should show the clinical significance of the results as previously commented placed in the context of the multiple variables that are present in the clinical situation. The authors showed based on the results of their study that the type of irrigating solution has an influence on measuring WL using these two apex locators? So what do the authors recommend? A particular sequence to use? A specific irrigant to use only during WL determination? In order to give value to results from laboratory studies their clinical significance must be shown.

In updated Version 3, Page: 15, Line: 396-400

The response to this comment again should be included in the discussion section.
- ○ There should also be a section on the limitations of the study such as the limitation of performing the study on single rooted teeth, the influence of curvatures, the influence of combining different irrigants, influence of embedding medium, volume of irrigant and other limitations that may influence the extrapolation of the results in a clinical sense. Especially since most of these points were not mentioned in the discussion and it is advisable to do so.

Thank you for adding limitations section

References

1. Ramezani M, Bolbolian M, Aliakbari M, Alizadeh A, et al.: Accuracy of Three Types of Apex Locators versus Digital Periapical Radiography for Working Length Determination in Maxillary Premolars: An In Vitro Study.*Clin Pract*. 2022; **12** (6): 1043-1053 PubMed Abstract | Publisher Full Text

2. Kaufman AY, Keila S, Yoshpe M: Accuracy of a new apex locator: an in vitro study.*Int Endod J*. 2002; **35** (2): 186-92 PubMed Abstract | Publisher Full Tex

Competing Interests: No competing interests were disclosed.

Reviewer Report 19 September 2023

? **Amira Salem** 🆔

¹ Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, Otago, New

Zealand

2 Blis Technologies Limited, Dunedin, New Zealand

3 Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, Otago, New Zealand

4 Blis Technologies Limited, Dunedin, New Zealand

The Revised version of the manuscript has been improved greatly from the first version, and many comments and confusions has been addressed and clarified.

However, there is still few comments I would like to add:

1- In the abstract: "The significant results were shown ..." word significant only doesn't indicate whether it is significantly accurate or significantly inaccurate. although it is understood from the results later in the article, it is better to make it more clear.

2-Hypothesis: the wording of the hypothesis can be improved, not in a past tense statement?! and more clearer

3-Methods: a repetition in the following sentence "Using freshly mixed alginate, each tooth was mounted at the cemento-enamel junction (CEJ) level using freshly mixed alginate.

4- Results:
a -"Moreover, the dry medium showed the most accurate WL with the Raypex 6, the NaOCl demonstrated the most accurate WL with the Raypex 6 " this is very confusing !

b- The authors indicated that the outliers was adjusted in the results. Does the authors think that this is an error during the readings? how can this be clinically avoided ?

c- the standard deviations in all the readings are quite high ranging from (+/- 0.4 to 0.7) difference from the accurate Working length this gives a low general confidence on the accuracy and significance of the difference between either the irrigants used or the type of the apex locator. The authors can reflect on this in their discussion and the limitations of solely relying on their use for determining the accurate working length.

d- "Figures 2 and 3 present the pre-flaring WL measurements for Root ZX and Raypex 6 for all irrigating solutions. The median lengths for dry medium and NaOCl were almost the same, whereas the median length for CHX significantly decreased and was closer to the actual WL. This was only observed with RoothZX only ? i.e figure 2 only.

e- "Moreover, the dry medium displayed the most accurate WL with Raypex 6, followed by NaOCl achieved the most accurate WL with Root ZX, and CHX showed the most accurate WL with Root ZX in all coronal pre-flaring conditions This is also very confusing.

f-"Figure 5 illustrates a similar pattern for the Raypax 6 apex locator"
They are not similar; the dry and chx are similar but different from the sodium hypochlorite!

5- Discussion:

"When NaOCl was used as an irrigant.." In this paragraph an emphasis on "no flaring" as an advantage of accuracy with the Raypex 6 and sodium hypochlorite while the authors have already described in the previous paragraph and in table 3 that no flaring was better among all irrigants and both EAL used. It is statistically significant in this condition but not an exceptional from other observations!

6- Conclusion:
"In conclusion, the study results suggest that adherence to the endodontic principles of conventional access opening, coronal pre-flaring….. " However, the results of this study indicated that the determination of WL will be more accurate before pre-flaring even though it might be necessary for other aspects of the successful RCT procedure. This is not reflected in the conclusion.!

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endodontics, microbiology, molecular biology, biotechnology, bioengineering, product development, drug delivery

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 Oct 2023

Shimaa Rifaat

The Revised version of the manuscript has been improved greatly from the first version, and many comments and confusions has been addressed and clarified.

However, there is still few comments I would like to add:

1- In the abstract: "The significant results were shown ..." word significant only doesn't indicate whether it is significantly accurate or significantly inaccurate. although it is understood from the results later in the article, it is better to make it more clear.

Thank you for the comment. This was adjusted in V3 Abstract for your kind review.

2-Hypothesis: the wording of the hypothesis can be improved, not in a past tense statement?! and more clearer

Thank you for the comment. This was adjusted in V3, Page: 6, Line: 62.

3-Methods: a repetition in the following sentence "Using freshly mixed alginate, each tooth was mounted at the cemento-enamel junction (CEJ) level using freshly mixed alginate.

Thank you for the comment. The repeated sentence has been removed.

4- Results:
a -"Moreover, the dry medium showed t̲h̲e̲ ̲m̲o̲s̲t̲ ̲a̲c̲c̲u̲r̲a̲t̲e̲ ̲W̲L̲ with the Raypex 6, the NaOCl demonstrated t̲h̲e̲ ̲m̲o̲s̲t̲ ̲a̲c̲c̲u̲r̲a̲t̲e̲ ̲W̲L̲ with the Raypex 6 " this is very confusing !

With Raypex 6 the most accurate results were shown with dry medium, NaOCl irrigant, and CHX respectively in all pre-flaring conditions.

b- The authors indicated that the outliers was adjusted in the results. Does the authors think that this is an error during the readings? how can this be clinically avoided?

The authors did not attribute the presence of any reading errors, but acknowledged that outliers may arise from various causes, including samples measurement and samples anatomy variations. Therefore, avoiding the outliers clinically could be achieved by using calibrated instruments for precision readings, training operators to use instruments correctly, adhering to standardized data collection procedures, carefully verifying data for errors prior to analysis, and employing statistical methods to detect and eliminate outliers resulting from measurement errors. These measures can lower the likelihood of measurement-induced outliers and enhance result accuracy.

c- the standard deviations in all the readings are quite high ranging from (+/- 0.4 to 0.7) difference from the accurate Working length this gives a low general confidence on the accuracy and significance of the difference between either the irrigants used or the type of the apex locator. The authors can reflect on this in their discussion and the limitations of solely relying on their use for determining the accurate working length.

The high standard deviations in the measurements indicate a considerable degree of variability in the data. This variability can be attributed to a range of factors, such as the inherent limitations of the apex locators, or potential errors in the experimental setup.

The results of the study indicate that EALs devices may not consistently provide precise measurements, as suggested by the wide standard deviations. EALs are valuable tools in root canal measurement, they should not be the sole basis for determining working length, and other complementary methods or clinical judgment should be used to enhance accuracy and reliability.

d- "Figures 2 and 3 present the pre-flaring WL measurements for Root ZX and Raypex 6 for all irrigating solutions. The median lengths for dry medium and NaOCl were almost the same, whereas the median length for CHX significantly decreased and was closer to the actual WL.
This was only observed with RoothZX only ? i.e figure 2 only.

Yes. This was observed for CHX irrigant when using Root ZX.

e- "Moreover, the dry medium displayed the most accurate WL with Raypex 6, followed by NaOCl achieved the most accurate WL with Root ZX, and CHX showed the most accurate WL with Root ZX in all coronal pre-flaring conditions. This is also very confusing.

With Raypex 6 the most accurate results were shown with dry medium, NaOCl irrigant, and CHX respectively in all pre-flaring conditions. Whereas Root ZX showed the most accurate results with CHX irrigant, dry medium, and NaOCl respectively in all coronal pre-flaring conditions.

f-"Figure 5 illustrates a similar pattern for the Raypax 6 apex locator"
They are not similar; the dry and chx are similar but different from the sodium hypochlorite!

Adjusted in updated V3 manuscript. Page:10, Line: 232-233.

5-Discussion:
"When NaOCl was used as an irrigant.." In this paragraph an emphasis on "no flaring" as an advantage of accuracy with the Raypex 6 and sodium hypochlorite while the authors have already described in the previous paragraph and in table 3 that no flaring was better among all irrigants and both EAL used. It is statistically significant in this condition but not an exceptional from other observations!

It has been demonstrated from previous studies that performing coronal pre-flaring has a substantial impact on enhancing the precision of measuring the working length of molars' root canals. This improvement is not solely attributed to better access but also to the reduction in file resistance, which, in turn, facilitates the smooth insertion of files into the root canal towards the tooth's apex. Additionally, it promotes improved circulation of irrigation within the root canal, thereby decreasing the likelihood of debris being pushed out towards the apex and reducing the risk of instrument breakage. It's worth noting that while previous studies utilized rotary files for canal preparation, the present study employed Gates Glidden instruments. (Discussed in the Introduction V3 Page: 5, Line: 48-54)

6- Conclusion:
"In conclusion, the study results suggest that adherence to the endodontic principles of conventional access opening, coronal pre-flaring….. " However, the results of this study indicated that the determination of WL will be more accurate before pre-flaring even though it might be necessary for other aspects of the successful RCT procedure. This is not reflected in the conclusion.!

The study's findings emphasize that sticking to established endodontic principles like conventional access opening, coronal pre-flaring, and maintaining patency are crucial for obtaining precise and consistent measurements of the working length (WL) with Root ZX and Raypex 6 electronic apex locators (EALs). However, the effect of coronal pre-flaring wasn't clear due to the use of Gates Glidden instruments instead of rotary instruments that showed better results.

Page: 13, Line: 302-307

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 19 June 2023

? **Rania El Backly** 🆔

[1] Endodontics, Conservative Dentistry Department and Tissue Engineering Laboratories, Faculty of Dentistry, Alexandria University, Alexandria, Alexandria Governorate, Egypt
[2] Endodontics, Conservative Dentistry Department and Tissue Engineering Laboratories, Faculty of Dentistry, Alexandria University, Alexandria, Alexandria Governorate, Egypt
[3] Endodontics, Conservative Dentistry Department and Tissue Engineering Laboratories, Faculty of Dentistry, Alexandria University, Alexandria, Alexandria Governorate, Egypt

In general:

The manuscript addresses an important topic relevant to the everyday practice of endodontics. However, it requires substantial language editing and rewriting to be more concise and focused. It is recommended to follow the PRILE 2021 guidelines for documenting laboratory studies in endodontics.

Title:

I suggest modification of the title to be as follows:

The influence of coronal pre-flaring and type of root canal irrigant on accuracy of working length determination using electronic apex locators

Abstract:

The abstract needs to be re-written to clearly demonstrate the specific objective of the study as well as the hypothesis. The methodology is unclear and non-specific. The groups are not clear. Results must be mentioned to clearly show quantitative data and show the differences between the groups used. Again, the conclusion must be more specific and clearly reflect the specific outcomes of this study.

Introduction:
- ○ The authors are advised to add a hypothesis statement at the end of the introduction section.

- ○ The rationale of the study is unclear. Coronal pre-flaring is a routine part of endodontic instrumentation and the irrigants used in this study are routinely used in everyday clinical practice in association with apex locators, so why are the authors assuming that these variables will affect the overall accuracy? This must be clearly shown.

Methods:
- ○ Sample size calculation: The exact number of teeth per group must be mentioned.

- ○ Sample selection and sample preparation sections contain repeated information.

- ○ The grouping is not clear. Upon reading the methodology it seems that the 120 teeth were subjected to all conditions of measurements? This must be clearly shown in a table and a flow chart should be included to show the exact procedure.

- ○ The experimental set-up needs to be more accurately described.

- ○ The settings used for each apex locator must be clearly defined. "mid-green area on the screen" is not precise.

- ○ How was irrigation done? The volume used? The type of needle and gauge used? And depth of insertion of the needle?

- ○ How long was the duration of irrigation?

- ○ Why did the authors not use EDTA? Additionally, the sequence of using EDTA and NaOCl is the most common combination of irrigating solutions used especially since working length is often verified in the middle of instrumentation procedures under the influence of mixtures of these irrigants together. It is well-known in endodontics that combinations of irrigants are often used so testing combinations of irrigants would have given higher validation of the results of this study.

- ○ There is a major concern that the same samples were used for all the different variables. Other studies separated the entire sample into groups to avoid the influence of all of the intermediate procedures on the measurements taken last. How do the authors justify this? It would have been better to divide the sample into sub-groups for each tested variable especially since each sample represents its own control and this division of the samples would not have influenced the results, on the contrary would have enhanced the precision of the results.

Results:
- ○ The tables and figures demonstrate that the values measured are the working length values in mm however, this is impossible since the values are even in the negative? So what do these values represent? Do they represent the values registered on the apex locator screens according to the pre-determined settings? I.e. the number registered on the screen when the device indicates that this is the CORRECT working length? If so these values are quite

misleading. Also, how is it possible that the ACTUAL working length is 0.087+-0.445? The authors should record the entire working length in mm from the coronal reference point to the apical constriction minus 0.5 mm as they mentioned in the methodology.

○ The frequency of the difference between the actual working length and that measured in each condition by the two apex locators should be shown as this is the true measure that would truly demonstrate which technique was of higher accuracy in determining the WL.

○ Check:
- ○ Ramezani, M.; Bolbolian, M.; Aliakbari, M.; Alizadeh, A.; Tofangchiha, M.; Faegh, S.M.; Patini, R.; D'Amato, G. Accuracy of Three Types of Apex Locators versus Digital Periapical Radiography for Working Length Determination in Maxillary Premolars: An In Vitro Study. *Clin. Pract.* **2022**, *12*, 1043-1053.
- ○ Kaufman AY, Keila S, Yoshpe M. Accuracy of a new apex locator: an in vitro study. Int Endod J. 2002 Feb;35(2):186-92.

○ The choice of 5.25% NaOCl should be justified. It would have been more practical to use 2.5% as this is the most commonly used concentration clinically.

○ It is recommended that diagnostic accuracy of the apex locators in presence of the different variables be recorded in terms of specificity and sensitivity as compared to the actual working length measured. This information would be a better guide for the clinician and as such authors can make a recommendation regarding the most "accurate" apex locator to use and in association with which conditions.

○ The results section should include a table for the demographic data of the sample including mean and SD of the types of teeth included, lengths of the teeth, number of canals (the authors mention single rooted but not the number of canals nor canal configurations), age of patients if present, gender….etc.

Discussion:
○ The discussion is well-written but the authors should show the clinical significance of the results as previously commented placed in the context of the multiple variables that are present in the clinical situation. The authors showed based on the results of their study that the type of irrigating solution has an influence on measuring WL using these two apex locators? So what do the authors recommend? A particular sequence to use? A specific irrigant to use only during WL determination? In order to give value to results from laboratory studies their clinical significance must be shown.

○ There should also be a section on the limitations of the study such as the limitation of performing the study on single rooted teeth, the influence of curvatures, the influence of combining different irrigants, influence of embedding medium, volume of irrigant and other limitations that may influence the extrapolation of the results in a clinical sense. Especially since most of these points were not mentioned in the discussion and it is advisable to do so. Additionally, a statement of accepting or rejecting the null hypothesis which should be mentioned in the introduction section is recommended.

References

1. Ramezani M, Bolbolian M, Aliakbari M, Alizadeh A, et al.: Accuracy of Three Types of Apex Locators versus Digital Periapical Radiography for Working Length Determination in Maxillary Premolars: An In Vitro Study.*Clin Pract*. 2022; **12** (6): 1043-1053 PubMed Abstract | Publisher Full Text
2. Kaufman AY, Keila S, Yoshpe M: Accuracy of a new apex locator: an in vitro study.*Int Endod J*. 2002; **35** (2): 186-92 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endodontics, regenerative endodontics, tissue engineering, regenerative medicine

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Jul 2023
Shimaa Rifaat

Dear reviewer (2), (Dr. Rania Al Backly)
Thank you very much for your valuable comments. They are a great addition to our research. Regarding the mentioned points, kindly check below for further details.

1) In general:
The manuscript addresses an important topic relevant to the everyday practice of endodontics. However, it requires substantial language editing and rewriting to be more concise and focused. It is recommended to follow the PRILE 2021 guidelines for documenting laboratory studies in endodontics.

Thank you for your comment and further editing and rewriting will be done in the updated version to make it clear for the readers.

2) Title: I suggest modification of the title to be as follows:
The influence of coronal pre-flaring and type of root canal irrigant on accuracy of working length determination using electronic apex locators.

Thank you for your comment. And I suggest modifying it too to be:
"The effect of coronal pre-flaring and type of root canal irrigant on working length accuracy using electronic apex locators."

3) Abstract:
The abstract needs to be re-written to clearly demonstrate the specific objective of the study as well as the hypothesis. The methodology is unclear and non-specific. The groups are not clear. Results must be mentioned to clearly show quantitative data and show the differences between the groups used. Again, the conclusion must be more specific and clearly reflect the specific outcomes of this study.

Updated Abstract:
Background: The success of root canal treatment relies on achieving proper root canal preparation and precise root canal filling, including reaching the full working length up to the apical constriction. Electronic apex locators (EALs) have been introduced to enhance the accuracy of working length determination. However, the optimal irrigation protocol for each generation of EALs remains unclear
Hypothesis: No significant difference in the accuracy of working length determination would be detected by coronal pre-flaring and the type of irrigant, varying with different generations of EALs
Methods: Actual working lengths of 120 single-rooted human teeth were measured and compared with working lengths determined by the 3rd generation EAL (Root ZX) and 6th generation EAL (Raypex 6). Measurements were taken in a dry medium, in the presence of 5.25% sodium hypochlorite, and 2% chlorhexidine. The teeth were evaluated both without coronal pre-flaring and after coronal pre-flaring using the same irrigating solutions. Data were collected, tabulated, and analyzed using one-way ANOVA with post-hoc tests to assess significant differences in average working length accuracy between the actual working length, Root ZX, and Raypex 6 EALs
Results: The results of the current study showed the best results with the Raypex 6 apex locator were in dry medium in all conditions of pre-flaring (without pre-flaring & with pre-faring) (T=3.8, P=0.001) and (T=5.4, P=0.001) respectively. Root ZX apex locator achieved significant results with the CHX in all conditions of pre-flaring (without pre-flaring & with pre-faring): (T=3.85, P=0.001) and (T=3.99, P=0.001) respectively
Conclusion: The study found that the Raypex 6 electronic apex locator (EAL) provided significantly more accurate readings closer to the actual working length compared to the Root ZX EAL. The best results with the Raypex 6 were obtained when measurements were taken in a dry medium, in all the pre-flaring conditions. The Root ZX EAL achieved significant results with the use of chlorhexidine (CHX) as an irrigant in all the pre-flaring conditions.

4) Introduction:

A) The authors are advised to add a hypothesis statement at the end of the introduction section.

Hypothesis: No significant difference in the accuracy of working length determination would be detected by coronal pre-flaring and the type of irrigant, varying with different generations of EALs.

B) The rationale of the study is unclear. Coronal pre-flaring is a routine part of endodontic instrumentation and the irrigants used in this study are routinely used in everyday clinical practice in association with apex locators, so why are the authors assuming that these variables will affect the overall accuracy? This must be clearly shown.

Thank you for your question. It is clear nowadays that some research studies recommended the Contracted endodontic cavities (CECs) that were developed from the concept of minimally invasive dentistry and provide an alternative to traditional endodontic cavities (TECs). They claimed that the CECs preserve the mechanical stability of teeth and preserve more of the dentin as discussed by Lovisi et al 2018, Weiqi et al 2022, Wang et al 2023. That's why the working length determination without coronal flaring was used in this study as it is a way some researchers recommended and supported by some of them.

Alovisi M, Pasqualini D, Musso E, Bobbio E, Giuliano C, Mancino D, Scotti N, Berutti E. Influence of contracted endodontic access on root canal geometry: an in vitro study. Journal of endodontics. 2018 Apr 1;44(4):614-20.

Weiqi Peng, Xuedong Zhou, Yuan Gao, Xin Xu, Effect of Access Cavity Preparation on Dentin Preservation, Biomechanical Property, and Instrumentation Efficacy: A Micro–Computed Tomographic Study, Journal of Endodontics, Volume 48, Issue 5,2022, 659-668, ISSN 0099-2399, https://doi.org/10.1016/j.joen.2021.12.012

*Wang, X., Wang, D., Wang, Yr. et al. Effect of access cavities on the biomechanics of mandibular molars: a finite element analysis. BMC Oral Health **23**, 196 (2023). https://doi.org/10.1186/s12903-023-02878-3*

In addition, the effect of the irrigant on the reading accuracy and the electrical conductivity of various irrigant used and how it affects the accuracy of electronic apex locators. This will work hand in hand with the most accurate determination of the WL. Therefore, it would ensure the root canal treatment outcome and prognosis.

5) Methods:

A) Sample size calculation: The exact number of teeth per group must be mentioned.

120 teeth were used. All the sample size experienced all the preparation scenarios and under all conditions to minimize the variables for among all the variables for each sample.

B) Sample selection and sample preparation sections contain repeated information.

Thank you for your comment. It will be revised and adjusted in the updated version.

C) The grouping is not clear. Upon reading the methodology it seems that the 120 teeth were subjected to all conditions of measurements. This must be clearly shown in a table and a flow chart should be included to show the exact procedure.

Yes, the 120 teeth were subjected to all conditions of measurements. This is supplied in the flow chart linked to the original manuscript on a figshare link.
For your kind review: *Rifaat S: The Effect of Coronal Pre-flaring and Root Canal Irrigant on Apex Locators Accuracy: In-Vitro Study. [Dataset]. figshare. 2023. 10.6084/m9.figshare.22492354.v4*

D) The experimental set-up needs to be more accurately described.

Thank you for your comment. It will be described clearly in the updated version.

E) The settings used for each apex locator must be clearly defined. "mid-green area on the screen" is not precise.

For Root ZX, working length determination was established at the point when the screen displays the line just in the middle of apex and 1 mark which corresponds to 0.5 mm short of radiologic apex. For Raypex 6, the third green line, just before the yellow lines, which corresponds to 0.5 mm short of radiographic apex was determined for working length. The measures were recorded as electronic measurement (EM) if they were stable for at least 5 seconds as described by Aydin et al 2015.

Moreover, When the file tip reaches or is close to the apical constriction, the apex locator's screen display typically indicates this by showing the file position within the mid-green area. Moreover, both Root ZX and Raypex 6 EALs also have an audio indicator that emits a sound, such as a continuous or intermittent beep, when the file approaches or reaches the apical constriction. The change in sound indicates that the file is close to the desired working length, assisting the clinician in achieving precise measurements. Furthermore, both Root ZX and Raypex 6 feature a digital display that shows the distance between the file tip and the apical constriction in millimeters as reported by Higa et al 2009. Besides, Calibration of EALs before each use, according to the manufacturer's instructions was performed to ensure precision of WL measurements as recommended by Cinar et al 2020.

Aydin U, Karataslioglu E, Aksoy F, Yildirim C. In vitro evaluation of Root ZX and Raypex 6 in teeth with different apical diameters. J Conserv Dent. 2015 Jan-Feb;18(1):66-9. doi: 10.4103/0972-0707.148899. PMID: 25657531; PMCID: PMC4313483"

Higa RA, Adorno CG, Ebrahim AK, Suda H. Distance from file tip to the major apical foramen in relation to the numeric meter reading on the display of three different electronic apex locators. Int Endod J. 2009;42(12):1065-1070. doi:10.1111/j.1365-2591.2009.01629.x"

"Çınar F, Üstün Y. Ex Vivo Evaluation of the Accuracy of 3 Electronic Apex Locators in Different

Environments: A Micro-Computed Tomography Study. Eur Endod J. 2020 Dec;5(3):226-230. doi: 10.14744/eej.2020.30633. PMID: 33353910; PMCID: PMC7881377."

F) How was irrigation done? The volume used. The type of needle and gauge used. And depth of insertion of the needle?

Irrigation was done with 23G with apical vented needle (Ultrdent Products, South Jordan, UT, USA). The needle was introduced until resistance was felt and then reduced 1 mm from the needle penetration depth to prevent obliteration of the root canal lumen as illustrated by Guerreiro et al 2013. 3 ml of 5.25% NaOCl and 2% CHX were used alternatively in the presence and absence of coronal pre-flaring as used by Spoorthy et al 2013.

Guerreiro-Tanomaru JM, Loiola LE, Morgental RD, Leonardo RD, Tanomaru-Filho M. Efficacy of four irrigation needles in cleaning the apical third of root canals. Brazilian Dental Journal. 2013;24:21-4.

Spoorthy E, Velmurugan N, Ballal S, Nandini S. Comparison of irrigant penetration up to working length and into simulated lateral canals using various irrigating techniques. International endodontic journal. 2013 Sep;46(9):815-22.

G) How long was the duration of irrigation?

It was for 2 minutes as suggested by Fernandes et al 2021.

Fernandes AL, da Silva Limoeiro AG, Kato AS, Pelegrine RA, de Martin AS, Rocha DG, da Silveira Bueno CE. Analysis of two irrigation methods in root canal disinfection against E. Faecalis biofilm under the influence of the concentration, volumen, and time in contact of the irrigant. Research, Society and Development. 2021 Jul 13;10(8):e32610817478.

H) Why did the authors not use EDTA? Additionally, the sequence of using EDTA and NaOCl is the most common combination of irrigating solutions used especially since working length is often verified in the middle of instrumentation procedures under the influence of mixtures of these irrigants together. It is well-known in endodontics that combinations of irrigants are often used so testing combinations of irrigants would have given higher validation of the results of this study.

Thank you for your valuable comment. In our research we preferred to use only one irrigant sequence to prevent the results bias. As if this recommended protocol used, the authors will not be able which irrigant electroconductivity is higher: the NaOCl or the EDTA. But as we have a benchmark after our study results, we can consider using combinations with the NaOCl in the future to evaluate their accuracy.

I) There is a major concern that the same samples were used for all the different variables. Other studies separated the entire sample into groups to avoid the influence of all of the intermediate procedures on the measurements taken last. How do the authors justify this? It would have been better to divide the sample into sub-groups for each tested variable especially since each sample represents its own control and this division of the samples

would not have influenced the results, on the contrary would have enhanced the precision of the results.

Thank you for the nice comment. We agree with you. But we tried to eliminate any variable that could take place among each group. That's why we subjected each tooth was its same anatomical variation, diameter, length, dentin thickness to all the study variables to be more precise while calculating the deviation from the actual working length. Because all the mentioned points above could contribute to many changes while collecting the study results and could affect the study accuracy.

6) Results:
A) The tables and figures demonstrate that the values measured are the working length values in mm however, this is impossible since the values are even in the negative? So what do these values represent? Do they represent the values registered on the apex locator screens according to the pre-determined settings? I.e. the number registered on the screen when the device indicates that this is the CORRECT working length? If so these values are quite misleading. Also, how is it possible that the ACTUAL working length is 0.087+-0.445? The authors should record the entire working length in mm from the coronal reference point to the apical constriction minus 0.5 mm as they mentioned in the methodology.

Thank you for your comment. Regarding the readings for our results, it is the difference between the mean WL determined by the 2 calibrated evaluators and the actual working length. Considering the working length is at zero difference and we are calculating the difference or deviation from the zero either increased by (+) or decreased by (-).

B) The frequency of the difference between the actual working length and that measured in each condition by the two apex locators should be shown as this is the true measure that would truly demonstrate which technique was of higher accuracy in determining the WL.

Thank you for your valuable comment. The below table shows the frequency of deviations from actual working length for both electronic apex locators with each irrigation medium in the absence and presence of coronal pre-flaring. The frequencies are presented in table below were calculated for the differences between the measured and the actual WL within the range of ±0.5 and greater than 1. In the absence of coronal pre-flaring the Root ZX (3rd generation) showed more accuracy as most of the values were within the range of ±0.5 in each irrigation system, whereas Raypex 6 (6th generation) showed less accuracy than Root ZX while having majority of the values greater than 1 which is showing the far distance from actual WL. A similar trend was observed in irrigation media used in the presence of coronal pre-flaring except in dry medium where Raypex 6 apex locator showed more accuracy with the most values near to ±0.5.

Medium	EAL	WL deviation in ±0.5		WL deviation > 1		WL deviation in ±0.5		WL deviation > 1	
		No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
Dry	3rd generation	111	94.1	2.5	2.5	99	83.9	4	3.4
	6th generation	102	86.4	10.2	10.2	104	88.1	14	11.9
NaOCl	3rd generation	104	88.1	2.5	2.5	102	86.4	7	5.9

	6th generation	100	84.7	12.7	12.7	84	71.2	32	27.1
CHX	3rd Generation	107	90.7	4.2	4.2	114	96.6	4	3.4
	6th Generation	96	81.4	15.3	15.3	101	85.6	17	14.4

C) The choice of 5.25% NaOCl should be justified. It would have been more practical to use 2.5% as this is the most commonly used concentration clinically.

A 2021 study by Fernandes et al 2021 showed that the 5.25% NaOCl showed a significantly greater reduction in E. faecalis than 2.5% NaOCl. Therefore, some researchers suggested the usage of the 5.25% NaOCl.

Fernandes AL, da Silva Limoeiro AG, Kato AS, Pelegrine RA, de Martin AS, Rocha DG, da Silveira Bueno CE. Analysis of two irrigation methods in root canal disinfection against E. Faecalis biofilm under the influence of the concentration, volumen, and time in contact of the irrigant. Research, Society and Development. 2021 Jul 13;10(8):e32610817478-.

D) It is recommended that diagnostic accuracy of the apex locators in presence of the different variables be recorded in terms of specificity and sensitivity as compared to the actual working length measured. This information would be a better guide for the clinician and as such authors can make a recommendation regarding the most "accurate" apex locator to use and in association with which conditions.

Thank you for your comment. The table below shows the relative error for each apex locator for accuracy of measurement of WL. Root ZX was found to be the most accurate measurement tool for WL in Dry (1.57%) and CHX (1.54%) irrigation system. Whereas with NaOCL, Raypax 6 showed high accuracy (1.76%) in teeth without coronal pre-flaring. However, in presence of pre-flaring, Root ZX showed high accuracy in NaOCL (2.21%) and CHX (1.93%) compared to Raypex 6.

Irrigation Solution	Apex Locator	Relative Error	
		Without Pre-flaring	With Pre-flaring
Dry	3rd generation	1.57%	2.48%
	6th generation	1.58%	1.74%
NaOCl	3rd generation	1.97%	2.21%
	6th generation	1.76%	2.61%
CHX	3rd Generation	1.54%	1.93%
	6th generation	2.03%	2.28%

E) The results section should include a table for the demographic data of the sample including mean and SD of the types of teeth included, lengths of the teeth, number of canals (the authors mention single rooted but not the number of canals nor canal configurations), age of patients if present, gender....etc.

Regarding the demographic data for the sample, it was selected as follows: single rooted

teeth with single canals of almost same length. Regarding the age and gender of the patient's, …. etc. We don't have this data as teeth were collected from the disposal of IAU dental hospital where patient identification were kept anonymous even after signing in the consent.

7) Discussion:
A) The discussion is well-written, but the authors should show the clinical significance of the results as previously commented placed in the context of the multiple variables that are present in the clinical situation. The authors showed based on the results of their study that the type of irrigating solution has an influence on measuring WL using these two apex locators? So, what do the authors recommend? A particular sequence to use? A specific irrigant to use only during WL determination? To give value to results from laboratory studies their clinical significance must be shown.

The author's suggestions are:
While using the 6th generation EAL (Raypex 6), dry medium is the best way to measure the most accurate WL. On the other hand, while using the 3rd generation EAL (Root ZX), it is better to use 2% CHX while measuring the WL to achieve the most accurate readings. Therefore, it is very important to know the specific irrigating medium used with each specific EAL to achieve the most accurate WL results during the root canal treatment.

B) There should also be a section on the limitations of the study such as the limitation of performing the study on single rooted teeth, the influence of curvatures, the influence of combining different irrigants, influence of embedding medium, volume of irrigant and other limitations that may influence the extrapolation of the results in a clinical sense. Especially since most of these points were not mentioned in the discussion and it is advisable to do so. Additionally, a statement accepting or rejecting the null hypothesis which should be mentioned in the introduction section is recommended.

Thank you for your comment.

-Regarding the limitation of the study:
Our study was conducted only on single rooted teeth with initial file size 15 while using one type of irrigant at a time to be used as a benchmark for further studies in the future. But our study limitations may include: the usage curved canals teeth with various degrees of curvature and with bigger apical foramen sizes may affect the given results. Furthermore, the usage of one irrgant at a time results may be different. Moreover, agitation of the irrigating solution may affect the electrical conductivity and hence affects the reading accuracy of EAL. Irrigation volume, concentration, temperature, and way of application may influence the reading accuracy as well. In addition, applying the current study in-vivo in the presence of the patient's body fluids may have an influence as well. All these points should be taken into consideration in any future studies.

-The null hypothesis was partially rejected due to the differences in the obtained results from the different irrigating solutions when used with each EAL. While it showed no significant difference between with/without coronal flaring groups.

Reviewer Report 05 June 2023

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The order of procedure?
Was CHX used on the same tooth as a last testing group, if so how do you justify that the results is not because multiple file insertions and a partial debridement specially after using sodium hypochlorite solution so it is giving more accurate results with this apex locator. Was there any testing group that has CHX used immediately after the dry group?

Table 3 label?
Check the label: all have Root ZX label

In all tables is the "mean" WL compared to the measured working length by microscope?
If so this means that either the positive or negative value closer to the zero is the most accurate? Please clarify

What are the numbers beside asterisks indicate? How many outliers were excluded from the mean measured.

In discussion: interpretation contradicts results "but the closest mean WL with actual WL was recorded in without coronal pre-flaring irrigation groups" comment on table 3.
"accurate results were observed in the canals that were prepared with coronal pre-flaring before working length (WL) determination in comparison to the canals that were prepared without coronal pre-flaring"? in discussion.

"However, it is contradicted by a previous study conducted by João Marcelo da Silva Teixeira *et al.* (2012) who concluded that the usage of Gates Glidden burs for cervical pre-flaring did not significantly influence the accuracy of the apical placement of the apex locator when determining the actual working length due to insufficient removal of coronal dentin when compared with the rotary system for preparing coronal pre-flaring" Not clear statement: was Gates Glidden burs better than rotary system or not, or there was no difference.

"Consequently, the difference was statistically significant in the roots with coronal pre-flaring prior to working length determination when using Raybex 6 apex locator and NaOCl as an irrigant" Compared to what?

"While comparing all the groups of Raypex 6 (with or without coronal pre-flaring) regardless of the type of irrigant used, significant results were seen when compared to the same groups of Root ZX (T=2.86, p=0.005)". More accurate? If so, not true statement related to table 3?

"In the case of using NaOCl as an irrigant, it showed significant results when used with Raypex 6 even without coronal pre-flaring" compared to what? To RootZX? With pre flaring it is less accurate.

The results showed that the readings when using Raypex 6 (6th generation electronic apex locator) are significantly closer to the actual working length than Root ZX (3rd generation electronic apex locator) for the with/without coronal pre-flaring groups. Doesn't match with the results of table 3.

In accordance with our study, Root ZX apex locator achieved significant results with the CHX : maybe due to that it is the last tested irrigant and could be due to more debris and tissues removed as it is also better Root ZX in all groups for after flaring.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endodontics, microbiology, molecular biology, biotechnology, bioengineering, product development, drug delivery

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Jul 2023
Shimaa Rifaat

Dear Reviewer (1), (Dr. Amira Salem)
Thank you very much for your valuable comments. They are a great addition to our research. Regarding the mentioned points, kindly check below for further details.

1) The order of procedure? Was CHX used on the same tooth as a last testing group, if so, how do you justify that the results is not because multiple file insertions and a partial debridement specially after using sodium hypochlorite solution so it is giving more accurate results with this apex locator. Was there any testing group that has CHX used immediately after the dry group?

Yes, CHX was used for the same tooth as the last testing group, without using the CHX immediately after the dry group.
A) We inserted the file only once for each medium (Dry, NaOCl, and CHX).
B) We used distilled water between each medium to wash, neutralize and stop the effect of each material used before moving to the next.
C) NaOCl is used routinely after access opening to dissolve all the pulp tissue remnants before the step of WL determination in all samples. Afterwards, the Dry, NaOCl, or CHX media was used to check the working length in all samples while using the electronic apex locators.
D) The materials were used as a transmitting media not a debridement agent.
E) The minimum exposure time for maximum effectiveness for the NaOCl is 20 minutes followed by 5minutes MTAD as a final rinse to ensure the complete debridement as stated by Lotfi et al 2012. Moreover, Bonnie et al 2009 reported that the maximum effectiveness for the 5% NaOCl as an antibacterial agent is at 40 minutes usage. In addition, Chen et al 2023 found that the application of NaOCl alone could be ineffective during chemical preparation of the canal and it should be agitated either by heat, ultrasonically, or followed by the usage of other materials. This concluded that only in this situation we can use NaOCl as a debridement agent otherwise it will not be an effective debriding agent, hence, the materials used in the current study was used only as transmitting medium for WL reading accuracy while using the EALs.

Lotfi M, Moghaddam N, Vosoughhosseini S, Zand V, Saghiri MA. Effect of Duration of Irrigation with Sodium Hypochlorite in Clinical Protocol of MTAD on Removal of Smear Layer and Creating Dentinal Erosion. J Dent Res Dent Clin Dent Prospects. 2012;6(3):79-84. doi:10.5681/joddd.2012.017

Bonnie Retamozo, Shahrokh Shabahang, Neal Johnson, Raydolfo M. Aprecio, Mahmoud Torabinejad, Minimum Contact Time and Concentration of Sodium Hypochlorite Required to Eliminate Enterococcus faecalis, Journal of Endodontics, Volume 36, Issue 3, 2010, 520-523, ISSN 0099-2399, https://doi.org/10.1016/j.joen.2009.12.005.

Chen Cai, Xuan Chen, Yang Li, Qianzhou Jiang, "Advances in the Role of Sodium Hypochlorite Irrigant in Chemical Preparation of Root Canal Treatment", BioMed Research International, vol. 2023, Article ID 8858283, 17 pages, 2023. https://doi.org/10.1155/2023/8858283.

Table 3 label? Check the label: all have Root ZX label

Thank you for your comment. Adjustments were done in the updated manuscript.

In all tables is the "mean" WL compared to the measured working length by microscope? If so this means that either the positive or negative value closer to the zero is the most accurate? Please clarify.

Yes, you are correct. The means that are either positive or negative value closer to zero are the most accurate values.

What are the numbers beside asterisks indicate? How many outliers were excluded from the mean measured.

A) The numbers beside Asterisks are the P value that indicates the significance in the given results.
B) The value of outliers was replaced with the median of the data. They were not deleted but adjusted.

In discussion: interpretation contradicts results "but the closest mean WL with actual WL was recorded in without coronal pre-flaring irrigation groups" comment on table 3. "accurate results were observed in the canals that were prepared with coronal pre-flaring before working length (WL) determination in comparison to the canals that were prepared without coronal pre-flaring"? in discussion.

Thank you for your comment. Adjustments were done in the updated manuscript.

"However, it is contradicted by a previous study conducted by João Marcelo da Silva Teixeira *et al.* (2012) who concluded that the usage of Gates Glidden burs for cervical pre-flaring did not significantly influence the accuracy of the apical placement of the apex locator when determining the actual working length due to insufficient removal of coronal dentin when compared with the rotary system for preparing coronal pre-flaring" Not clear statement: was Gates Glidden burs better than rotary system or not, or there was no difference.

Thank you for your comment. Rephrasing the statement to make it clearer were done as follows and modified in the text. "However, a prior study conducted by João Marcelo da Silva Teixeira *et al.* (2012) reported that the usage of Gates Glidden burs for cervical pre-flaring

did not significantly influence the accuracy of the apical placement of the apex locator when determining the actual working length. This may be due to the insufficient removal of coronal dentin when G.G. were used. On the contrary, the usage of rotary system for preparing coronal pre-flaring can significantly influence the WL determination.

"Consequently, the difference was statistically significant in the roots with coronal pre-flaring prior to working length determination when using Raypex 6 apex locator and NaOCl as an irrigant" Compared to what?

Consequently, the difference was statistically significant in the roots with coronal pre-flaring prior to working length determination when using Raybex 6 apex locator and NaOCl as an irrigant (T=4.5, p=0.0001) when compared with roots without pre-flaring and using NaOCl.

"While comparing all the groups of Raypex 6 (with or without coronal pre-flaring) regardless of the type of irrigant used, significant results were seen when compared to the same groups of Root ZX (T=2.86, p=0.005)". More accurate? If so, not true statement related to table 3?

Thank you for your comment, it is adjusted in the discussion.

"In the case of using NaOCl as an irrigant, it showed significant results when used with Raypex 6 even without coronal pre-flaring" compared to what? To RootZX? With pre flaring it is less accurate.

In case of using NaOCL as an irrigant the mean score was significantly higher in Raypex 6 apex locator for coronal pre-flaring (0.464±0.64, p-<0.001)

The results showed that the readings when using Raypex 6 (6th generation electronic apex locator) are significantly closer to the actual working length than Root ZX (3rd generation electronic apex locator) for the with/without coronal pre-flaring groups. Doesn't match with the results of table 3.

Thank you for your comment. Rephrasing was done as follows to make it clearer. The results showed that the readings when using Raypex 6 (6th generation electronic apex locator) are significantly closer to the actual working length than Root ZX (3rd generation electronic apex locator) for the with/without coronal pre-flaring in CHX irrigant system and in overall comparison when irrigant systems were not considered.

Competing Interests: N/A

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