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Article

In Vitro Accuracy of Two Different Electronic Apex Locators Under Various Conditions

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Abstract: Background. The purpose of this study was to analyze the accuracy of the two apex locators using seven different canal irrigants. Methods. 40 multi-rooted extracted teeth were included in this study. The actual canal length (ACL) was determined using a #10 file until the tip was visualized (12× magnification) just within the apical foramen (AF). The teeth were placed in a conductive medium of alginate to test the ACL and electronic canal length (ECL) using both apex locators in various conditions. Seven irrigant solutions were used in the root canal: 0.9% saline, FileEZE EDTA 19%, Glyde EDTA 17%, Consepsis V Chlorhexidine 2% CHX, Chlorcid 3%, citric acid 20%, and EDTA 18%. Results. Within a range of ± 0.5 mm from the ACL, the Raypex showed an accuracy of 87.5% with 0.9% saline solution, 82.5% with Chlorcid 3%, and 75% with Consepsis V CHX 2% solution. The Ipex showed an accuracy of 82.5% with 0.9% saline solution, 80% with Chlorcid 3%, and 70% with Consepsis V CHX 2% solution. The differences among the canal irrigants were significant for both the Raypex ($p = 0.021$) and Ipex ($p < 0.0001$) devices. The mean values showed the greatest variations in ECL accuracy with the combination of the Raypex and EDTA 18% ($p = 0.042$) and with the Ipex and Glyde EDTA 17% ($p = 0.026$). Conclusions. Canal irrigants have an impact on the accuracy of apex locators. The apex locators showed an accuracy of 80% and greater when 0.9% saline and Chlorcid 3% solutions were used.

Keywords: apex locator; working lengths; irrigation solutions; sodium hypochlorite; chlorhexidine; endodontics

1. Introduction

Determining the correct working length (WL) of a root canal is essential and an important factor for successful endodontic treatment. Underestimation of the WL can lead to insufficient debridement of the root canal, while overestimation may result in damage to the periapical tissues, which will delay or prevent healing [1]. Although the apical constriction may vary widely in shape, it is generally defined as the narrowest portion of the canal that has the smallest diameter blood supply. It creates the smallest wound site and provides optimal healing conditions [2]. According to histological studies, the apical constriction is often at some distance (0–2 mm) from the radiographic apex [3]. Another study showed distances from 0.5 to 0.75 mm from the anatomical to apical constriction, with great variations between the anterior and posterior teeth [4]. Therefore, locating the exact terminus of the canal at the apical constriction is an important clinical factor [5].

The development of electronic apex locators (EALs) stemmed from the findings of Suzuki in the year 1942 [6]. It was established that the electrical resistance between the periodontal ligament and the oral mucosa of dogs in vivo was a constant value. The first generation EALs were resistance-based, the second generation impedance-based, and the third generation frequency-based [7]. They had many limitations, so mostly fourth and fifth generation devices are employed currently. What is typical for fourth generation devices is that they measure and compare the complex electrical characteristics of the root canal using two or more frequencies of electrical impulses [8]. Nowadays, there is also sixth generation of devices, which represent modified version of fifth generation [9].

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Conductive solutions used as canal irrigants inside the root canal have been reported to affect the accuracy of EALs in vivo, in vitro and ex vivo [8,10–34]. It is also reported that different irrigants and methods also affect the accuracy of EALs under in vitro conditions [16,18–24,26–34], but some studies showed no impact on the performance of apex locators [24–28].

Sodium hypochlorite (NaOCl) is used in many situations during endodontic treatment with concentrations from 0.5% to 6% because of its antibacterial properties and ability to rapidly dissolve remains of pulpal tissue. Also, chlorhexidine (CHX) is used as antimicrobial agent (exhibit bacteriostatic or bactericidal) in forms of solution or gel. Ethylenediaminetetraacetic acid (EDTA) is chelating agent, used to remove mineral debris and smear layer in endodontic treatment. The varying accuracy of different available electronic apex locators under various canal irrigation solutions is problematic for clinicians. Moreover, a comparative evaluation of different apex locators under similar simulated clinical conditions is deficient in the literature, as well as the influence of different root canal irrigants. Also, there are many commercial root canal irrigation products on the market with different chemical compositions that can affect the accuracy of EALs differently. However, a limited number of studies have evaluated the influence of different root canal irrigants on the accuracy of different EALs.

The aim of this study was to determine the impact of seven different root canal irrigation preparations used in clinical practice on the accuracy of two different EAL devices. The null hypotheses tested were as follows: (i) the accuracy of both EALs would not depend on the type of root canal irrigation agent used and (ii) there is no difference in the accuracy of measurement between the two EALs

2. Materials and Methods

Teeth Selection and Preparation

This study design was similar to previously published studies and well described [7,18–20].

The study protocol has been evaluated and approved by the Local Ethics Committee of School of Dentistry University of Zagreb. Forty multi-rooted human teeth extracted and collected for orthodontic or surgical reasons used in this study. The number of samples used in the study was determined by the number of samples in previous studies, which is usually around 40 samples for *in vitro* research of this type. The teeth were stored in 2% chloramine solution. The teeth were radiographed, and those with complicated anatomy, external root resorption, immature roots, root fractures, and severe curvature were excluded from the study. The remaining tissues on the external root surface were removed using a periodontal curette. All teeth were cut horizontally with a high-speed diamond burr to provide a consistent reference point for future measurements. In each multi-rooted tooth, one canal was chosen and marked with nail polish.

The root canals were prepared by one operator (W.D.), experienced in clinical practice. After access to the cavities were made, the canal orifices were enlarged with a Gates Glidden drill, type G ISO 50-150 (Komet Dental, Lemgo, Germany), and a Protaper Hand Use/Shaping File SX (Densply/Maileffer, Mont-sur-Lausann, Switzerland) in a crown-down technique. Canals were irrigated with 1% sodium hypochlorite and the excess solution was removed with cotton pellets and paper points.

Length Measurement

The canal length and canal patency up to the apical foramen (AF) were determined by introducing a #10 or #15 K-type file (WDW, VDW, Műnchen, Germany) into the root canal until the tip was level with the apical foramen under 12× magnification with an Olympus SZX-10 microscope (Olympus Europa Holding GMBH, Hamburg, Germany). Then, a S1 Protaper hand file with two silicone stops was introduced into the root canal until the tip was seen to be level with the apical foramen under 12× magnification with the Olympus microscope. The silicone stop was stabilized at the reference point on the tooth. The S1 file was removed, and the distance between the silicone stop

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and the file tip was measured with a digital caliper (±0.01 mm). This measurement in millimeters was the actual canal length (ACL) and was repeated for all tested teeth three times. This method of measurement of the canal length was taken as the gold standard (GS).

The teeth were placed in a conductive medium of alginate (GC Aroma Fine Normal Set, GC Corporation, Tokyo, Japan) to simulate periodontium. The alginate was mixed according to the manufacturer's instructions and packed in a plastic box (Figure 1). The teeth were kept in position until the alginate had set completely. When not in use, the model was wrapped in a wet paper towel and refrigerated to provide a moist environment throughout the experiment.

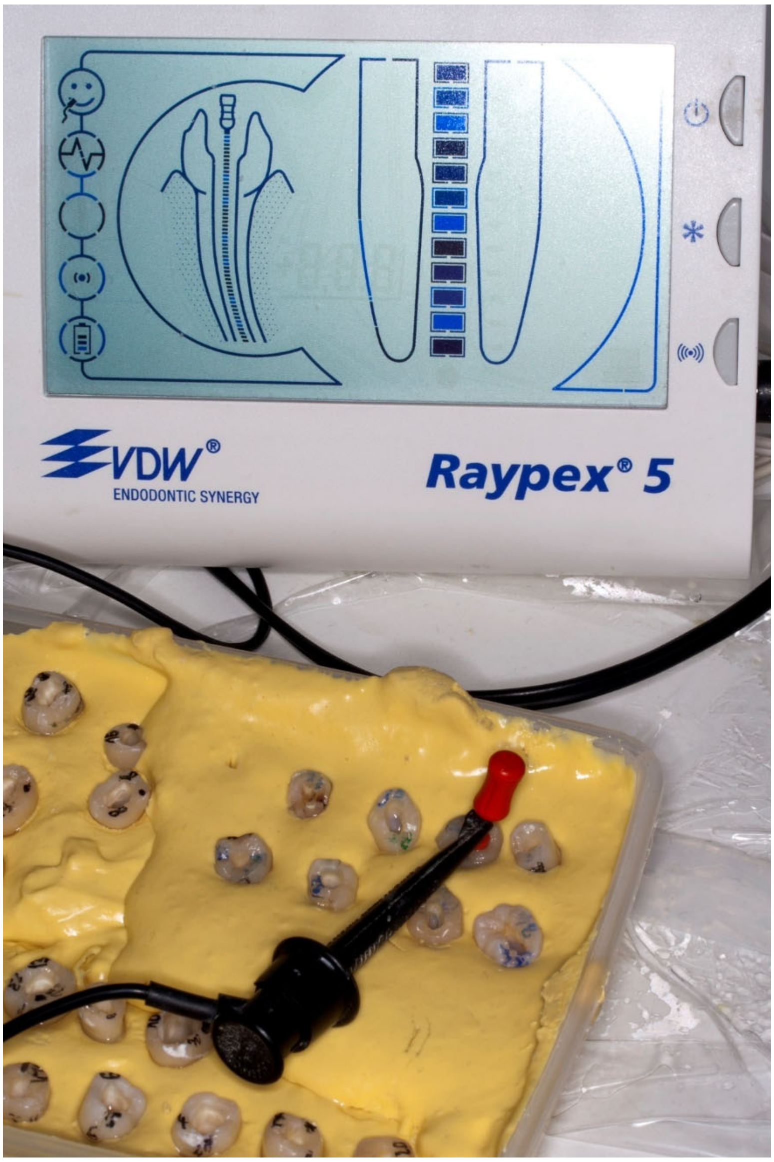

Figure 1. Alginate measuring model with Raypex EAL showing last red line as EAL/AF.

Electronic Length Measurement

The EALs used in this study were the Raypex 5 (VDW, Munchen, Germany) and the Ipex (NSK, Tokyo, Japan), which were used in accordance with the manufacturer's instructions. Raypex belongs to the 5th generation of EAL, and Ipex to the 4th.

The clip of EAL was attached to the alginate model and the electrode was connected to the Protaper S1 endodontic file with canal irrigant solution inside. In order to determine the canal length from a reference point to the supposed AF, for the Raypex was the red line on the display, and for the Ipex was the “0.0” mark on the display. The measurements were considered correct if the

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instrument remained stable for at least 5 s. The two silicone stoppers on the S1 file were carefully positioned with respect to the reference point on the tooth. The file was removed and the distance was measured with a digital caliper under 2.5 × magnifying glasses. This measurement represented the electronic apical length (EAL) and was repeated three times. For each EAL, measurements were taken with seven different irrigant solutions in the root canal using a Navitips cannula (Ultradent Products, South Jordan,UT, USA):

- 0.9% saline (Pliva Hrvatska, Zagreb, Croatia),
- Ultradent FileEZE EDTA 19%,
- Glyde EDTA 17% (Densply products, York, PA, USA),
- Ultradent Consepsis V Chlorhexidine 2% CHX,
- Ultradent Chlorcid 3%,
- Ultradent Citric Acid 20%,
- Ultradent EDTA 18%.

After applying each irrigant for measuring EALs, excess solution was removed from the cut surface of the dental specimen and pulp chamber with cotton pellets, but without an attempt to dry the canal or the surrounding area. The canals were thoroughly rinsed with distilled water and dried with paper points before applying another irrigant solution.

Each canal irrigation solution (EALs measurements), will be compared to the gold standard ACL

Statistical Analysis

The statistical analysis was performed with the PASW Statistics 17.0 (SPSS, Chicago, USA) software package. The ACL and ECL were compared for each tooth, as well as for every irrigant solution and both EALs. The differences between the ACL and ECL were calculated for every irrigant solution and both EALs with an analysis of variance (ANOVA) test.

The Kolmogorov–Smirnov test showed that all variables were normally distributed ($p > 0.05$); therefore, for the analysis of statistical differences between irrigant solutions, a one-way ANOVA test was performed for repetitive measurements. The differences between the groups were identified by Bonferroni corrections. A t-test was used to analyze the differences between the two apex locators ($p = 0.05$).

3. Results

The discrepancies of both EALs were set to the ± 0.5 mm range, resulting in six groups increasing from negative to positive values, according to the distance from the AF (Groups A to F). A negative value means that the EAL measurement was shorter than the ACL measurement, and a positive value means that the EAL measurement was longer than the ACL measurement. Tables 1 and 2 show the discrepancies between ACL and EAL values for different canal irrigants.

An ANOVA test with a Greenhouse-Geisser correction showed that the differences between the canal irrigants were statistically significant ($F(3.766; 55.328) = 10.779$, $p < 0.0001$) for the Ipex EAL, and a post hoc Bonferroni correction showed which canal irrigants were different among the groups; the measurements and p-values are shown in Table 3. The results for the Ipex EAL showed statistically significant differences among all the canal irrigants, and showed the largest discrepancy with Glyde EDTA 17%.

A paired t-test was used to analyze both EALs with the same canal irrigant. A statistically significant difference was found with the combinations of File EZE EDTA 19% for the Raypex and Ipex EALs ($p = 0.001$) and for Glyde EDTA 17% with the Raypex and Ipex ($p = 0.015$) devices. The Raypex EAL was more accurate with File EZE EDTA 19%, Glyde EDTA 17%, Consepsis V 2% CHX, and EDTA 18%. The Ipex EAL was more accurate with Chlorcid 3%, 0.9% saline, and citric acid 20%. The smallest discrepancies were found with 0.9% saline, Chlorcid 3%, and Consepsis V 2% CHX (Figure 2).

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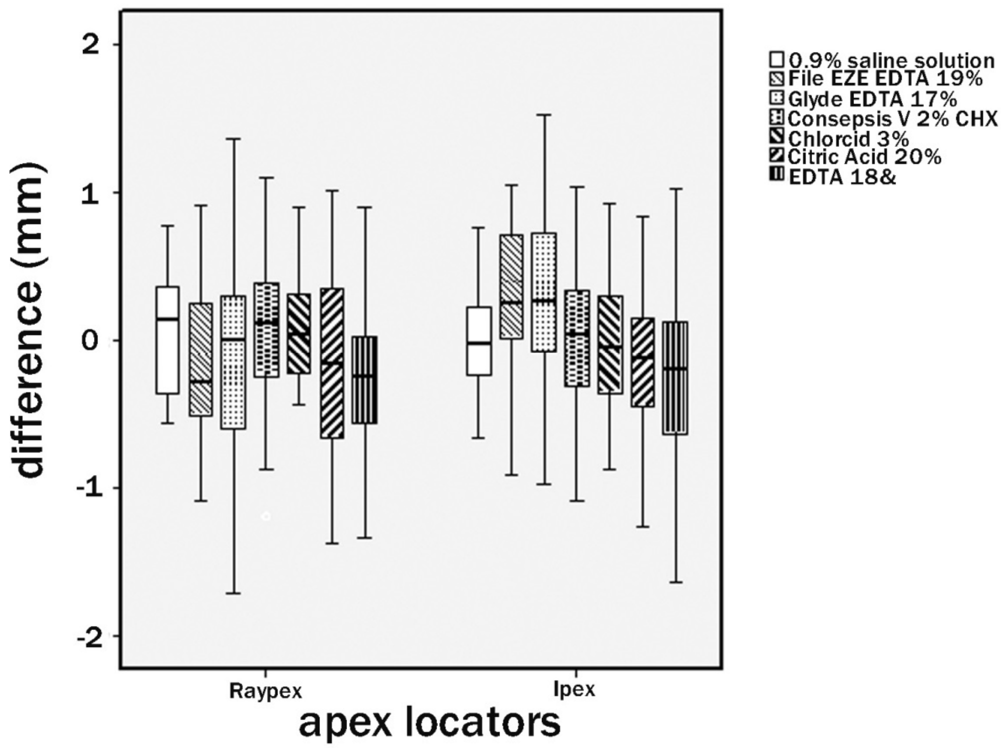

Figure 2. Discrepancies between the ACL and ECL for both EALs and different canal irrigants.

Table 1. Discrepancies for the Raypex 5 EAL with different canal irrigants.

	Raypex 5						
Groups (mm)	**0.9% saline**	**File EZE EDTA 19%**	**Glyde EDTA 17%**	**Consepsis V 2% CHX**	**ChlorCid 3%**	**Citric Acid 20%**	**EDTA 18%**
<-1 (A)	0(0%)	1(2.5%)	9(22.5%)	1(2.5%)	0(0%)	4(10%)	1(2.5%)
-1.00 to -0.5 (B)	2(5%)	10(25%)	1(2.5%)	4(10%)	0(0%)	9(22.5%)	10(25%)
-0.5 to 0.0 (C)	**15(37.5%)**	**14(35%)**	**10(25%)**	**12(30%)**	**17(42.5%)**	**11(27.5%)**	**18(45%)**
>0.0 to 0.5 (D)	**20(50%)**	**11(27.5%)**	**12(30%)**	**18(45%)**	**16(40%)**	**11(27.5%)**	**7(17.5%)**
0.5 to 1 (E)	3(7.5%)	4(10%)	5(12.5%)	4(10%)	7(17.5%)	4(10%)	4(10%)
> 1 (F)	0 (0%)	0(0%)	3(7.5%)	1(2.5%)	0(0%)	1(2.5%)	0(0%)
Total	40	40	40	40	40	40	40

C and D measurements mean clinically acceptable accuracy of EALs.

Table 2. Discrepancies for the Ipex EAL with different canal irrigants.

Groups (mm)	Ipex						
	0.9% saline	**FileEZE EDTA 19%**	**Glyde EDTA 17%**	**Consepsis V 2% CHX**	**ChlorCid 3%**	**Citric Acid 20%**	**EDTA 18%**
<-1 (A)	0(0%)	0(0%)	0(0%)	2(5%)	0(0%)	3(7.5%)	2(5%)
-1.00 to -0.5 (B)	3(7.5%)	4(10%)	2(5%)	3(7.5%)	4(10%)	5(12.5%)	10(25%)

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-0.5 to 0.0 (C)	**18(45 %)**	**5(12.5%)**	**10(25%)**	**11(27.5%)**	**19(47.5%)**	**17(42.5%)**	**15(37.5%)**
>0.0 to 0.5 (D)	**15(37.5 %)**	**17(42.5%)**	**13(32.5%)**	**17(42.5%)**	**13(32.5%)**	**10(25%)**	**11(27.5%)**
0.5 to 1 (E)	4(10%)	12(30%)	12(30%)	6(15%)	4(10%)	5(12.5%)	1(2.5%)
> 1 (F)	0(0 %)	2(5%)	3(7.5%)	1(2.5%)	0(0%)	0(0%)	1(2.5%)
Total	40	40	40	40	40	40	40

C and D measurements mean clinically acceptable accuracy of EALs.

Table 3. Differences between the canal irrigants and EALs.

	Mean value	SD	N
Raypex 0.9% saline[1]	0.038	0.362	40
Raypex File EZE EDTA 19%[2]	-0.165	0.503	40
Raypex Glyde EDTA 17%	-0.083	0.837	40
Raypex Consepsis V 2% CHX	0.047	0.484	40
Raypex ChlorCid 3%[2,3]	0.087	0.381	40
Raypex Citric Acid 20%	-0.183	0.649	40
Raypex EDTA 18%[1,3]	-0.239	0.488	40
Ipex 0.9% saline[4]	0.017	0.351	40
Ipex File EZE EDTA 19%[5,6]	0.266	0.513	40
Ipex Glyde EDTA 17%[7,8,9,10]	0.299	0.549	40
Ipex Consepsis V 2% CHX[4]	0.048	0.519	40
Ipex ChlorCid 3%[8]	-0.017	0.433	40
Ipex Citric Acid 20%[5,9]	-0.141	0.533	40
Ipex EDTA 18%[4,6,10]	-0.253	0.520	40

*Statistical difference Raypex: [1]p = 0.042, [2]p = 0.027, [3]p = 0.034. Statistical difference Ipex: [4]p = 0.005, [5]p = 0.008,[6]p = 0.001, [7]p = 0.009, [8]p = 0.026, [9]p = 0.001, [10]p < 0.0001.

4. Discussion

Studies have reported the accuracy of EALs for in vitro models using various canal irrigants [7,17–20,22,24–30]. In the presence of dried canals and a tolerance level of ±0.5 mm, the Raypex showed an accuracy of 80%, 82.07%, and 85.59% with instrument sizes 08, 10, and 15, respectively [31]. Measurements within the ±0.5 mm clinical tolerance range are considered to be highly accurate. We agree that a tolerance of ±0.5 mm can be permitted, because this accuracy level cannot be reached with any other working length determination method in clinical practice [34]. Our results showed that the Raypex EAL was accurate in the range of ±0.5 mm between 55% and 87.5%, depending on canal irrigant. Additionally, the Ipex showed a similar accuracy of 55% to 82.5% within the ±0.5 mm range, depending on canal irrigant.

If a tolerance of ±1 mm was allowed, the accuracy reached 96.5%, 95.14%, and 96.88% with instrument sizes 08, 10, and 15, respectively [34]. No statistically significant differences were observed between the Raypex 5 and ApexDal for all intracanal media tested (2% chlorhexidine solution, gel and 2% NaOCl). In this study, good results were achieved only when canals were filled with CHX solution or CHX gel. Measurement accuracy was 79.3% and 86.2%, respectively, for the devices. Measurements that were made in canals irrigated with 2% NaOCl were correct only in 53.2% and 48.2% of cases for Raypex 5 and ApexDal EAL, respectively [35].

In the presence of saline and 17% EDTA, measurements were closer to the AL, while those performed in dry canals or in the presence of xylol were shorter than the AL [18]. The authors concluded that EDTA and saline can be considered reliable solutions for electronic measurements. In the present study, 0.9% saline solution and 3% NaOCl (Chlorcid) showed the most accurate readings

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within a tolerance of ±0.5 mm for both EALs. Chlorcid, or 3% NaOCl according to manufacturer's composition data, also showed the smallest discrepancies for both EALs between the ACL and ECL. Another study concluded that NaOCl concentration had no effect on the accuracy of the Root ZX EAL [29]. One study reported that chlorhexidine gave the least variability in EAL performance [20]. Another study concluded that there were no differences in canal measurements in the presence of chlorhexidine (0.2%) or NaOCl . One published paper showed that the accuracy of neither the Ipex nor the Root ZX EAL was affected by 2.5% NaOCl or 2% CHX. Yet, study reported that chlorhexidine digluconate (2%) showed the best results, in contrast to 3% NaOCl and 2% lidocaine, but the results were not significant [21]. In newer generations of EALs, multiple frequencies are used to determine WL, resulting in a more precise WL measurement in the presence of different electrolytes. However, the presence of highly electroconductive irrigants, such as saline, local anesthetic solution, CHX, blood, hydrogen peroxide, EDTA, irrigating fluids, and NaOCl, may compromise the accuracy of the EAL's performance [33]. However, significant differences were observed between the readings of the Ipex and Root ZX, irrespective of whether 2.5% NaOCl or 2% CHX was used as the irrigant [36]. These results were not similar to those found in our study, but can be explained by the different chlorhexidine concentrations (0.1% and 0.2% versus 2%) or by the in vivo or in vitro conditions used. Chlorhexidine digluconate is an antiseptic and has an affinity for hydroxyapatite. Another study showed that 0.8% CHX and RC Prep may not be clinically acceptable because of measurement error, in contrast to NaOCl and EDTA [7]. Despite the fact that third generation EALs can work under chelating canal irrigants, measurement errors are still possible. An explanation could include dentine conductivity changes from the chelating ability of canal irrigants, which could have an impact on impedance of the EALs. One study utilized various irrigant solutions, including NaOCl, CHX, ozonated water, EDTA (gel and solution), and distilled water. The findings showed that the use of NaOCl resulted in less accurate results in determining WL compared to CHX. However, there was no statistically significant difference for both EALs when used with NaOCl [24]. Regarding the concentration of NaOCl solution, they did not significantly affect the performance of EALs. The best results were obtained with Root ZX II for 2% NaOCl and Dual Pex for 5.25% NaOCl, but without statistically significant difference [26]. Another study showed influence of heated and nonheated canal irrigants. The accuracy in the presence of 0.1% Octenidine dihydrochloride and 2% CHX was higher than 5% NaOCl heated and nonheated in both the EAL.

One paper reported accuracy of different EALs and effect of variations in 5.25% NaOCl temperature. With a tolerance range of ± 0.5 mm, Raypex 6 displayed variable accuracy measuring 95% at 19.4 °C (± 1.5 °C), decreasing to 80% at 36 °C, before returning to 95% at 70 °C. [37]

Also, the EAL overestimation was seen in the presence of 0.1% OCT and 2% CHX and underestimation was seen in the presence of 5% sodium hypochlorite heated and nonheated. [22] The presence of the 2.5% sodium hypochlorite (NaOCl), 0.9% saline solution (NaCl), and 17% EDTA, affected the accuracy of Dentaport ZX and Rootor EAL, and most accurate measurements were obtained in dry canals. [23]

In one study, with two apex locators, saline and EDTA gave precise results close to the actual length. Thus, these irrigants can be considered as reliable solutions for electronic measurements. Large deviation occurred with the more conductive solutions such as NaOCl and Chlorhexidine digluconate. [28] The meta-analysis from fifteen studies regarding accuracy of different generations of apex locators in determining working length showed that there was no significant difference among the generations of EAL in determination of working lenght. [9]

Also, another study compared the accuracy of working length of two generations of EAL (3rd vs 4th). Statistically, both third generation and fourth generation apex locators are equally accurate for determination of working length. [38] However, the type of irrigant appears to affect the accuracy of the EAL with respect to its generation, so it is very important to know the specific irrigating medium used with each specific EAL to achieve the most accurate ACL results during the root canal treatment. [9]

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When using tolerance ±0.5mm, the Raypex and Ipex showed accuracy of 64.29% under 2.5% NaOCl, which were less then results in our study. [40] Nasiri et al. reported accuracy of 90.9% for Raypex 6 EAL using tolerance ±0.5mm and under 0.9% NaCl irrigant. [41]

Ipex II, also a 4th generation EAL like Ipex, showed an accuracy of 90% with a tolerance of ±0.5mm with canal irrigation agent 5.25% NaOCl. [42]. Even with artificially created canal perforations and various canal irrigation agents, Ipex and other EALs showed clinically acceptable results, although they were better in dry canals. [43] Based on our work and on the basis of numerous other researches, the exact conclusions about the difference in the accuracy of different EALs with regard to their generation. Therefore, we can conclude that 4th and 5th generations of EALs used in this study have similar results and are quite accurate.

The measured canal length was adversely affected by different canal irrigant solutions. The Raypex EAL showed an accuracy of 87.5% for 0.9% saline solution and 82.5% for Chlorcid (3% NaOCl), and the Ipex EAL showed an accuracy of 82.5% with 0.9% saline solution and 80% for Chlorcid (3% NaOCl). The greatest variations in accuracy were for the combinations of the Raypex and EDTA 18% and for the Ipex and Glyde EDTA 17% ($p = 0.026$). It can be concluded that all canal irrigant solutions have a greater or lesser impact on the accuracy of the EAL. Some authors suggest that for clinicians, most appropriate root canal irrigation's protocol with the least negative impact on the accuracy of EALs could be combination of 17% EDTA after NaOCl or CHX in contrast to use NaOCl alone due to its high electroconductivity.[44]

Solutions with high electrical conductivity such as saline, anesthetic solution and hypochlorite may affect the accuracy of 4th generation of EALs. 1% hypochlorite with electrical conductivity 66 ms , pH 11.72 and EDTA with electrical conductivity 40 ms, pH 7.1 caused shortening of the canal length measured with EAL. CHX 2% with electrical conductivity 1ms and pH6.5 caused longer canal length. We can conclude that electroconductive solutions significantly reduce the canal impedance and can lead to shorter measurements due to high electroconductivity. [45,46]

Therefore, we agree based on the results of our study that the different electroconductivity of root canal irrigation solutions, among other factors, affects the accuracy of EALs measurements. We must keep in mind that this and other *in vitro* studies were performed under ideal conditions, therefore the accuracy of EALs may be significantly different than in clinical work, mainly due to factors such as complex root canal morphology, debris and pulp remains, saliva contamination, and other factors. Most commercial canal irrigant solutions have an impact on measurements due to the materials conductivity. An explanation could be that the complex composition of these solutions has an influence on hard dental tissue as well as on the EAL reading. Correct and detailed chemical compositions of canal irrigants are scarce, and most canal irrigant manufacturers consider it confidential data, making it hard to analyze or determine which chemical compounds have an influence on EALs. Also, the clinician should know which generation of EAL he is working with, in order to know their performance in different canal irrigants.

5. Conclusions

Canal irrigants have an impact on the accuracy of both apex locators. The apex locators showed an accuracy of 80% and greater when 0.9% saline and Chlorcid solutions were used. The limitations of this research are that the experimental model cannot fully duplicate the biological tissue and complexity of root canals in clinical work, therefore greater deviations in accuracy are likely to occur in clinical work. Despite the EAL manufacturer's claims of accurate and precise measurements with various canal irrigants and selling them on the dental market today, we suggest more studies analyzing the accuracy of different EALs under various conditions.

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Abbreviations

The following abbreviations are used in this manuscript:

ACL	Actual Canal Length
EALs	Electronic Apex Locators
AF	Apical Foramen
ECL	Electronic Canal Length
WL	Working Length
GS	Gold standard

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