

Influence of Embedding Medium on iPex Electronic Apex Locator Accuracy: An *Ex Vivo* Comparative Study Using Alginate, Saline, and Gelatin Models

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ABSTRACT

Aim: Accurate working length determination during root canal treatment is essential for achieving successful outcomes. This study aimed to evaluate the impact of embedding medium on the accuracy of iPex electronic apex locator (EAL).

Materials and methods: Sixty-one extracted single-rooted teeth were decoronated and coronally flared with Gates-Glidden burs. Actual canal length (ACL) was obtained by introducing a size 8 K-file until its tip reached the most coronal border of the apical foramen. This step was performed thrice and then averaged. Subtracting 0.5 mm from the ACL provided the working length (WL). The teeth were randomly placed in plastic containers filled with freshly mixed alginate, gelatin, or saline, with the lip clip placed in the medium. The blinded operator obtained electronic measurements using iPex by advancing a K-file with a size compatible with the canal attached to the file clip and advanced until the 0.0 mark, then withdrawn to the 0.5 mark. This step was performed thrice and then averaged. Data were analyzed using ANOVA and Tukey's *post hoc* test, with significance level set at 5% ($\alpha = 0.05$).

Results: The mean difference between WL and iPex length obtained in the gelatin model was significantly longer than the difference with mean iPex length in alginate ($p = 0.005$) and in saline ($p < 0.001$). There was no significant difference between iPex readings obtained in alginate and saline ($p = 0.249$).

Conclusion: The use of freshly mixed alginate or saline for *ex vivo* assessment of iPex is recommended, whereas the use of gelatin could increase the chances of readings longer than looked for.

Clinical significance: Identifying the optimum embedding medium for *ex vivo* testing of EALs permits the comparison and assessment of several factors affecting EALs' precision under standardized conditions. This helps in understanding EAL performance *in vivo* and in optimizing its clinical utilization.

Keywords: Apex locators, Electronic apex locator, Electronic working length, Embedding medium, Endodontics, iPex, Working length determination.

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INTRODUCTION

The most favorable outcome of root canal treatment relies on cleaning and shaping of the entire root canal system to its full length, followed by sealing with appropriate root filling.¹ Thus, accurate working length determination is a pivotal step during root canal treatment. According to the latest glossary of endodontic terms by the American Association of Endodontists, working length is defined as "The distance from a coronal reference point to the point at which canal preparation and obturation should terminate".² The determination of the apical termination point of the root canal system can be a challenging step for any clinician. The ideal end-point to terminate the root canal treatment is at the apical constriction, which is typically found 0.5–1 mm coronal to the radiographic apex.³ Any instrumentation beyond this point can lead to overinstrumentation, overfilling, mechanical or chemical irritation of the periapical tissue, and postoperative pain. All these factors increase the chances of root canal treatment failure. Similarly, short working length terminating before the apical constriction can result in underinstrumentation, incomplete cleaning and shaping, and possibly leaving remnants of pulpal tissue or microbial infection, all of which might compromise the success of root canal treatment.⁴

Studies have shown that the use of electronic apex locators (EALs) along with periapical radiographs for working length

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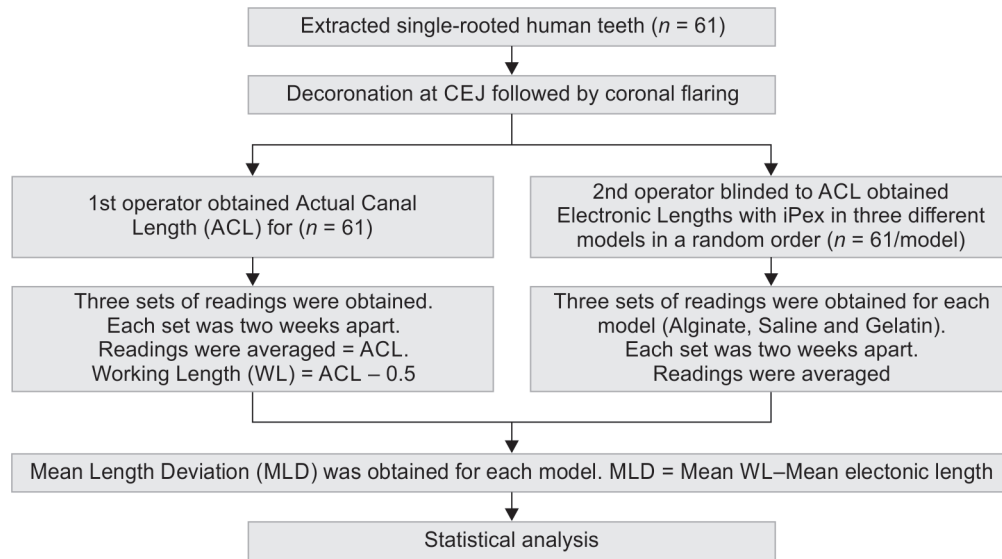
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measurement has significantly increased the chances of terminating the root canal treatment in proximity to the apical constriction.^{5–8} Hence, the use of EALs for working length determination during root canal treatment is considered a standard of care.⁹ The literature is replete of studies that have assessed and compared the performance of different EALs.^{10–13} Electronic apex locators were evaluated either *in vivo* or *ex vivo*.^{10,14} *Ex vivo* EAL assessment involves placing extracted teeth in a conductive medium that conveys the electric current and closes the circuit.^{15,16} Despite the fact that the passage of electrical current *in vivo* would be affected by parameters

Flowchart 1: Study design and steps



that might not be exactly replicated under *ex vivo* conditions,¹⁷ the *ex vivo* approach permits the comparison of different EALs under standardized conditions. This allows for a better understanding of the performance of the devices.¹⁸ Further, several studies have reported that *ex vivo* EAL readings are comparable to *in vivo* ones without any significant differences between them.^{19,20}

Different embedding media have been utilized to evaluate the precision of EALs.¹⁴ A thorough search of the relevant literature yielded a very few studies that have investigated the influence of embedding media on the accuracy of EALs.^{16,21–23} Root ZX EAL's readings varied when the same teeth were placed in alginate, gelatin, or saline, but the differences were not significant.²¹ However, Root ZX measurements were significantly longer when obtained with teeth placed in electroconductive gel compared with alginate.¹⁶ Readings of EALs from the 3rd-generation, Dentaport ZX, and the 4th-generation, Raypex 5, were affected by the type of embedding medium with Raypex 5 registering more accurate measurements compared with Dentaport ZX regardless of the type of the embedding medium.²² From the conflicting results of previous studies, it seems plausible that the embedding medium could have an impact on the accuracy of EALs.

Limited *ex vivo* studies have evaluated the accuracy of the 4th generation's multifrequency iPex EAL. These studies found that between 55.3% and 90% of the readings were within ± 0.5 mm from WL.^{24–27} All these studies utilized the alginate model to simulate the periodontium. However, none of these studies investigated the impact of different embedding media on the precision of iPex. Hence, an assessment of the effect of different embedding media on iPex is warranted. Thus, the aim of this *ex vivo* study was to investigate whether embedding teeth in different media, namely alginate, gelatin, or saline, affects the accuracy of iPex. The null hypothesis was that the precision of the iPex was not affected by the type of embedding medium used.

This is the first study that investigates the influence of different embedding media on the accuracy of the 4th-generation iPex electronic apex locator. The results of current research will significantly contribute to standardizing an important parameter for the future *in vitro/ex vivo* studies assessing the accuracy of EALs with the same electrical principles.

MATERIALS AND METHODS

Study Design and Setting

This *ex vivo* study was an experimental laboratory study (Flowchart 1). It was carried out at the Department of Endodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia, between December 2022 and April 2023. The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia (Approval Number: 133-10-22, date of approval 15 November, 2022).

Sample Calculation

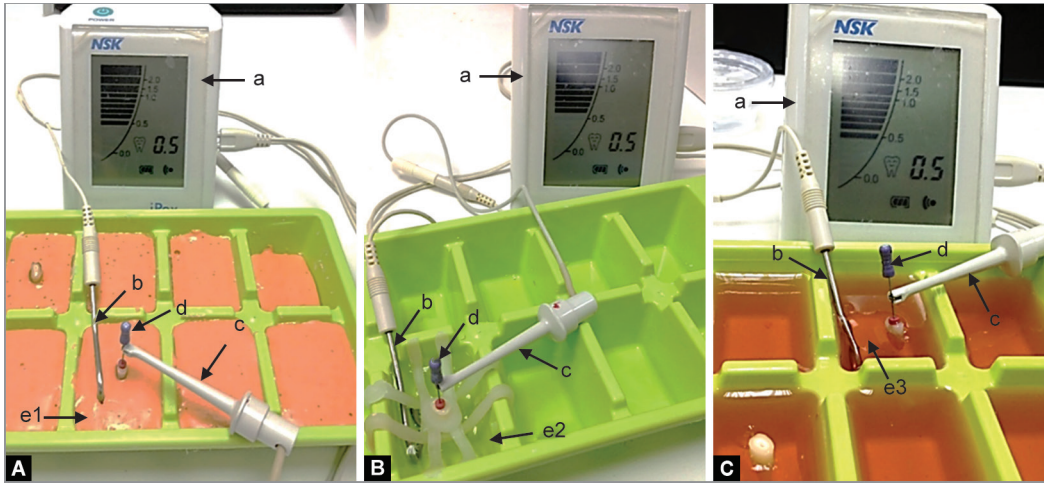
For this study, a priori power analysis was conducted utilizing the G*Power software (v3.1.9.6) to determine the appropriate sample size. Based on the findings of Chen et al.²² on premolars, an anticipated effect size of 0.24 was used. It was estimated that a minimum of 54 teeth in each of the three groups would be necessary to reach a statistical power of 80% and 0.05 alpha-error probability. Thus, the number of teeth included in this study was 61 in each group.

Sample Selection

This investigation was conducted on 61 single-rooted human teeth that were extracted for periodontal or orthodontic reasons. All extracted teeth were selected based on specific inclusion criteria. Included teeth were free of caries, restorations, root fillings, and root resorption. Furthermore, all teeth exhibited fully formed intact straight roots and had single canals based on buccolingual and mesiodistal periapical radiographs. The teeth that lacked previous characteristics were excluded. The samples were placed in 5% sodium hypochlorite (NaOCl) for 24 hours and stored in numbered bottles filled with saline until use.

Sample Preparation

All teeth were decoronated at the level of the cemento-enamel junction using diamond discs (Meisinger, Neuss, Germany) to produce a flat coronal reference point followed by coronal flare utilizing Gates-Glidden burs sizes 2 and 3 (Dentsply, Maillefer, Switzerland).^{28,29} Canals were irrigated with 5% NaOCl (2 mL)³⁰



Figs 1A to C: Experimental setup for the three tested models. (A) Alginate model; (B) Saline model; (C) Gelatin model. Model components: (a) iPex electronic apex locator; (b) Lip clip; (c) File clip; (d) K-file inserted in extracted tooth to acquire electronic working length; (e1) Alginate embedding medium; (e2) Saline-embedding medium; (e3) Gelatin-embedding medium

with a 27-gauge irrigation needle attached to a disposable syringe inserted in the canals as deep as possible without binding. The canals were then dried with paper points.

Determination of Actual Canal Length (ACL) and Working Length (WL)

Under 3.5 \times magnification, one operator passively inserted a size 8 K-file (Dentsply, Maillefer, Switzerland) until the file tip was apparent at the most coronal border of the apical foramen.^{18,31} The rubber stopper was adjusted to the coronal reference point, and the file was withdrawn and measured using a digital caliper (Mitutoyo Corp., Tokyo, Japan) to an accuracy of 0.01 mm. This step was performed three times, and the lengths were averaged to obtain the ACL. The first reading was obtained for the entire sample, then the second and third readings were recorded in a similar manner. Each reading was two weeks apart. The WL was determined by subtracting 0.5 mm from ACL.

Determination of iPex Electronic Lengths in Three Different Models

Another operator blinded to the WL utilized iPex (NSK, Tochigi, Japan) to obtain electronic readings. Plastic containers were filled with one of the following media: freshly mixed alginate (ChemPoint, Bellevue, WA, USA), saline (Bram-Cor, Parma, Italy), or gelatin (Foodmate Co., Ltd., Jiujiang City, China). The teeth were placed in the models, with a lip clip inserted into the medium. A fresh mix of alginate was poured every 30 minutes.²⁶ iPex was used according to the manufacturer's recommendations. The canals were frequently irrigated with 2 mL of 5% NaOCl,³⁰ as previously mentioned. The excess irrigant was soaked with cotton pellets. A K-file with a size compatible with the canal size¹⁸ was attached to the file clip of the iPex and introduced into the canal until the 0.0 mark, then the file was withdrawn to the 0.5 mark as per the manufacturer's recommendation (Fig. 1).

If the meter gauge was stable for 5 seconds, the rubber stopper was adjusted to the coronal reference point, the file was withdrawn, and the length was measured using a digital caliper (Fig. 2). To reduce bias, the sequence of the three models was randomized,



Fig. 2: Length measurement using digital caliper

and the operator obtained the first reading for all teeth, followed by the second and third readings, in a similar manner. Each set of readings was 2 weeks apart. The three readings were then averaged to obtain the electronic length. The external surfaces of the teeth were thoroughly washed with running water before being placed in the next medium.

Statistical Analysis

Data were collected using an Excel spreadsheet (Microsoft Corp., Washington, USA). The mean length deviation (MLD), between the electronic measurements of the iPex and the WL, was calculated by subtracting the mean electronic length of iPex in each medium from the mean WL. Analysis of variance (ANOVA) was utilized to determine if there were any significant differences in the MLD among the three distinct media. Tukey's *post hoc* test was employed to identify intergroup variations. The statistical analyses were carried out using IBM SPSS software (SPSS version 23, IBM). The significance level was set at 5% ($\alpha = 0.05$).

RESULTS

The mean iPex length was shorter than (coronal to) mean WL by 0.2 ± 0.45 mm when the measurements were done in saline

Table 1: Comparisons of the groups' means with mean WL (mm)

Comparison	Sample		Mean length		Common SD
	size	Mean	SD	difference	
WL	61	14.45	2.00	0.07	0.46
Alginate	61	14.38	2.04		
WL	61	14.45	2.00	0.20	0.45
Saline	61	14.25	2.07		
WL	61	14.45	2.00	-0.19*	0.43
Gelatin	61	14.64	2.00		

*Negative sign indicates that mean iPex length was longer than mean WL. SD, standard deviation

Table 2: Comparison of iPex readings with ACL and WL (%)

Model	Longer than ACL	Shorter than ACL	Longer than WL	Shorter than WL
Alginate	3.3	96.7	37.7	62.3
Saline	3.3	96.7	27.9	72.1
Gelatin	4.9	95.1	86.9	13.1

ACL, actual canal length; WL, working length

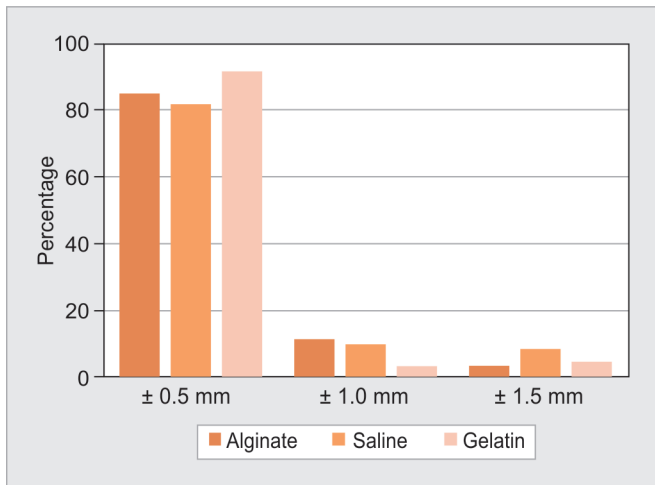


Fig. 3: Frequency distribution of iPex readings in the three models (%)

model. The mean iPex length was longer than (apical to) mean WL by 0.19 ± 0.43 mm when recordings were done in the gelatin model. When the device was operated in the alginate model, the mean iPex length was shorter by 0.07 ± 0.46 mm (Table 1). ANOVA test revealed a significant difference between mean differences of WL compared with iPex length in the three media ($p < 0.001$). *Post hoc* Tukey test showed that the mean difference between WL and iPex length obtained in the gelatin model was significantly longer than the difference with mean iPex length in alginate ($p = 0.005$) and in saline ($p < 0.001$). There was no significant difference between iPex readings obtained in alginate and saline ($p = 0.249$). Interestingly, iPex failed to exactly match WL in any tooth (Table 2). Figure 3 illustrates the frequency distributions of iPex readings for the three models.

DISCUSSION

As per existing research, this is the first study to investigate whether iPex EAL accuracy is affected by the placement of the same teeth in three different embedding media namely alginate, saline, and

gelatin. The results showed that the most accurate readings were obtained when iPex was tested in the alginate and saline models, whereas testing it in gelatin resulted in significantly long recordings. Hence, the null hypothesis was rejected.

The accuracy of EALs investigated in *ex vivo* settings is influenced by several factors, with the type of embedding medium used in the experimental setup being one of the important aspects. However, to the best of available knowledge, this has been infrequently investigated.^{16,21-23} Chen et al.²² utilized 27 teeth to compare the readings of Dentaport ZX and Raypex 5 when teeth were placed in alginate, saline, or gelatin. Baldi et al.²¹ added two more media, agar and flower sponge soaked with saline, with a total sample size of 30 teeth. Both studies evaluated a limited number of teeth and did not randomize the sequence of use of the embedding medium. This study was designed to overcome these limitations and provide a better insight into the accuracy of iPex. In addition, the operator who utilized iPex was unaware of the WL, with measurements performed over several days and in triplicate and then averaged to avoid any bias. The selection of three media was based on the fact that they are the most commonly used media for *in vitro/ex vivo* EAL assessments.¹⁴

According to Baldi et al.,²¹ the accuracy of Root ZX was not affected by the type of embedding medium, and the device recorded a mean length shorter than the mean WL when operated in gelatin, saline, and alginate. None of the recordings were longer than the level of the apical foramen. Readings were obtained with K-file size 30 extended to the 0.5 mark of the digital display of the EAL. On the other hand, Chen et al.²² reported that Dentaport ZX, a variant of Root ZX, gave significantly more accurate readings when it was operated in alginate, while its readings in gelatin and saline were not significantly different. The three electronic means were shorter than the mean WL values. For Raypex 5, readings were significantly different across the three media. They did not specify which digital display mark was adopted, and the file size selection was dependent on the size of the canal. A recent study reported that the precision of measurements obtained with Root ZX II, Canal Pro, and Apex ID was negatively affected by embedding teeth in the electroconductive gel compared with the use of alginate.¹⁶ Nevertheless, the precision of the Elements Diagnostic Unit was not affected by the type of embedding media used.²³ The findings of this study revealed that the alginate and saline models were the best setups for iPex testing, whereas the chance for long readings with iPex would increase if it was operated in gelatin. The differences in results between the aforementioned studies could be attributed to the different methodologies followed and different EALs used. It must be noted that the performance of one EAL under certain conditions should not be generalized to other EALs.³²

The electrical resistance of alginate increased over time, affecting its conductive properties.³³ Several studies have reported that the alginate mix was refreshed every 2 hours.^{27,34-37} One study utilized the same alginate mix for 45 days. The setup was kept moist and refrigerated while not in use.³⁸ Others did not mention the duration for which the alginate mix was used.^{24,25,39,40} In this study, the mix was refreshed every 30 minutes in accordance with the recommendations of de Vasconcelos et al.²⁶ This could explain the current results obtained with alginate model. Nevertheless, the ideal time for the use of alginate mix during EAL assessment is unknown and requires further investigation. Consistent with earlier findings, no adverse effects of irrigation with NaOCl on alginate integrity were observed in this study.^{39,40}



The highest frequency of readings shorter than the WL was recorded in the saline model (Table 2). Saline could seep into the canal through the apical foramen, which might lead to a short EAL recording.³² However, this did not significantly affect the precision of iPex (Table 1), indicating that the potential for this seepage to affect the device's accuracy is limited. The significantly long MLD in the gelatin model reflects the high frequency of long readings when the device was operated in this setup (Table 2). Possibly, gelatin's electrical conductivity was not high enough to allow current passage between the lip clip and file tip until the file touched the gelatin present outside the tooth, leading to longer-than-desired readings. Indeed, the highest frequency for readings longer than ACL was acquired in the gelatin model (Table 2). None of the previous studies have evaluated the performance of iPex in saline or gelatin setups. When operated in the alginate model, 85.2% of iPex readings were within ± 0.5 mm. This is within the previously reported iPex accuracy of 55.3–90%.^{24–27}

Diligent efforts were made in the current investigation to ensure the acquisition of valid and reliable results by controlling the *ex vivo* variables. The sample size used was notably larger than what had been reported previously. Utilizing 5% NaOCl as an irrigant had no discernible impact on the accuracy of EAL readings.³⁰ Furthermore, file sizes were tailored to match the canal, based on findings from a prior study, suggesting that the accuracy of iPex EAL remains consistent, regardless of the file size used for determining working length.¹⁸ As mentioned earlier, the operator who obtained the electronic lengths was blinded to WL, and all measurements were done in triplicate and then averaged to reduce the chance for bias. Nevertheless, it is important to acknowledge the inherent complexities associated with the *in vivo* scenarios. In such settings, numerous uncontrolled variables can adversely influence the precision of EALs' electronic length determination. These unaccounted factors highlight the importance of conducting comprehensive clinical studies to validate the reliability and accuracy of EALs in clinical practice.

Based on the results of this investigation, alginate and saline model setups are simple, affordable, and provide reliable results with iPex EAL. Thus, a recommendation is made to use freshly mixed alginate or saline as the embedding medium of choice for future studies investigating the precision of iPex EAL in laboratory settings. Furthermore, they can be used as an effective teaching model for dental students during preclinical endodontic courses.

CONCLUSION

From the results obtained and within the limitations of this study, it can be concluded that freshly mixed alginate and saline are the preferred embedding media for *ex vivo* assessment of iPex EAL accuracy in root canal working length determination. In contrast, the use of gelatin as an embedding medium should be avoided in such *ex vivo* set-ups because that could affect the performance of iPex and increase the chance of erroneous electronic readings.

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