Author’s response to reviews

Title: The influence of two forms of chlorhexidine on the accuracy of contemporary electronic apex locators

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Author’s response to reviews:

On behalf of the authors, thank you very much for a thorough reviews, which significantly helped to improve the article. All comments of the reviewers have been taken into account.

Answer to the comments of Reviewer #1

1. Reviewer concern:

There is no novelty in this study. There are numerous similar in vitro studies performed.

Our response:

Although there are a lot of study evaluating the accuracy of root canal length measurements in different environments and with using different apex locators, there is still a need to carry out such research in relation to the placing on the market of new apex locators and new agents used during endodontic treatment. At the beginning of the research, there was no study evaluating the ApexDal, so it was decided to compare the accuracy of measurements obtained using this new EAL with another, well-tested and reliable Rayex 5. Also the studies evaluating the accuracy of endodontic measurements in the environment of various irrigation fluids was also limited. Only one study evaluating the effect of CHX in the form of a gel on the accuracy of endometric measurements was available. Shin HS, Yang WK, Kim M, Ko HJ, Cho KM, Park SH, Kim JW. Accuracy of Root ZX in teeth with simulated root perforation in the presence of gel or liquid type endodontic irrigant. Restor Dent Endod. 2012 Aug;37(3):149-154 Due to appearing this form of chlorhexidine on the market in our country, it seemed justi- lied to carry out such studies.
Revised text:
Not applicable

2. Reviewer concern:
English needs to be improved significantly.
Our response:
The English language was proofed by American Journal Experts
Revised text:
Not applicable

3. Reviewer concern:
Introduction, Page 4, line 10: What is the meaning of CHX could complete the NaOCl during endodontic treatment
Our response:
Due to sodium hypochlorite (NaOCl) and chlorhexidine gluconate (CHX) wide spectrum antimicrobial activity, an irrigation regimen has been proposed, in which NaOCl would be used throughout instrumentation, followed by EDTA, and CHX would be used as a final irrigant, especially during retreatment. The combination of NaOCl and CHX has been advocated to enhance their antimicrobial properties.
Revised text:

Irrigation is presently the best method for the removal of tissue remnants and dentine debris during instrumentation [20]. All over the years, many materials have been used to the root canal irrigation, and certainly, the sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA) and chlorhexidine gluconate (CHX) are the most popular solutions used and most reliable ones. Due to their wide spectrum antimicrobial activity, an irrigation regimen has been proposed, in which NaOCl would be used throughout instrumentation, followed by EDTA, and CHX would be used as a final irrigant. The combination of NaOCl and CHX has been advocated to enhance their antimicrobial properties [21, 22, 23, 24].

4. Reviewer concern:
Methods: Mention the manufacturer’s details of diamond flame and diamond round bur
Our response:
The manufacturer’s details of burs used in the study were placed in the text.
Revised text:

First the crowns have been cut horizontally with the high speed diamond flame bur REF F 0250 343, no 16 (Dentsply-Maillefer, Ballaigues, Switzerland) at 2-mm coronal to the CEJ. Special edges resembling a cube were made with round diamond bur REF F 0001 343, no 18 (Dentsply-Maillefer, Ballaigues, Switzerland) placed in a turbine.

5. Reviewer concern:
A clinical study of the same nature would have been more relevant than in vitro study.
Our response:
Of course, clinical research have the greatest cognitive significance, but the literature of the subject also presents many in-vitro studies that inspired the authors to design the presented study.
The present study is an introduction for further in vivo study which the aim is to de-terminate the accuracy of EAL in teeth with wide apical foramen (research in progress). The study attempts to compare the obtained results also with the results achieved in in vivo stud-ies. Revised text:

Khandewal et al. [14] conducted comparative evaluation of accuracy of Raypex5 and Apex NRG XFR EALs with conventional radiography in ex vivo study. They obtained the same accuracy in determining the WL for both EALs when compared with radiography.

6. Reviewer concern:
Page 4, line 27
What is the rationale of using CHX solution or gel and testing its effect on working length determination using EALs? Working length is determined before cleaning and shaping of the root canal. CHX is used as a final rinse to disinfect the canal after shaping of the canal. Hence, there won’t be any effect of CHX on the working length determination using EALs
Our response:
Working length is determined before cleaning and shaping of the root canal, but also during endodontic treatment stages (initial, preflared, and concluded). The extend of the root canal length may be reduced, thereby control of the apical limit during instrumentation might be necessary to avoid over-instrumentation. (J Endod. 2016 Nov;42(11):1683-1686.)Therefore it seems noteworthy to know the effects of various solutions applied during treatment on EALs accuracy, especially NaOCl and CHX solutions. CHX is used as a final rinse to disinfect the canal after shaping of the canal, but also it might be used as an alternative to NaOCl, especially in cases of open apex, root resorption, foramen enlargement and root perforation, due to its biocompatibility, or in cases of allergy related to bleaching solutions, so it is worth to explore it’s influence on the accuracy of EALs.
Revised text:
Although CHX is recommended as a final rinse, after shaping of the canal, it also might be considered as an alternative to NaOCl, especially in cases of open apex, root resorption, foramen enlargement and root perforation, due to its biocompatibility, or in cases of allergy related to bleaching solutions.

7. Reviewer concern:
Mention the full manufacturer’s details of EALs used in this study
Our response:
The manufacturer’s details of EALs used in the study were placed in the text.
Revised text:
Raypex 5 is apex locator that uses two separate frequency (400 Hz and 800 Hz) [14]. This device makes use of the same frequencies of alternating currents but bases the measurement on the mean square root values of the electrical signals. [14]. ApexDal is a new digital apex locator. It also uses two separate frequency.

8. Reviewer concern:
Which type of needle was used for the irrigation of the test agents?
Our response:
For the irrigation needle 0,3 mm (30ga) (Appli-Vac Irrigating Needle Tip; Vista Dental, Racine, WI, USA) was used.

Revised text:
Irrigants were placed into the canal using irrigating syringe and needle 0,3 mm (30ga) (Appli-Vac Irrigating Needle Tip; Vista Dental, Racine, WI, USA).

9. Reviewer concern:
Mention the sample size in each group.
Our response:
Sample size was 29 in each of three groups

Revised text:

10. Reviewer concern:
How was irrigation solution placed inside the root canal without enlarging the canals?
Our response:
Irrigation solution was placed inside the pleflaring part of canal with needle and syringe, api-cal part of canal was filled during inserting the K-file. Other studies used the same methodol-ogy i. e. the authors enlarge 1/3 of the canal with gates glidden burs and irrigated them using irrigation needle. Jung IY., Yoon BH., Lee SJ., Lee SJ. Comparison of the reliability of ‘0.5’ and ‘APEX’ ark measurements in two frequency-based electronic apex locators. J Endod 2011 37, 1, 49-52. Hor D., Krusy S., Attin T. Ex vivo coparison of two electronic apex loca-tors with different scales and frequencies Int Endod J 2005 38, 855-859. Khattak O., Raidullah E., Francis ML. A comparative assessment of the accuracy of electronic apex loca-tor (Root ZX) in the presence of commonly used irrigating solution J Exp Dent 2014 6, 1: e41-e46.
Revised text:
Not applicable

11. Reviewer concern:
For how long the test agents were placed inside the root canal before determining the WL?
Our Response:
All measurements were made directly after placement the testing agent into the canal.

Revised text:
All measurements were made directly after placement the gel or irrigants into the canal.

12. Reviewer concern:
Why root canals were not cleaned and enlarged?
Our response:
The canals were not enlarge due to afraid of damaging apical constriction and apical foramen. As it is know these two anatomical structures are very important for proper EAL measure-ments. Also the size of root canal is another factor which affect on the accuracy of the EALs Ebrahim AK, Yoshioka T, Kobayashi C, Suda H. The effects of file size, sodium hypochlorite and blood
on the accuracy of Root ZX apex locator in enlarged root canals: an in vitro study. Aust Dent J. 2006 Jun;51(2):153-7. Authors reported that as the diameter of the root canal increased, the electronically measured length with smaller size files became shorter.

Revised text:
Not applicable

13. Reviewer concern:
Mention the volume of irrigating solution and gel placed inside the root canal.

Our response:
The volume of irrigant was 2ml and the volume of gel was 0.2ml.

Revised text:
Each canal was filled with 2ml irrigant, the excess of fluid was drained with cotton pellet. Chlorhexidine gel was introduced directly from syringe using needle 0.3mm (30ga) (Appli-Vac Irrigating Needle Tip; Vista Dental, Racine, WI, USA), the apical part of the canal was filled with chlorhexidine gel using #10 K-file in counterclockwise movement. Each canal was filled with 0.2 ml of gel.

14. Reviewer concern:
In the same tooth all the three test agents were used? Or separate tooth was used for each test agent? Its not clear. Mention in detail.

Our response:
Yes, in the same tooth all three test agents were used.

Revised text:
Six endodontic measurements were taken for each tooth: two measurements in a presence of 2% NaOCl; one measurement using Raypex 5 and one measurement using ApexDal, another two measurements in a 2% CHX solution environment using Raypex 5 and ApexDal and last measurements in a 2% CHX gel environment with both devices respectively. Measurements were performer until all irrigants and gel were tested with both devices in one tooth. In total, 6 measurements were taken in each tooth.

15. Reviewer concern:
How was the normality of the data tested?
Response:
The normality of the distribution was checked by the Shapiro-Wilk test. Due to the lack of normality of distribution, nonparametric tests were used.

Revised text:
Not applicable

Answer to the comments of Reviewer #2
1. Reviewer concern:
To make the reader better understand the subject and its clinical relevance, authors should focus
in the discussion section on the overall precision of EALs.
Revised text:
Some studies differ in establishing the reference point from which measurement accuracy is
determined. Some authors measured from the minor diameter (apical constriction) whereas
others measured from the major diameter or apical foramen (56). It is worth to pay attention on
fact that EALs are highly accurate in determining the location of minor constriction, but the
mean distance from the file tip to the minor constriction always has a positive value (56, 57). It's
mean that mostly EALs overestimate WL. In this research position of the tip file was located
more apical relative to apical constriction. Such position of the file tip resulted from the fact that
the measurements were made until the “apex” was marked on the devices.

2. Reviewer concern:
Author should have considered a different experimental design, both from the use of alginate
(t hat in few minutes changes its chemical properties, thus modifying the impedance re-sult),
and the actual WL measurement of the specimens.
Our response:
When designing the experiment, we based on our previous research on the possibility of using
alginate mass during in vitro endodontic measurement described in study : Lipski M, Trąbska-
Świstelnicka M, Woźniak K, Dembowska E, Drożdżik A. Evaluation of alginate as a substitute
Working length measurements were performed within 15 min in one tooth. However, in a study
assessing the stability of a model manufactured from plastic dental jaw, natural teeth and
alginate, the length measurements did not change significantly over 45 h for teeth with foramina
of 0.3 mm or less, and teeth with foramen diameters of 0.45 mm proved stable up to 28 h (Tinaz
AC, Alacam T, Topuz O. A simple model to dem-onstrate the electronic apex locator. Int Endod
J 2002;35: 940–5.)
Revised text:
Not applicable

3. Reviewer concern:
Finally, along the text there are several typos that should be revised prior the article to be ac-
cepted
Response:
The text was carefully reviewed, and the typos were corrected. In addition, The English lan-
guage was proofed by American Journal Experts
Revised text:
Not applicable