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Autoclaving and battery capacity influence on laser fluorescence measurements

RENATA MACIEL ROCHA-CABRAL[1], FAUSTO MEDEIROS MENDES[2], FABIANA MIURA[2], ADRIANA DA COSTA RIBEIRO[3], MARIANA MINATEL BRAGA[2] & DENISE MARIA ZEZELL[1]

[1]*Centro de Lasers e Aplicações, Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil,* [2]*Department of Pediatric Dentistry, School of Dentistry, Universidade de São Paulo, Brazil and* [3]*Department of Restorative Dentistry, School of Dentistry, Universidade de São Paulo, São Paulo, Brazil*

Abstract

Aim. To evaluate the influence of probe tip autoclaving and depleted alkaline batteries on laser fluorescence (LF-DIAGNOdent) device performance. ***Material and methods.*** One-hundred-and-twenty occlusal sites were analyzed with an LF device in nine conditions: No autoclaved probe and probe after 5, 10, 15, 20, 25, 30, 40, and 50 autoclaving cycles. Subsequently, the performance of the device was analyzed with: New batteries, batteries with 1.49/1.39 V, 1.38/1.37 V, 1.36/1.34 V, 1.33/1.32 V, and lower than 1.32 V. LF values, sensitivity, specificity, accuracy, and area under the ROC curve were compared. ***Results.*** In the probe study, sensitivity was lower after 50 sterilizing cycles, though specificity was higher than the assessment performed using a new tip. In the batteries study, specificity was higher for depleted batteries, but LF performance did not differ significantly among the groups. ***Conclusion.*** Batteries do not significantly influence device performance, but consecutive sterilization of probes in autoclave alters readings, downgrading its performance.

Key Words: *Dental caries, laser fluorescence, occlusal surfaces, primary teeth*

Introduction

Fluorescence is used to detect caries, since this emission is known to differentiate between sound and carious tissue [1]. The excitation source provides the light partially absorbed by the dental substance and the absorbed light is re-emitted by chromophores within the dental tissue in a longer wavelength while excitation occurs. The approach has led to the development of new technologies. A laser fluorescence (LF) method, named DIAGNOdent (Kavo, Biberach, Germany), uses a diode laser emitting a red light ($\lambda = 655$ nm) as excitation. The device measures the amount of fluorescence emitted from the organic content of the caries lesions [2–4] and in the presence of a caries lesion, the fluorescence signal increases [2].

Good performance and excellent reproducibility have been achieved with the LF method in occlusal caries detection [5]. None the less, factors that can have an influence on the measurements produced by new technologies include optical fibers, ageing of light sources, and instability of the power source [6]. In fact, potentially confounding factors that complicate the LF interpretation are bacterial dental plaque [7], humidity [7,8], and stains [9,10]. In a previous pilot study, we observed that the LF readings were significantly decreased after 50 autoclaving cycles [11]. However, in this earlier study, we did not investigate intermediate numbers of autoclaving cycles. Furthermore, as only one tip was used [11], this could have limited the generalizability of the results. Another unclear aspect is the effect of depleted batteries on the LF readings.

The aim of this *in vitro* study was to evaluate the influence of successive probe tip autoclaving and gradually depleted alkaline batteries on measurements and performance of the LF device in detecting occlusal caries lesions in primary molars.

Correspondence: Denise Maria Zezell, Centro de Lasers e Aplicações – Instituto de Pesquisas Energéticas e Nucleares, Av. Lineu Prestes, 2242 05508-900, São Paulo, SP, Brazil. Tel: +55 11 3816 9313. Fax: +55 11 3816-9315. E-mail: zezell@usp.br

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Material and methods

The study was approved by the Ethics Committee in Research in Humans (Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil).

One-hundred-and-nineteen suspect occlusal sites (without frank cavitation) were selected from 66 recently exfoliated primary molars. All teeth were donated by the Bank of Teeth (School of Dentistry, University of São Paulo) and stored in saline solution for up to 3 months before measurements were taken. Photographs were taken of each tooth using a digital camera (CEOS Digital Rebel XTi; Canon Inc., Tokyo, Japan) (×10) and the site to be analyzed was indicated by black dots, which allowed reproducible positioning of the probe. The teeth were polished with water and non-fluorescent pumice (Dentsply, Rio de Janeiro, Brazil) slurry and rinsed in tap water.

LF measurements

One examiner took the LF device (DIAGNOdent 2095, KaVo, Biberach, Germany) readings in accordance with the manufacturer's instructions. Probe tip A (for occlusal surfaces) was selected for all measurements. The device was calibrated against a ceramic standard prior to examination, and then recalibrated after every 10th tooth. It was also calibrated on a sound surface of each tooth. After these procedures, teeth were removed from the solution and dried in compressed air from a 3-in-1 syringe for 10 s. The probe was then placed perpendicular to the site and rotated. Three measurements were taken at each site and the mean value was recorded.

The presence or absence of caries lesions was determined using the cut-off limits described in a previous study in primary teeth [10]: 0–4 = sound/early enamel caries lesions; 5–12 = advanced enamel caries; >12 = dentinal caries.

Probe tips autoclaving study

Measurements using the LF device were taken at the same sites under nine different conditions in the following sequence using one new tip (named tip 1 in the present study): 1) No autoclaved probe. The sites were measured with a new probe. The same probe was used for the entire sample without autoclaving. 2) Probe after five autoclaving cycles. The sites were measured with the same probe after five sterilizing cycles in autoclave. 3) Probe after 10, 15, 20, 25, 30, 40, and 50 autoclaving cycles, subsequently.

Each sterilizing cycle consisted of 12 min at 121°C and 16 psi pressure in one autoclave (Speedclave, Odontobrás, Brazil) and then an interval (at least 30 min) allowing the probe to return to room temperature after each cycle.

In order to check generalizability of the results obtained using tip 1, another sample comprising 39 primary molars was selected. One suspected site per tooth in the occlusal surface was chosen. New measurements were carried out using two new tips (named tips 2 and 3) in the same way as described above. In this experiment, the assessments were made with the new tips, and after 10, 20, 30, 40, and 50 autoclaving cycles.

Depleted alkaline batteries study

The LF readings were performed in teeth under different conditions: 1) New batteries. The sites were measured with new batteries (~1.60 –1.50 V). 2) Depleted batteries at 1.49–1.39 V. 3) Depleted batteries at 1.38–1.37 V. 4) Depleted batteries at 1.36–1.34 V. 5) Depleted batteries at 1.33–1.32 V. 6) Depleted batteries at <1.32 V. At this voltage, the low-battery signal appeared in the device.

A new probe (not autoclaved) and new alkaline batteries (Duracell; Gillete Company, Bethel, USA) were used at the beginning of this experiment. The power of these batteries was gradually spent by the probe being positioned on a carious site for several minutes in order to distinguish and create the different groups listed above. A digital multimeter was used (Fluke 867B; Fluke Corporation, Everett, USA) to measure the precise voltage of the batteries.

Histopathological analysis

Following examination, buccolingual sections with a thickness of about 300 μm were cut perpendicular to the suspected occlusal sites with a water-cooled diamond blade (Labcut 1010; Extec, Enfield, USA). Lesion depth was examined under the stereomicroscope (Olympus SZ-PT; Olympus Optical Co., Japan) at a magnification of ×16–32 by two examiners in joint session until they reached consensus. A 5-point scale was used to classify the lesions: D0 = no caries; D1 = enamel caries limited to the outer half of enamel; D2 = caries extending into the inner half of the enamel but not to the amelodentinal junction; D3 = caries limited to the outer half of the dentin; and D4 = caries involving the inner half of the dentin.

Statistical analysis

As the values were not normally distributed, differences were analyzed by means of a non-parametric test. Thus, a Friedman one-way ANOVA test was used to compare the mean values under the different conditions of sterilizing cycles to which the probes were exposed. The Wilcoxon test was employed to compare the results between the values obtained with tips 2 and 3 in the same sample and after the same number of autoclaving cycles. Sensitivity,

specificity, and accuracy were calculated at the D2 and D3 thresholds using the cut-off points previously described [10]. These values were compared among the groups using the McNemar change test. ROC analysis was also applied to compare diagnostic performance at both the D2 and D3 threshold. A non-parametric statistical test was used to estimate the difference among the areas under the ROC curves. The level of significance for all tests was taken as $p < 0.05$.

Results

Histopathological examination showed that of 120 sites evaluated, 46 were sound surfaces (D0), 29 had initial enamel caries lesions (D1), 24 had advanced enamel caries lesions (D3), 19 had caries lesions in the outer half of the dentin (D3), and two sites had deep dentin caries lesions (D4).

Probe tips autoclaving study

The measurements taken under different conditions of sterilizing cycles in autoclave in occlusal surface sites of primary molars are shown in Figure 1. There were statistically significant differences among groups ($p < 0.05$). Tip 1 was used in the first group of samples, and after 10 autoclave sterilizing cycles LF mean values decreased significantly, showing a higher decrease rate after 30 cycles. With tips 2 and 3, a group of samples with 39 teeth was used; a significant decrease in the values was observed, and both tips presented a similar trend until 40 cycles. After 50 cycles, tip 3 presented significantly lower values than tip 2 (Figure 1).

Sensitivity was higher in the readings obtained with a new probe compared to the most autoclaved probe at the D2 threshold. At the D3 threshold, sensitivity was significantly higher for the group in which the new probe was used (0.77) and lower in the group in which the most autoclaved probe was used (0.37). Nevertheless, specificity was statistically significantly higher in teeth measured with the most autoclaved probe at two thresholds (D2 and D3), 0.80 and 0.96, respectively. Accuracy did not change significantly among the groups for D2, but, for D3, accuracy was significantly higher when the probe was more autoclaved. The area under the ROC curve did not change significantly among the groups (Table I).

Depleted alkaline batteries study

LF readings taken under different voltages of batteries in occlusal surface sites of primary molars are given in Table II. When the batteries had lost 25% of their total power (<1.32 V), and the low battery signal appeared, there was a statistically significant difference ($p < 0.05$) between this group and the one in which new batteries were used. Regarding performance in detecting occlusal caries lesions in this experiment, at the D3 threshold the specificity was significantly higher in the readings obtained when depleted batteries were used (0.86) than in the readings taken with new batteries (0.74). Sensitivity, accuracy, and area under the ROC curve did not change significantly among the groups in both the D2 and D3 thresholds (Table III).

Discussion

The protocols for use of the LF system differ among studies and some points remain unclear. There are no studies stipulating the ideal conditions of the probe, i.e. resulting in the most precise measurements.

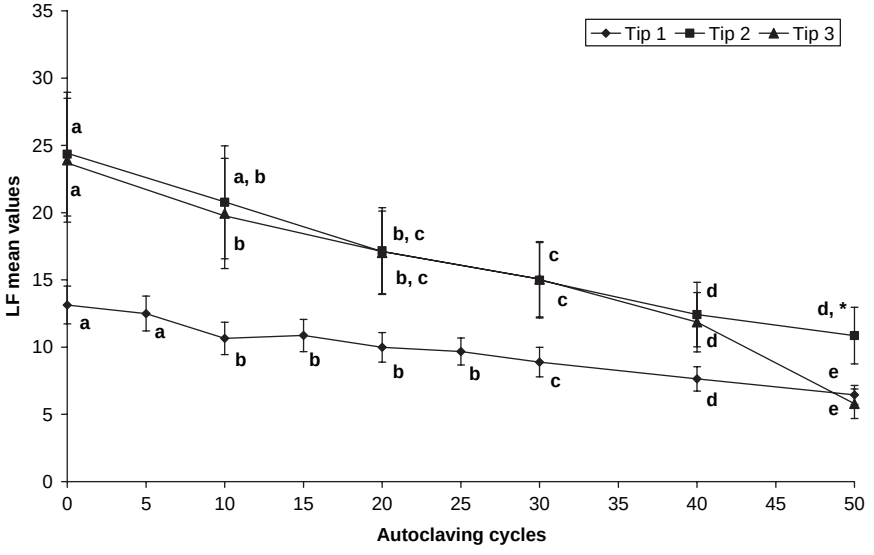

Figure 1. Mean of laser fluorescence (LF) values versus the subsequent sterilizing cycles. Different letters express statistically significant difference among values using the same tip ($p < 0.05$). Tip 1 was used in a different group of samples ($n = 120$) from tips 2 and 3 ($n = 39$). An asterisk indicates statistically significant difference ($p < 0.05$) between tips 2 and 3 after the same number of autoclaving cycles. Vertical bars represent the standard error.

Table I. Performance of the laser fluorescence device under different conditions of autoclaving probe, expressed in sensitivity, specificity, accuracy, and area under the ROC curve (A_z), in detecting occlusal caries lesions affecting enamel (D2) and dentin (D3).

	Sensitivity		Specificity		Accuracy		A_z	
Autoclaving	D2	D3	D2	D3	D2	D3	D2	D3
×0	0.88[a]	0.77[a]	0.63[a]	0.74[a]	0.78[a]	0.75[a]	0.82[a]	0.86[a]
×5	0.88[a]	0.73[a]	0.53[a]	0.78[a,b]	0.73[a]	0.77[a]	0.81[a]	0.86[a]
×10	0.84[a,b]	0.67[a]	0.67[a,b]	0.86[b,c,d]	0.77[a]	0.81[a,b]	0.82[a]	0.89[a]
×15	0.86[a]	0.73[a]	0.63[a,b]	0.84[b,c]	0.76[a]	0.81[a,b]	0.82[a]	0.88[a]
×20	0.88[a]	0.70[a]	0.63[a,b]	0.89[c,d]	0.78[a]	0.84[b]	0.82[a]	0.90[a]
×25	0.90[a]	0.67[a,b]	0.60[a]	0.92[d,e]	0.78[a]	0.86[b]	0.83[a]	0.89[a]
×30	0.80[a,b]	0.60[a,b]	0.71[b]	0.94[e]	0.76[a]	0.86[b]	0.84[a]	0.90[a]
×40	0.86[a]	0.47[b,c]	0.71[b]	0.96[e]	0.80[a]	0.84[b]	0.84[a]	0.90[a]
×50	0.74[b]	0.37[c]	0.80[c]	0.96[e]	0.77[a]	0.81[a,b]	0.82[a]	0.89[a]

D2: D0, D1 = sound, D2–D4 = decayed.
D3: D0–D2 = sound, D3, D4 = decayed.
Different letters express statistically significant difference ($p < 0.05$) among values within the same column.

To the best of our knowledge, the use of depleted alkaline batteries has not been questioned in the literature.

Sterilization in autoclave has been considered one of the best choices in an asepsis protocol against cross-infection [12]. The manufacturer of the LF device asserts that if the probe is used correctly it can be sterilized more than 500 times with no distortion effect on results. Nevertheless, in the present study, we found significant differences after autoclaving sterilizing cycles of the LF probe tip. After 10 autoclaving sterilizing cycles, LF mean values decreased significantly with a more significant reduction after 30 cycles (Figure 1). This trend was observed for all tips in a previous study [11] and in the present study. However, susceptibility to degradation seems to differ between tips; tip 3 showed a higher decrease after 50 cycles than tip 2 did.

Therefore, using a sequentially autoclaved probe may possibly lead to substandard clinical recommendations, as postulated in a study by Cabral et al. [11]. In relation to the performance of the method in the present study, sensitivity decreased successively when the probe was exposed to more cycles in the autoclave, mainly at the D3 threshold. Some authors have affirmed that, for scientific purposes, the probe should be new to prevent variations in the readings due to wear of the fibers [13]. Our results concur with this recommendation. Due to the fact that sensitivity was poorer after successive cycles, the number of false-negative diagnoses increased. We therefore believe that constant sterilization of these probes under autoclave should be avoided, and that the use of autoclaved probes can worsen the performance of the device.

Table II. Laser fluorescence (LF) readings according to battery level.

Battery voltage	LF readings Mean (SD)
New batteries	13.3 (15.3)[a]
1.49–1.39 V	12.6 (14.8)[b]
1.38–1.37 V	12.0 (14.0)[b,c]
1.36–1.34 V	12.2 (15.0)[b,c]
1.33–1.32 V	11.4 (13.4)[c]
Depleted batteries (<1.32 V)	11.2 (14.0)[c]

Superscript letters express statistically significant difference among different groups ($p < 0.05$); ($n = 120$).

It is possible that the heat under pressure is capable of damaging the fibers, with the resultant fluorescence partially lost during the course of the fibers, thereby promoting a reduced signal. Thus, as caries lesions are underestimated, specificity values increase. An alternative to infection control is utilization of PVC seal wrap as a protection barrier, since this procedure does not significantly change the LF readings [11]. Although the LF device has been used previously in clinical studies, the authors did not mention anything about the infection control procedure used [2,4,13–17]. Considering the results of the present study and of the earlier one [11], however, this issue must be addressed in further *in vivo* studies.

The new LF device designed for occlusal and approximal caries detection, the DIAGNOdent pen, was introduced recently [18,19]. Despite the promising results obtained from *in vitro* studies in detecting approximal [18] and occlusal caries lesions [19], the effect of tip autoclaving of this new device remains unclear. Thus, further study of the DIAGNOdent pen is required.

The LF device requires five 1.5 V standard AA batteries in order to work. The manufacturer recommends the use of alkaline batteries but does not offer guidelines about improving the performance of the device.

According to the manufacturer, the battery charge status is shown by an indicator flashing at approximately 20% power. When the power falls below 20%, a low-battery signal is emitted and the unit switches off. However, this was not observed in our experiment. The new batteries used in our study showed an output voltage of approximately 1.6 V. After losing only 25% of their initial power (~1.2 V), the equipment signaled low energy, although it

Table III. Performance of the laser fluorescence device under different conditions of depleted alkaline batteries, expressed in sensitivity, specificity, accuracy, and area under the ROC curve (A_z), in detecting occlusal caries lesions affecting enamel (D2) and dentin (D3).

	Sensitivity		Specificity		Accuracy		A_z	
Battery voltage	D2	D3	D2	D3	D2	D3	D2	D3
New batteries	0.88[a]	0.77[a]	0.63[a]	0.74[a]	0.78[a]	0.75[a]	0.82[a]	0.86[a]
1.49–1.39 V	0.92[a]	0.67[a]	0.56[a]	0.79[a,b]	0.77[a]	0.76[a]	0.82[a]	0.87[a]
1.38–1.37 V	0.86[a]	0.73[a]	0.63[a]	0.81[a,b]	0.76[a]	0.79[a]	0.81[a]	0.87[a]
1.36–1.34 V	0.90[a]	0.70[a]	0.61[a]	0.83[b]	0.78[a]	0.80[a]	0.83[a]	0.88[a]
1.33–1.32 V	0.92[a]	0.73[a]	0.61[a]	0.82[a,b]	0.79[a]	0.80[a]	0.83[a]	0.87[a]
Depleted batteries (<1.32 V)	0.86[a]	0.67[a]	0.63[a]	0.86[b]	0.76[a]	0.81[a]	0.83[a]	0.88[a]

D2: D0, D1 = sound, D2–D4 = decayed.
D3: D0–D2 = sound, D3, D4 = decayed.
Different letters express statistically significant difference among values within the same column ($p < 0.05$).

was capable of operating for several minutes thereafter.

Concerning the batteries charge study, it could be seen that as the battery depleted the LF readings decreased (Table II), which in a clinical situation could lead to underestimating caries lesioning. However, the values for sensitivity, area under the ROC curve, and accuracy showed no statistically significant differences among the groups. Although a significant increase of specificity for the D3 threshold was observed (Table III), it was shown that alkaline batteries do not significantly influence the performance of the device. Thus, the cut-off which indicates the low battery charge established by the manufacturer is appropriated to prevent loss of performance.

Owing to the several possible confounders that could affect the performance of the LF device, clinicians should be careful about using additional technical support for caries detection. Treatment decisions must not be made based on readings obtained by devices alone. In clinical practice, the diagnosis of caries should include analysis of the signs and symptoms in each patient, with the LF and other devices being used as adjuncts of visual inspection.

It is concluded that consecutive sterilization of the probe in the autoclave influences LF readings, downgrading its performance in detecting occlusal caries lesions in primary teeth. On the basis of these findings, another method for infection control of probes should be chosen.

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