The influence of pvc seal wrap and probe tips autoclaving on the *in vitro* performance of laser fluorescence device in occlusal caries in primary teeth

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The goal of this study was to evaluate the influence of a PVC Seal Wrap as barrier protection and the influence of autoclaving on the in vitro performance of a laser fluorescence device in detecting occlusal caries lesions in primary teeth. The clinical significance of the experiment is to create a protocol to avoid the possibility of underestimating caries in clinical setting.
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INTRODUCTION

Since tooth is known to fluoresce, the properties of this fluorescence emission have been studied in order to differentiate sound from caries tissue, thus improving caries detection.[1] Using these properties new devices based on this principle have been developed.[2] Quantitative laser/light-induced fluorescence measures intrinsic fluorescence of the teeth[2] and has been mainly used in smooth surfaces to quantify minor mineral changes.[3] Another Laser Fluorescence device (LF) consists of a diode laser that emits a red light (l=655nm), which is absorbed by dental substance. Part of this light is absorbed and then reemitted by chromophores within the dental tissue in a longer wavelength at near-infrared while excitation works. This optical phenomenon is called fluorescence. The system, named DIAGNOdent, measures the amount of fluorescence coming from the organic content of the caries lesions.[4] In the presence of a caries lesion in occlusal or smooth surface, the signal increases and a number scaled from 0 to 99 is displayed.[4-6]

While evidence of the promising results has been shown,[7] a number of potential problematic findings that complicate the LF interpretation have been pointed out. Bacterial dental plaque, stains, restorations have been shown to interfere with the obtained values.[6,8-11] Other concerns which remain unclear against cross-infection is the influence of the use of PVC seal wrap as barrier protection and the sterilization of the tips in autoclave in the readings.

Thus the aim of this study was to evaluate the influence of the PVC seal wrap and the autoclave sterilization of the tips on the measurements and performance of the LF method in suspected occlusal sites in primary molars.

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Materials and Methods

The research protocol received previous consent from the Research Ethics Committee of the School of Dentistry, University of São Paulo (report No. 64 /04 and 153/04).

Sample preparation

Forty primary molars recently exfoliated and presenting at least two thirds of root resorption were donated by the tooth Bank (Faculty of Dentistry, University of São Paulo). All teeth had at least one suspected site (absence of frank cavitation) with occlusal caries. Specimens were stored in saline solution for at least 3 months. Overall, 50 suspected sites on the occlusal surfaces of primary molars were obtained. Photographs (x10) were taken of each tooth, and the area to be detected was indicated by black dots, which allowed reproducible positioning of the probe. The teeth were polished with water and pumice slurry and rinsed with tap water.

LF Measurements

One examiner performed the LF (DIAGNOdent, KaVo, Biberach, Germany) readings following the manufacturer's instructions. Probe tip A (for occlusal surfaces) was selected. The device was calibrated against a ceramic standard prior to examination, and then was recalibrated after every 10th tooth. After the initial calibration, teeth were taken out of the solution and dried for 10seconds. The laser device was calibrated on a sound surface of every tooth, as provided in the manufacturer's calibration procedure, prior to the examination of the suspect site. The baseline value of the fluorescence of each tooth was subtracted electronically from the maximum value recorded for sound surface on the occlusal site under examination. The tip was placed perpendicularly to the site and rotated. Three measurements

were taken for each site and the mean value was recorded for each designated site.

The cut-off limits between sound and carious surfaces was determined from a previous study in primary teeth:[12] 0-9= sound/early enamel caries; 10-17=enamel caries; 18-99=dentinal caries.

PVC Seal wrap study

Measurements using the LF device were performed in teeth under different conditions:

1. Without PVC. LF calibrated against the porcelain standard without PVC seal wrap on its tip, and samples analyzed in this condition.
2. Calibrated with one layer. LF calibrated against the porcelain standard with one layer of the seal wrap on its tip, which was carefully positioned, and samples analyzed in this condition.
3. Calibrated with three layers. The same procedure for the last group, but the tip was covered with three layers of the PVC seal wrap.
4. Calibrated without one layer. LF calibrated against the porcelain standard without PVC seal wrap and calibration of a sound area with one layer of this film on its tip, and then samples were analyzed.
5. Calibrated without three layers. The same procedure for the last group, but the tip was involved with three layers of the PVC seal wrap.

Influence of autoclave study

Measurements using the LF device were performed in teeth under different conditions:

1. New tip. The sites were measured with a new tip, without previous use.
2. Old tip. The sites were measured with the same tip after 50 cycles in autoclave. Each cycle consisted of 15 minutes at 132ºC in one autoclave (M7 SpeedClave, Dabi Atlante, Brazil). Each cycle was initiated after an interval that allowed the tip return to ambient temperature.

Histopathological Analysis

Following examination and scoring, teeth were embedded in clear resin and buccolingual sections with a thickness of about 300mm were cut perpendicular to the suspected occlusal sites with a water-cooled diamond blade (0.3 mm thickness) (Labcut 1010, Extec, Enfield, USA). Most extensive areas with changes were chosen for analysis. The response variable was the presence or absence of caries and the depth of the lesions were examined under the stereomicroscope Olympus SZ-PT (Olympus Optical Co., Japan) at a magnification of 16-32 X by 2 examiners in a joint session until they reached a 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; D4= caries involving the inner half of the dentin.

Statistical Analysis

Mean of LF values were assessed with the Friedman one-way ANOVA test to compare these values under different conditions in which all teeth were exposed in each study. A non-parametric test was used because the LF values did not achieve a normal distribution. Sensitivity, specificity and accuracy were calculated at the D2 and D3 thresholds for all studies, using the cut-off points described for primary teeth.[12] The McNemar change test was used to compare the LF performance in different conditions. Receiver operating characteristics (ROC) analysis was also applied to compare the diagnostic performance, at both D2 and D3 thresholds. A nonparametric statistical test was used to estimate the difference among the areas under the ROC curves.[13] The level of significance for all the tests was taken at $p<0.05$.

Results

PVC seal wrap study

The measurements performed under different conditions of PVC seal wrap in occlusal surface sites of primary molars are shown in table 1. There are statistically significant differences among the results found for groups 1x3; 1x4; 1x5; 2x5 ($p<0.05$).

The sensitivity was higher in the measurements obtained without PVC seal wrap at two analyzed thresholds (D2 and D3), 0.93 and 0.73, respectively. This measurement was smaller in teeth that were calibrated without seal wrap and samples analyzed with three layers of the film at D3 threshold. Nevertheless, the specificity was statistically significantly higher for all other conditions eg.(calibrated with one layer, calibrated with three layers, calibrated without one layer, and calibrated without three layers). The accuracy did not change significantly among the groups at D2 threshold, but decreased in teeth that were analyzed without PVC seal wrap at D3 threshold. The area under ROC curves showed smaller values for the groups that used three layers of the seal wrap (table 1).

Influence of autoclave study

The measurements performed before and after autoclaving cycles in occlusal surface sites in primary molars are shown in table 2. There was a statistically significant difference between the groups ($p<0.05$).

The sensitivity was higher in the readings obtained with a new tip at two analyzed thresholds (D2 and D3), 0.91 and 0.67, respectively, and smaller in the group that used an old tip at D2 and D3 threshold, 0.50 and 0.11, respectively. Nevertheless, the specificity was statistically significantly higher in teeth that were measured with the old tip at two threshold (D2 and D3), 0.86 and 0.95, respectively. The accuracy and the area under ROC curve did not change significantly between the groups (table 2).

Table 1. Mean of Laser Fluorescence values (LF) and the performance of the device under different conditions of PVC seal wrap expressed in sensitivity, specificity, accuracy and the area under the ROC curve (Az) in detecting occlusal caries lesions affecting enamel (D2) and dentin (D3).

Discussion

The protocols for utilization of the LF system vary among studies and some points remain unclear. The use of the PVC seal wrap, as barrier protection has not been standardized in the literature. There are no studies that relate the ideal conditions of the tip to obtain the mostly correct measurements.

We observed that the values related to the performance of the method (sensitivity, specificity, accuracy and area under ROC curves) differed significantly across the different conditions with the use of the seal wrap. LF device has achieved the best performance when one layer of the PVC seal wrap covered the tip. This condition is difficult to achieve since the wrap is thin and tears easily. That is

Table 1. Mean of Laser Fluorescence values (LF) and the performance of the device under different conditions of PVC seal wrap expressed in sensitivity, specificity, accuracy and the area under the ROC curve (Az) in detecting occlusal caries lesions affecting enamel (D2) and dentin (D3).

	LF	Sensitivity		Specificity		Accuracy		Az	
	values	D2	D3	D2	D3	D2	D3	D2	D3
Without	21.7 [a]	0.93 [a]	0.73 [a]	0.56 [a]	0.60 [a]	0.71 [a]	0.62 [a]	0.84 [a]	0.78 [a]
Cal. With-1 layer	18.2 [b]	0.89 [a]	0.64 [a,b]	0.59 [a]	0.73 [b]	0.71 [a]	0.71 [a,b]	0.84 [a]	0.76 [a]
Cal. With-3 layers	13.2 [c]	0.74 [a]	0.36 [a,b]	0.77 [b]	0.80 [b,c]	0.76 [a]	0.73 [a,b]	0.81 [a]	0.67 [b]
Cal. Without-1 layer	11.5 [c]	0.74 [a]	0.46 [a,b]	0.74 [a,b]	0.84 [c,d]	0.74 [a]	0.77 [b]	0.85 [a]	0.78 [a]
Cal. Without-3 layers	8.3 [d]	0.52 [b]	0.27 [b]	0.82 [b]	0.86 [d]	0.70 [a]	0.80 [b]	0.82 [a]	0.73 [a,b]

Different letters express statistically significant difference among values within the same column ($p < 0.05$)

Table 2. Mean of Laser Fluorescence values (LF) and the performance of the device with a new tip and one sterilized 50 times, expressed in sensitivity, specificity, accuracy and the area under the ROC curve (Az), in diagnosing occlusal caries lesions affecting enamel (D2) and dentin (D3).

	LF	Sensitivity		Specificity		Accuracy		Az	
	values	D2	D3	D2	D3	D2	D3	D2	D3
New	18.7 [a]	0.91 [a]	0.67 [a]	0.54 [a]	0.61 [a]	0.70 [a]	0.62 [a]	0.83 [a]	0.76 [a]
After 50 cycles	6.3 [b]	0.50 [b]	0.11 [b]	0.86 [b]	0.95 [b]	0.70 [a]	0.80 [a]	0.78 [a]	0.83 [a]

Different letters express statistically significant difference among values within the same column ($p < 0.05$)

why many clinicians use more than one layer of the seal wrap to protect the patients against cross-infection. The performance is improved as well when the tip is calibrated in the ceramic standard in this same condition. On the other hand, conditions that used three layers of the film showed the worst results, inducing a decrease in readings by the device and consequently a decrease in the values of sensitivity. It is possible that these layers promote a higher reflection of the light that should interact with the dental tissue and then the resultant signal is smaller. Thus, as caries lesions are subestimated, the values for specificity increase. There were no significant changes in accuracy. The area under ROC curve did not change among the groups. The area under the ROC curve analysis does not take a stipulated cut-off point. This kind of analysis investigates several cut-off points, and an area is computed according to these results.[14-16]

Dental instruments classified into non critical category (low risk of transmitting infection) can be reprocessed between patients with intermediate-level or low-level disinfection or detergent wash and rinse, depending on the nature of the surface and the degree and nature of the contamination.[17, 18] The use of clear plastic wrap is recommended to cover surfaces that may be contaminated by saliva. The covering should be removed, discarded and then replaced with clean material between patients. In dentistry, it has been used mostly to cover surfaces of devices such as light handles, x-ray unit heads.[17, 19] Since this film is transparent and thin/smooth it does not interfere with radiographic images.[20] This situation cannot be generalized for studies that use light for diagnosing caries lesions. Our study shows that three layers of the PVC seal wrap provoked a reduction in the mean values obtained with LF device, which in a clinical setting can give rise to sub-treatments.

According to LF manufacturer's, the sterilization of the tips of the device is the best choice against cross-infection. The effectiveness of autoclaving will depend on following autoclave manufacturer's instructions.[17] With no detailed information, it was reported that the tip of the device should be new to avoid variation from the wear of the fibers caused by multiple applications.[21] Concerning the performance of the method, sensitivity decreased after the tip was exposed to 50 cycles in autoclave, mainly at D3 threshold (0.11). The specificity at two thresholds after 50 cycles was improved and the area under ROC curves did not change significantly between groups. Our results are in agreement with the statement that the tip should be new. Due to fact that the sensitivity was poorer after 50 cycles, the number of false negative readings is increased. From this perspective, we believe that the constant sterilization of these tips under autoclave must be avoided and the use of old tips could worsen the performance of the device. Nevertheless, further studies must be performed in order to determine the number of cycles where the decreased values commenced.

In daily practice, many dentists do not have free schedules for sterilizing LF tips, neither they dispose of a quantity of tips to attend the demand of the patients. They utilize, therefore, PVC seal wrap as a barrier protection. We recommend to calibrate following the same condition in which the measurements will be achieved. The PVC seal wrap must cover the tip completely with only one layer, whereas three layers must be avoided. We have seen, therefore, that the utilization of the PVC seal wrap is recommended provided that this protocol is followed.

In conclusion, our results suggest that the LF device can be useful for *in vitro* and *in vivo* studies once standardized criteria is adopted. The sterilization process performed in autoclave is not recommended, since this process interferes with the LF readings and performance. We recommend that the PVC seal wrap used as barrier protection cover the tip carefully, only once and the device be calibrated in the same condition.

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