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Critical steps in electronic volume quantification of gingival crevicular fluid: the potential impact of evaporation, fluid retention, local conditions and repeated measurements

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Background and objectives: Various methodological factors may operate during clinical gingival crevicular fluid (GCF) sampling, volume quantification or subsequent laboratory analysis. For precise volume quantification, specific concern for generation and maintenance of a reliable calibration curve, the potential risk of GCF loss as a result of evaporation or fluid retention on actual volume and the impact of local conditions is needed because each of these factors may act as a source of subsequent volumetric distortions. Thus, the present study aimed to analyse the impact of sample transfer time on the rate of evaporation and the possibility of fluid retention, and the impact of local conditions and number of replicated measurements on the reliability of calibration data.

Materials and methods: To analyse evaporative errors, standardized Periopaper® strips provided with known test volumes (0.1 μl, 0.2 μl, 0.5 μl and 0.6 μl) were transferred to Periotron 8000® with different time intervals (immediately, 5 s, 30 s and 60 s). For fluid retention, after quantifying the actual volume of the strips provided with known volumes (0.1 μl and 0.6 μl) of two test fluids, a second set of measurements was performed using dry strips. To determine the impact of local conditions (temperature and humidity) and the validity of 3, 5 and 20 replications (0.0–0.6 μl with 0.1-μl increments) on device calibration for 20°C and 25°C, electronic readings were obtained from three devices at three different locations. Differences in volumetric data in each experimental design were statistically analysed.

Results: No significant fluid loss was observed within 5 s, but evaporation clearly led to volumetric distortions with extending transfer times (30 s or 60 s) ($p < 0.05$). Measurable amounts of fluid retention were found for both volumes and both test fluids, but not with identical patterns. Local conditions resulted in unique calibration data for each test volume and for each device.

Although a 5°C increase generally provided higher readings, this was not observed for all devices at all volumes. Additional replicates ($n = 5$ or $n = 20$) did not seem to add any further reliability to the triplicate scores for the given test volumes.

Conclusion: The findings of the present study confirm the reliability of triplicate readings, and uniqueness of each device and electronic data and the distinct impact of local environmental conditions on the generation/maintenance of calibration scores for each particular device. Furthermore, they underline time-dependent evaporation and fluid retention as additional technical concerns and once again highlight the importance of methodological standardization of the electronic volume quantification process.

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A considerable part of the interest in gingival crevicular fluid (GCF) seems to be devoted to the unique volumetric features of this biological fluid (1–7). In fact, GCF's specific volumetric features such as the presence of resting volume and flow rate (2, 3, 8, 9), individual and site-specific variations in fluid quantity (4–6, 8, 10–14) and the intensity of the factors with the potential to alter volumetric measures (1, 2, 4, 5, 7, 8, 11, 15–19), make GCF a unique fluid. However, several basic issues still seem to be unclear (20). Various factors that operate during clinical GCF sampling are well defined and to reduce their impact the need to develop an ideal clinical sampling strategy is frequently mentioned (1, 4, 5, 7, 9, 12, 13, 20). Because different sampling techniques, even the placement of paper strips with different extensions (21, 22), and the sampling time (6, 8, 18, 23–28) have been shown to alter actual volume/flow of GCF to various magnitudes (22, 29), methodological considerations mainly emphasize the importance of the clinical sampling technique and sampling time (2, 3, 21, 22, 29). However, if not followed by precise volume quantification, a successful clinical GCF sampling on its own may not ensure a reliable outcome (2, 5, 8, 11, 15, 16) due to additional factors that operate following *in vivo* GCF sampling and that have the potential to interfere with the accuracy of the process of volume quantification (2, 10, 16, 30–35). Thus, an ideal methodological standardization with the most sensitive approach is necessary at both stages (2, 3).

When compared to other available methods, electronic volume quantification provides certain clear advantages (2, 32, 36–38). The Periotron 8000® (Ora Flow Inc., Amityville, NY, USA), which is the latest version of the device, quantifies the volume of GCF or saliva collected on filter papers by measuring the electrical capacitance of a wet paper strip and has the capability to interface the machine with a computer for automatic data recording and output, where the inputted data can also be converted to volumes (30, 37). Thus, by adding computer capability, it further improves the reliability of the process of volume quantification (2, 30, 32, 36–38). However, the possible impact of the room temperature, humidity, sample evaporation, strip location within the machine, fluid retention, reliability/maintenance of calibration data and the viscosity/ionic strength of the calibration fluid deserve a particular interest due to the potential of each of these factors to cause volumetric distortions in electronic readouts (2, 10, 16, 30–35).

As environmental factors can affect the rate of fluid evaporation from paper strips (16), evaporation seems to be an important factor that can cause variations in Periotron® readout measurements (2, 10, 31, 34). Since in an early study, with a previous version of the device (HAR-600), increased room temperature resulted in higher readings, volume determination was claimed to be as important as performing a sensitive *in vivo* GCF sampling (16). Fluid evaporation, room temperature and humidity are suggested to cause shifts in the calibration curve, creating an error of 10% (35) and leading the electronic device to reproduce scores in an error range between 5% and 11% (10). Evaporative losses because of delays in volume quantification were claimed to distort the actual volume and such small errors were reported to become magnified in percentage terms with small volumes, which in fact may be the case in many of the GCF sampling sites (2). Further, evaporation was addressed as a technical problem because it was also shown to affect the content of GCF, leading to erroneously high GCF content at sites with limited quantity of GCF (5, 15, 39, 40). Based on such an understanding, short *in vivo* sampling time (15, 39, 40), immediate transfer of the sample papers to the device and sealing of tubes after the introduction of each sample strip (40) were suggested as a precaution (34) to minimize errors due to evaporation.

Changes in the room conditions may interfere with the process of calibration of the device and the maintenance of the reliability of the calibration curve, which are also crucial issues for accurate volume quantification (2, 16, 21, 22, 28, 30–35, 40–43). In fact, when Suppipat & Suppipat (16) comparatively analysed the effect of local conditions on electronic readings, increased room temperature was found to result in higher readings, either due to the viscosity of the fluid or to a higher evaporation rate at higher temperature. On the other hand, to generate a reliable calibration curve with an appropriate calibration liquid (e.g. saliva, 0.9% saline solution, guinea-pig or human serum, sterile distilled water) (16, 32, 34, 38), each volume is advised to be repeated at least in triplicates (34, 44). However, recommendations for the repetition of each particular volume for three or five times are also

available, indicating the lack of a general consensus (16, 30–32, 38). Since the initially very high digital score with a very wet paper was shown to fall rapidly after a few seconds, as a result of the fluid moving beyond the periphery of the electrodes and depending on the viscosity of the fluid (34), loss of GCF may be assumed to occur due to retention of some of the fluid between the upper and lower counterparts of the device during electronic volume quantification.

The intensity and variety of factors with the potential to interfere with precise electronic volume quantification brings out the necessity for further focusing on development of an appropriate methodological standardization for this step in GCF-related studies. Thus, the present study aims to analyse the impact of transfer time on evaporative losses, the possibility of fluid retention during volume quantification, the potential effect of local conditions on electronic volume readings and the possibility of improvement of the reliability of calibration scores through increased number of repeated measurements.

Material and methods

General preparation protocol

Periotron 8000® was switched on and allowed to warm up before placing a blank Periopaper strip (#593525, Ora Flow Inc., Amityville, NY, USA) between the counterparts of the device and setting the reading dial to zero (31, 34). To prevent variations in actual fluid volume, Periopaper strips® (Ora Flow Inc.) with standardized dimensions, absorbance capacity and surface texture were used. A standardized syringe (Hamilton 0–1.0 μl micro syringe, Hamilton 80100, Hamilton Company, Reno, Nevada, USA) graduated with 10 nl markings was used for calibration of the device. During calibration, an average of every three readings for each volume of distilled water (ranging from 0.0 to 0.6 μl, with 0.1-μl increments) was used to calibrate the machine as recommended (34, 38). The test fluid was repeatedly (three times) drawn into the syringe then dispensed into the vial to ensure that the inner walls of the syringe barrel were coated with fluid, and then the syringe was held at eye level to the fluid meniscus to minimize the pipetting error. The device was placed next to two investigators (one filling the syringe with known amounts of the test fluid, and the other holding and placing the paper strips between the counterparts of the Periotron 8000®), which enabled them to immediately transfer paper strips to Periotron 8000® (31, 45). Special care was taken to ensure that the strip was located at a standard distance between the counterparts of the device (16, 20, 30, 31, 34) and the digital readout was obtained within 16 s. The mean values for each volume were recorded to the computer by the MLCONVRT.EXE program provided by the manufacturer, where a calibration curve was achieved for the conversion of each unit to microlitres (μl). To eliminate the risk of any contamination, after each measurement the electrodes were dried by use of sterile cotton tissue. This preparation protocol was standard for all experimental designs.

Analysis of the possibility, presence and the extent of evaporation

To analyse the presence and the extent of evaporation and to test the potential variations between rather smaller and larger fluid quantities, known test volumes of distilled water (0.1 μl, 0.2 μl, 0.5 μl and 0.6 μl) were included. For the transfer of fluid sample to the device, four experimental time points (baseline – immediate transfer, 5, 30 and 60 s) were determined. To obtain the baseline readings (first set of readings), paper strips provided with a known amount of test volume were immediately transferred between the counterparts of the device. For the second set of readings, the paper strips were again provided with known test volumes; however, before transfer, they were kept within local room conditions for 5 s. The same procedure was repeated for the third set and fourth set of readings, with waiting times of 30 s and 60 s, respectively, prior to the transfer of the paper strips to the device for electronic volume determination. These procedures were performed for each of the mentioned test volumes, and for a given test volume 15 repetitions and a total of 60 measurements were achieved. An additional time point, 10 s, was also tested; however, only five repetitions were performed for each test volume and further readings for 10 s were quitted as a result of the similar readings achieved with 5 s. To eliminate the risk of any fluid retention, the counterparts of the device were dried by use of sterile cotton tissue after each measurement. All measurements were performed on the same day (temperature 20°C, humidity 55%).

Analysis of the possibility, presence and the extent of fluid loss as a result of retention

The possibility and the extent of the retained fluid within the counterparts of the device were analysed by use of two different test fluids with different physiochemical properties, human serum and distilled water. To obtain the serum samples, human venous blood, which was allowed to clot, was centrifuged at 3000 *g* for 5 min (31). Paper strips were provided with either distilled water or human serum for two different test volumes, 0.1 μl and 0.6 μl, representing rather smaller or larger volumes, and strips with known amounts of test fluids were immediately transferred between the counterparts of the device for volume determination. After completion of the first measurement, the measured strip was removed and a dry strip was immediately placed between the counterparts of the device to determine the quantity of the retained distilled water or human serum, and the second electronic measurement indicating the quantity of the retained fluid was achieved as retained fluid reading. Following the quantification of the retained distilled water by use of this second measurement; the counterparts of the device were dried by use of sterile cotton tissue to eliminate a possible contamination with the subsequent measurements. The counterparts of the device were cleaned with an alcohol soaked swab and dried with

Table 1. Statistical data regarding the mean Periotron units (PU) and corresponding microlitre (µl) values for all test volumes at baseline, 5, 30 and 60 s (arithmetic mean ± SEM) (n = 15)

	0.1 µl		0.2 µl		0.5 µl		0.6 µl	
	PU	µl	PU	µl	PU	µl	PU	µl
Baseline	20.27 ± 0.64	0.089 ± 0.003	43.27 ± 0.60	0.213 ± 0.0042	74.00 ± 0.80	0.481 ± 0.0083	85.67 ± 0.96	0.603 ± 0.0087
5 s	19.87 ± 1.21	0.088 ± 0.006	39.07 ± 0.88	0.187 ± 0.0053	73.00 ± 1.23	0.471 ± 0.0129	83.87 ± 0.87	0.587 ± 0.0089
30 s	19.40 ± 0.92	0.0833 ± 0.0044	37.33 ± 1.37	0.176 ± 0.0077	70.80 ± 0.75	0.447 ± 0.007	81.80 ± 0.70	0.567 ± 0.733
60 s	6.60 ± 0.82	0.029 ± 0.004	22.87 ± 1.32	0.101 ± 0.006	65.60 ± 1.65	0.396 ± 0.017	78.93 ± 1.34	0.535 ± 0.015
Baseline *U*	111.000	112.000	35.000	38.000	100.500	100.500	83.000	82.500
vs. 5 s *p*	0.967	1.000	**0.001***	**0.001***	0.624	0.624	0.233	0.217
Baseline *U*	103.000	96.000	43.500	39.000	49.500	47.000	47.500	47.500
vs. 30 s *p*	0.713	0.512	**0.003***	**0.002***	**0.008***	**0.006***	**0.006***	**0.006***
Baseline *U*	0.0001	0.0001	0.0001	0.0001	26.500	26.500	35.000	35.000
vs. 60 s *p*	**0.0001***	**0.0001***	**0.0001***	**0.0001***	**0.0001***	**0.0001***	**0.0001***	**0.0001***

Levene's test was used for the equality of variances. Since data was not normally distributed, Kruskal–Wallis analysis was used for differences among all test periods and Mann–Whitney *U*-test [with Bonferroni correction (α/iteration number = 0.00833)] was used for bilateral comparisons [0.1 PU ($\chi^2 = 33.057$, $p = 0.0001$*), 0.1 µl ($\chi^2 = 33.123$, $p = 0.0001$*), 0.2 PU ($\chi^2 = 40.356$, $p = 0.0001$*), 0.2 µl ($\chi^2 = 40.930$, $p = 0.0001$*), 0.5 PU ($\chi^2 = 17.550$, $p = 0.001$*), 0.5 µl ($\chi^2 = 17.778$, $p = 0.0001$*), 0.6 PU ($\chi^2 = 16.256$, $p = 0.001$*) and 0.6 µl ($\chi^2 = 16.280$, $p = 0.001$*)].
*Significant.

a cotton tissue during human serum assay (31, 34). All measurements were performed on the same day (temperature 20°C, humidity 55%), and 20 repetitions for each test volume with each test fluid were performed.

Analysis of the impact of local conditions and number of volumetric repetitions

The calibration data reported were collected from three Periotron 8000® devices at three different locations (in periodontology departments of three dental schools at three different cities). The above-mentioned general preparation protocol was applied in a strict standard methodology for all devices, expressed as Periotron-1, -2 and -3. Periotron readings and corresponding microlitre values were determined for both 20°C and 25°C, and the devices were calibrated for each temperature as previously described. Electronic measurement for each test volume was repeated 20 times, and a total of 120 readings were achieved (ranging from 0.0 to 0.6 µl, with 0.1-µl increments) for each temperature with each device. In order to evaluate the potential value of increased number of repetitions for a given test volume for the reliability of calibration, mean values were also calculated for 3, 5 and 20 × readings for each volume. For a given temperature, all measurements were performed on the same day.

Statistical analysis

SPSS (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis.

Quantification of fluid evaporation — Levene's test was used for the equality of variances (46). As data was not normally distributed, Kruskal–Wallis analysis was performed for among group (time intervals) comparisons (47) and Mann–Whitney *U*-test with Bonferoni correction (α/iteration number = 0.00833) was used for bilateral comparisons (48, 49).

Quantification of fluid retention — Levene's test was used for the equality of variances (46). When data was

normally distributed, differences between the two test fluids were analysed by *t*-test for independent samples (50). When data was not normally distributed, Mann–Whitney *U*-test was used (48). *t*-test was used for the statistical analysis of the two test volumes (50).

Quantification of the impact of local conditions and number of volumetric repetitions — The data (a total of 720 Periotron readings and the corresponding microlitre values) from three devices at three dental schools were pooled at one of the dental schools for statistical analysis. Levene's test was used for the equality of variances (46). When data was normally distributed, one-way ANOVA (46) was performed for among device comparisons and bilateral comparison of the devices was utilized by Tukey's HSD test (51) at 20°C and 25°C. When the data was not normally distributed, Kruskal–Wallis analysis (47) was performed for among device comparisons and Mann–Whitney *U*-test (with Bonferoni correction) was done for bilateral comparison of devices at 20°C and 25°C (48, 49). Same statistical analysis was performed for the comparison of 20°C and 25°C for each individual device at intra-machine level.

Results

Presence and extent of fluid loss as a result of evaporation

A total of 260 Periotron readings and the corresponding microlitre values were obtained. Mean Periotron unit (PU) scores, corresponding microlitre values for each experimental time point and actual *p*-values are shown in Table 1. The results clearly demonstrated that evaporation took place during volume quantification. There was a general trend for evaporation to increase in accordance with the extension of the transfer time for all test volumes, as diagrammatically demonstrated in Fig. 1.

Baseline vs. 5-s measurements — Volumetric features of baseline and 5-s measurements were mostly similar and a statistically significant loss of fluid was not noticed ($p > 0.05$), except 0.2 µl. Measurements for 10 s provided very similar results to 5 s for all test volumes (data not shown).

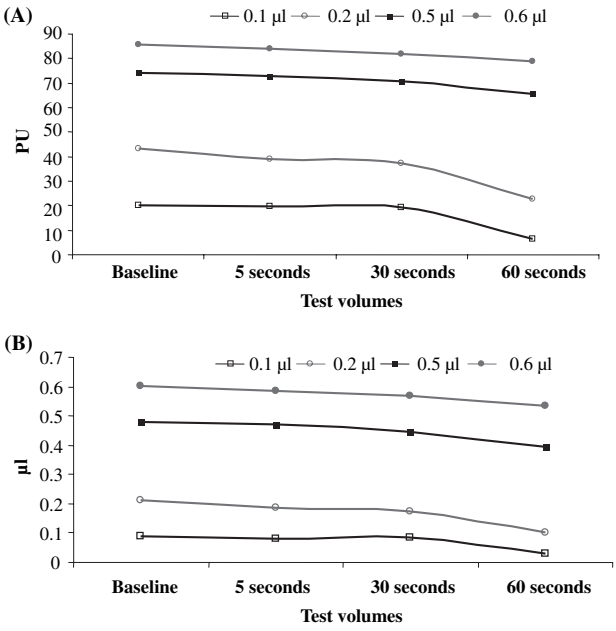

Fig. 1. Diagrammatic presentation of extent of evaporation expressed as Periotron units (PU) and microlitre (µl) values based on sample transfer time. (A) PU scores; (B) µl values.

Baseline vs. 30-s measurements — A general trend of reduction in the initial volume was observed for all of the test volumes at 30 s. This reduction was significant for all test volumes ($p < 0.05$), except 0.1 µl. A 4.3% of fluid loss for 0.1 µl, a 13.73% of loss for 0.2 µl, a 4.32% of loss for 0.5 µl and a 4.52% of loss for 0.6 µl was noticed. There was no specific trend of fluid loss for either relatively smaller (0.1 µl and 0.2 µl) or larger volumes (0.5 µl and 0.6 µl).

Baseline vs. 60-s measurements — The extent of evaporation and loss of fluid reached to the highest rate at 60 s. The loss of fluid was 67.44% for 0.1 µl, 47.15% for 0.2 µl, 11.36% for 0.5 µl and 7.87% for 0.6 µl, and was significant for all test volumes ($p < 0.0005$). Further, the amount of evaporation showed different trends for the relatively smaller or the larger volumes. The rate of evaporation was significantly higher [67% (0.1 µl) and 47% (0.2 µl)] for the relatively smaller volumes compared to the larger volumes [11% (0.5 µl) and 7.87% (0.6 µl)].

Presence and extent of the loss of fluid as a result of retention

A total of 80 Periotron readings and corresponding microlitre values were obtained. The actual volume as determined by the first set of measurements and the quantity of retained fluid as determined by the second set of measurements for both volumes and test fluids and actual *p*-values are shown in Table 2. With both of the test fluids, some amount of fluid was retained between the counterparts of the device during the process of electronic volume determination. For a given test volume, electronic readings significantly differed between the two test fluids and this was true for the quantity of both the actual and the retained fluid ($p < 0.05$). In general the actual reading for distilled water was higher for both test volumes (0.1 µl and 0.6 µl) compared to human serum. When the amount of retained fluid was concerned, distilled water showed a different pattern than human serum. For the relatively smaller volume (0.1 µl), the amount of retained fluid

Table 2. Statistical data regarding the actual fluid (first reading) and retained fluid (second reading) for both test fluids and test volumes (arithmetic mean ± SEM) (n = 20)

	Actual fluid				Retained fluid			
	0.1 µl		0.6 µl		0.1 µl		0.6 µl	
	PU	µl	PU	µl	PU	µl	PU	µl
Water	26.65 ± 0.36	0.11 ± 0.001919	90.95 ± 0.82	0.6270 ± 0.009655	1.10 ± 0.32	0.003 ± 0.001051	2.30 ± 0.42	0.008 ± 0.001717
Serum	24.95 ± 0.56	0.1125 ± 0.003234	84.05 ± 1.02	0.5755 ± 0.0115	2.05 ± 0.49	0.0055 ± 0.001983	0.65 ± 0.29	0.0015 ± 0.1094
Water vs. serum								
t/*U*	2.561	0.665	5.260	3.421	1.620	$U = 181.0$	$U = 87.5$	$U = 102.0$
p	**0.015***	0.510	**0.0001***	**0.002***	0.114	0.532	**0.001***	**0.002***
0.1 µl vs. 0.6 µl								
t	4.645	53.250	2.303	2.364	48.520	38.833	2.252	1.633
p	**0.0001***	**0.0001***	**0.033***	**0.029***	**0.0001***	**0.0001***	**0.036***	0.119

Water vs. serum: Levene's test was used for the equality of variances. When data was normally distributed, *t*-test for independent samples was used. When data was not normally distributed, Mann–Whitney *U*-test was used for analysis of two test fluids. 0.1 µl vs. 0.6 µl: *t*-test was used for analysis of two test volumes.
*Significant.

was lower for distilled water, whereas the amount of retained fluid for the larger volume (0.6 µl) was higher than human serum. The amount of retained distilled water for 0.1-µl volume was significantly less than 0.6 µl. This situation was opposite for human serum and was significant only at the Periotron unit (PU) level ($p < 0.05$). The amount of retained fluid of distilled water for 0.6 µl was significantly higher than human serum ($p < 0.05$). There was an 8.21% loss of human serum for the smaller volume (0.1 µl), whereas this amount was 4.12% for distilled water. However, at a higher volume (0.6 µl), less serum (0.77%) was lost compared to distilled water (2.52%).

Analysis of the impact of local conditions and number of volumetric repetitions

Estimated marginal means of Periotron unit and microlitre values for three devices — **Periotron unit:** All devices demonstrated the similar linear trend at 20°C for all calibration volumes as shown in Fig. 2(A). However, the same pattern was not observed at 25°C, where Periotron-1 provided the least linear measurements and Periotron-3 provided the highest measurements (Fig. 2B). For Periotron-2, a sharp increase was noticed between 0.3 µl and 0.4 µl, and 0.4 µl and 0.5 µl.

Microlitres: As shown in Figs 3(A and B), all three devices represented more linear curves at 20°C compared to 25°C. Periotron-1 values presented a straight linear distribution. However, sharp increases were observed between 0.3 µl and 0.4 µl for Periotron-1, and between 0.4 µl and 0.5 µl for Periotron-3 at 25°C.

Comparison of calibration scores at within-machine and between-machine level — **20°C:** Data including the mean, minimum and maximum values of each device at 20°C and actual *p*-values are presented in Table 3. The room conditions were standard for the temperature; however, the level of humidity differed as being 55% for Periotron-1, 72% for Periotron-2 and 49% for Periotron-3. There were significant differences in both PU and µl

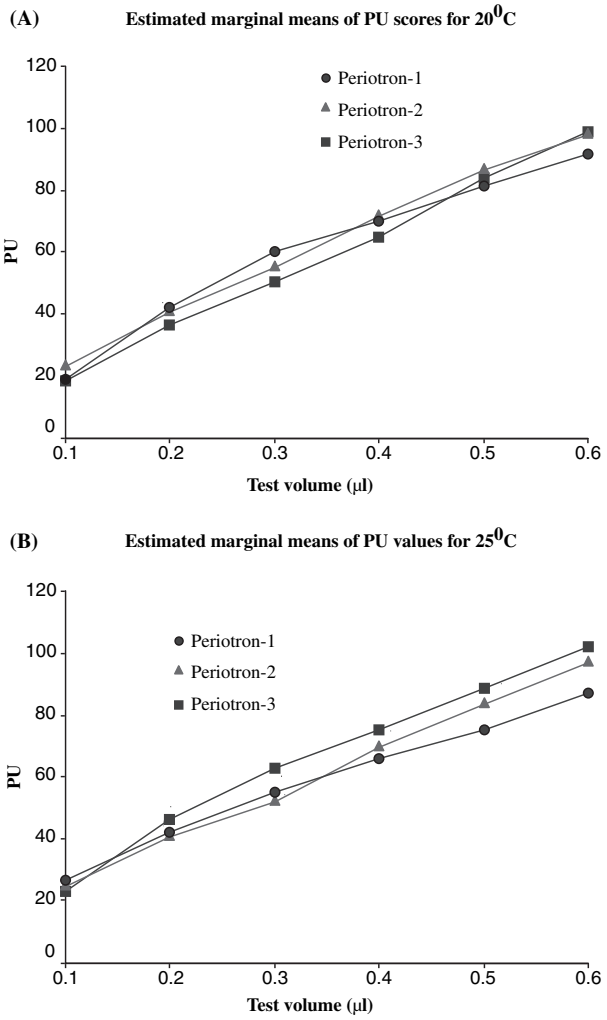

Fig. 2. Plots of Periotron units (PU) scores vs. tested volume for three devices at 20°C and 25°C. (A) 20°C; (B) 25°C.

values for each test volume among all devices at 20°C, indicating the presence of a unique calibration data and calibration curve for each Periotron ($p < 0.05$). The variability for minimum and maximum values was within a range of 5–26 PU. However, this range did not reveal an increase from relatively lower (0.1 µl) to higher (0.6 µl) calibration volumes. The significant differences among the three devices for µl values were more notable than PUs.

25°C: Data including the mean, minimum and maximum values of each device at 25°C and actual *p*-values are presented in Table 4. At this standard temperature, the level of humidity was 56% for Periotron-1, 72% for Periotron-2 and 39% for Periotron-3. Similar to 20°C, there were statistically significant differences between machine readouts at 25°C ($p < 0.05$), where these significances for PU values were more remarkable than µl values. When all machines were accounted together, the calibration values including minimum, maximum and mean values of each Periotron were also different from each other at 25°C. The variability for minimum and maximum values was within a range of 8 to 32 PU.

20°C vs. 25°C: Statistical comparisons of the volumetric data for 20°C vs. 25°C and actual *p*-values are shown in Table 5. There were within machine significances between 20°C and 25°C comparisons for all devices ($p < 0.05$). As a general trend, the range of minimum and maximum PU values at 25°C was higher than 20°C values, and 55.5% of the mean PU readings at 25°C were higher than the 20°C values. Nevertheless, all PU values for all volumes in Periotron-3, which had the least and the most stabile humidity at 25°C, were higher at 25°C than at 20°C, whereas Periotron-1 and -2 provided higher values for relatively smaller volumes (0.1 µl and 0.2 µl) and reduced readouts between 0.3 and 0.6 µl at 25°C compared to 20°C data. Consequently, compared to 20°C values, 25°C values were similar to or higher than each other for relatively smaller volumes (0.1 µl and 0.2 µl), whereas higher volumes (≥ 0.3 µl) demonstrated lower values (Fig. 4).

The possible impact of the number of repetitions for each calibration volume — Table 6 shows the mean, minimum and maximum values for the first three and the first five Periotron readouts of three devices for each volume that were selected from 20 × analysis. Although statistical analysis could not be performed, when all devices and both temperatures were considered together, 3 ×, 5 × and 20 × readouts were found to be almost similar to each other. The sets repeated for five or 20 times demonstrated a similar trend with triplicate readings at both temperatures (Figs 5 and 6), indicating that 20 × did not represent any additional trend of sensitivity or reliability compared to three or five replications at Periotron 8000®.

Discussion

Evaporation is widely considered as a technical problem that could interfere with both *in vivo* GCF sampling (15, 39) and with volume quantification processes (2, 10, 16, 31, 34, 52), and act as a source of volumetric error, particularly for small GCF volumes (10, 15, 31, 34). If a wet filter paper strip was left on a balance without being included in a sealed container, evaporation was observed by following the decrease in weight recorded on the balance (2). In a similar manner, as the moisture on the strip was reduced through evaporation the

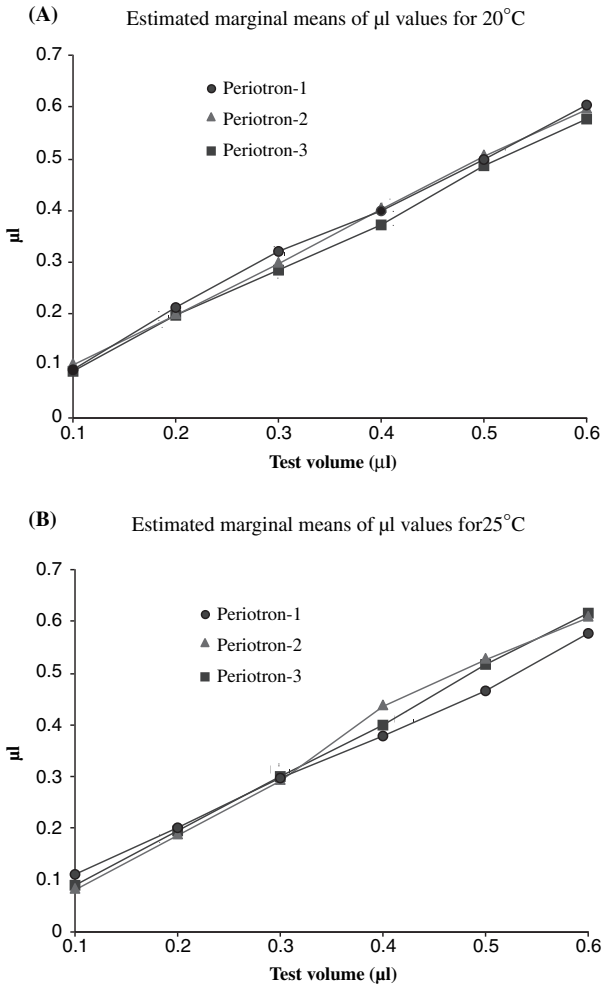

Fig. 3. Plots of corresponding microlitre (µl) values vs. tested volumes for three devices at 20°C and 25°C. (A) 20°C; (B) 25°C.

electronic reading was shown to reduce (16). Evaporation, due to inevitable delay in measuring the strip, was listed among the disadvantages of the staining techniques and in methods where wet strips are weighed (2). The measurement technique and problems with evaporation, rather than Periotron 6000® itself, were shown to produce errors, especially for volumes < 0.2 µl (mean error due to evaporation 18.7%), and it was calculated that 0.1 µl of GCF would only have to lose 10 nL through evaporation in order to incur a 10% error, and evaporation was also accounted as the main source of within machine variances (31). In another study, the range of error due to evaporation was reported to be between 5% and 11% (10) and evaporative errors were especially considered to be crucial for sites harbouring only small amounts of GCF (10, 31, 34). Volumetric errors leading to small losses of fluid have also been reported to affect the concentration of various GCF constituents, including antibiotics, enzymes and total protein levels (5, 15, 39, 40). Small losses of fluid are suggested to result in erroneously high antibiotic concentration within GCF (39, 40). Sekellari *et al.* (39) suggested that the high tetracycline concentration of GCF could be attributed to concentration of antibiotics on paper strips as a result of evaporation due to the long sampling duration at healthy sites. Lamster *et al.* (5) reported that small errors in volume quantification could lead to 50% or greater errors in calculation of enzyme concentration in sites harbouring limited quantity of GCF but measurable amounts of enzyme. The same situation was observed for the total protein levels of GCF, and the wide range of GCF protein levels in different studies was attributed to the evaporative losses that occurred prior to volume quantification, which could lead to greater underestimation of total sample (where the initial volume was low) and to an overestimation of the concentration of GCF components (e.g. total protein) (15).

Evaporation was further suggested to be related with the room conditions (10, 16, 30, 53) and it was shown that an increase in room temperature (from 20°C to 37°C) led to a fast evaporation (53). At constant temperature, higher electronic readings, longer reading time and slow evaporation rate were observed, whereas at constant humidity, the higher room temperatures providing higher volumetric readings and requiring less time for measurement were attributed to the viscosity of the fluid or to a higher evaporation rate at higher temperature (16). When different test fluids were concerned, different solutions were shown to consume different reading times due to slower rates of diffusion and evaporation (16). However, the extent of evaporation during volume quantification and the impact of sample transfer time have not been clarified.

The results of the present study confirmed that a time-dependent evaporation took place during sample transfer for electronic volume quantification, which is in agreement with the previous studies addressing evaporation as a technical concern (2, 10, 16, 30, 31, 34, 52–54). Based on the fact that significant losses in the actual fluid volume occurred within 30 s and 60 s, then 5 s may be suggested as a relatively safe transfer time. Although statistical analysis was not performed, a prominent fluid loss was not observed within 10 s (data not shown). However, our results should be considered with precaution because it probably would be applicable only to the given test fluid and room conditions. It can be assumed that there would naturally

Table 3. Statistical data for Periotron units (PU) and corresponding microlitre (μl) values for Periotron 8000®-1, -2 and -3 at 20°C [arithmetic mean ± SEM and range (min–max)] (n = 20)

		Periotron 8000®			1 vs. 2		1 vs. 3		2 vs. 3	
Volume		1(55%)	2(72%)	3(49%)	*U*	*p*	*U*	*p*	*U*	*p*
0.1	PU	19.15 ± 0.39	23.40 ± 1.20	18.75 ± 0.89	86.000	**0.002***	35.000	**0.0001***	102.500	0.007
	(min–max)	(17–22)	(12–32)	(13–26)						
	μl	0.0925 ± 0.002036	0.1030 ± 0.006329	0.0915 ± 0.005041	160.000	0.289	117.500	0.024	143.000	0.127
	(min–max)	(0.08–0.11)	(0.05–0.15)	(0.06–0.13)						
0.2	PU	42 ± 0.62	40.45 ± 1.12	36.45 ± 1.13		0.863		**0.001***		**0.044***
	(min–max)	(37–46)	(31–50)	(26–47)						
	μl	0.2120 ± 0.003044	0.1995 ± 0.007199	0.1975 ± 0.006916	142.000	0.121	83.500	**0.001***	200.000	1.000
	(min–max)	(0.19–0.23)	(0.14–0.26)	(0.13–0.26)						
0.3	PU	60.25 ± 0.60	55.25 ± 1.42	50.65 ± 0.87	81.000	**0.001***	10.500	**0.0001***	72.500	**0.0001***
	(min–max)	(53–65)	(37–63)	(42–57)						
	μl	0.3225 ± 0.003965	0.2965 ± 0.009127	0.2845 ± 0.005452	109.000	0.013	58.000	**0.001***	134.000	0.076
	(min–max)	(0.28–0.36)	(0.18–0.35)	(0.23–0.32)						
0.4	PU	70.30 ± 0.87	71.45 ± 0.84	64.75 ± 1.05	160.000	0.289	170.500	0.429	53.500	**0.0001***
	(min–max)	(63–77)	(62–77)	(58–72)						
	μl	0.3985 ± 0.007444	0.404 ± 0.005777	0.3725 ± 0.006604	164.000	0.341	79.500	**0.001***	80.500	**0.001***
	(min–max)	(0.34–0.46)	(0.34–0.44)	(0.33–0.42)						
0.5	PU	81.55 ± 1.12	86.45 ± 1.11	84.00 ± 1.16		**0.020***		0.592		0.592
	(min–max)	(75–91)	(76–94)	(76–99)						
	μl	0.50 ± 0.01138	0.5055 ± 0.007996	0.4880 ± 0.007056		0.998		0.922		0.705
	(min–max)	(0.44–0.60)	(0.43–0.56)	(0.44–0.58)						
0.6	PU	91.45 ± 0.88	97.70 ± 0.62	98.75 ± 1.04		**0.0001***		**0.0001***		0.961
	(min–max)	(84–96)	(93–103)	(89–107)						
	μl	0.6025 ± 0.009621	0.5945 ± 0.005915	0.5765 ± 0.006580	148.500	0.165	176.500	0.529	130.500	0.060
	(min–max)	(0.52–0.65)	(0.55–0.65)	(0.52–0.63)						

Levene's test was used for the equality of variances. When data was normally distributed, one-way ANOVA was performed for among device comparisons and bilateral comparison of the devices was utilized by Tukey's HSD test including 0.2 PU ($F = 10.694$, $p = 0.0001$*), 0.5 PU ($F = 18.601$, $p = 0.0001$*), 0.5 μl ($F = 6.28$, $p = 0.0001$*) and 0.6 PU ($F = 36.777$, $p = 0.0001$*). When data was not normally distributed including 0.1 PU ($\chi^2 = 51.522$, $p = 0.0001$*), 0.1 μl ($\chi^2 = 24.107$, $p = 0.0001$*), 0.2 μl ($\chi^2 = 15.030$, $p = 0.0101$*), 0.3 PU ($\chi^2 = 72.641$, $p = 0.0001$*), 0.3 μl ($\chi^2 = 23.146$, $p = 0.0001$*), 0.4 PU ($\chi^2 = 49.847$, $p = 0.0001$*), 0.4 μl ($\chi^2 = 38.882$, $p = 0.0001$*) and 0.6 μl ($\chi^2 = 19.993$, $p = 0.001$*), Kruskal–Wallis analysis was used for among device comparisons and Mann–Whitney *U*-test [with Bonferroni correction (α/iteration number = 0.00333)] was used for bilateral comparison of the devices.*Significant.

be variances in room conditions among studies and changes in room temperature and humidity in a clinical setting would not be as great as the artificial conditions set in an experimental design (16). Further, standardization of the room conditions would not always be feasible in the clinical setting and, therefore, results would not pertain to clinical environment (38). Thus, to minimize evaporative losses, we support the previous studies recommending immediate transfer (within 0–2 s) (31, 45) of sample strips to the device, standardization of room conditions (whenever possible) (16, 31) and placement of the sample strips within sealed containers (40).

The user manual of Periotron® and most previous studies recommend cleaning or drying of the counterparts of Periotron® after each measurement with alcohol or cotton tissue (18, 31, 34). In a previous study, being much less viscous than serum, 0.9% saline solution was suggested to more readily diffuse out of the area enclosed by the counterparts of the device (34). If significant amounts of the fluid are to be retained within the device, it can be assumed that this fluid loss could serve as a source of a methodological error. Our findings revealed that a measurable amount of both test fluids was retained between the counterparts of the device after each measurement, depending on the quantity and properties of the test fluid. The amount of fluid retention was generally found to be higher for small test volumes compared to larger ones for both fluids. The percentage of the retained fluid for 0.1 μl of serum or of water was 8.21 and 4.12, respectively, and the amount of retained fluid for 0.6 μl of serum or of water was 0.77 and 2.52, respectively. These findings may suggest fluid retention as an additional methodological concern, especially for small volumes.

For a given test volume electronic scores significantly differed between the two test fluids (higher for distilled water), which was true for both the quantity of the actual volume and the retained fluid volume. Readings for equal volumes of various fluids (e.g. deionized water, serum, distilled water, saline, tryptic soy broth, ethanol, methanol) were shown to differ (10, 31, 54) and the variances were attributed to the influence of viscosity (16, 31, 38), differences in dissipation constant (10), composition and pH (31, 38), some other physiochemical effects, perhaps the adsorption of ions and/or proteins on the electrode surface (30), and volume delivery systems (31, 54). Our findings generally are in accordance with the previous studies reporting different reading scores for equal quantities of various solutions (16, 30,

Table 4. Statistical data for Periotron units (PU) and corresponding microlitre (µl) values for Periotron 8000®-1, -2 and -3 at 25°C [arithmetic mean ± SEM and range (min–max)] (n = 20)

Volume		Periotron 8000®			1 vs. 2		1 vs. 3		2 vs. 3	
		1(56%)	2(72%)	3(39%)	*U*	*p*	*U*	*p*	*U*	*p*
0.1	PU	26.75 ± 0.64	24.70 ± 0.80	23.40 ± 0.65	137.000	0.091	82.500	**0.001***	153.000	0.211
	(min–max)	(23–31)	(18–32)	(18–29)						
	µl	0.1105 ± 0.003283	0.0825 ± 0.003898	0.0895 ± 0.003033	43.500	**0.0001***	64.000	**0.0001***	150.500	0.183
	(min–max)	(0.09–0.13)	(0.05–0.12)	(0.07–0.11)						
0.2	PU	42.10 ± 0.56	40.70 ± 1.14	46.15 ± 1.01		0.906		**0.040***		**0.001***
	(min–max)	(38–48)	(32–53)	(30–51)						
	µl	0.2005 ± 0.003871	0.1855 ± 0.009854	0.1945 ± 0.004892	113.000	0.018	177.000	0.547	125.500	0.043
	(min–max)	(0.17–0.24)	(0.12–0.30)	(0.12–0.22)						
0.3	PU	55.25 ± 0.54	52.25 ± 0.68	62.95 ± 1.00	93.500	**0.003***	31.000	**0.0001***	11.000	**0.0001***
	(min–max)	(52–61)	(48–58)	(54–73)						
	µl	0.2960 ± 0.004	0.2925 ± 0.006762	0.3010 ± 0.007251	181.000	0.620	170.000	0.429	171.000	0.445
	(min–max)	(0.27–0.34)	(0.25–0.35)	(0.24–0.38)						
0.4	PU	65.75 ± 0.70	69.30 ± 0.71	75.15 ± 1.5	70.000	**0.0001***	33.500	**0.0001***	64.000	**0.0001***
	(min–max)	(61–74)	(63–79)	(59–91)						
	µl	0.3795 ± 0.006003	0.4355 ± 0.004946	0.3985 ± 0.013	25.000	**0.0001***	144.000	0.134	84.000	**0.001***
	(min–max)	(0.34–0.45)	(0.39–0.50)	(0.27–0.54)						
0.5	PU	75.30 ± 1.03	83.65 ± 0.95	88.75 ± 1.06		**0.0001***		**0.0001***		**0.013***
	(min–max)	(66–85)	(75–94)	(77–98)						
	µl	0.4650 ± 0.009305	0.5260 ± 0.005867	0.5160 ± 0.009015		**0.0001***		**0.001***		0.963
	(min–max)	(0.38–0.55)	(0.47–0.59)	(0.41–0.59)						
0.6	PU	87.20 ± 0.81	96.70 ± 0.95	102.15 ± 0.97		**0.0001***		**0.0001***		**0.0001***
	(min–max)	(82–94)	(90–106)	(93–109)						
	µl	0.5780 ± 0.009614	0.6055 ± 0.006261	0.6165 ± 0.006294	130.500	0.06	91.000	**0.003***	143.000	0.127
	(min–max)	(0.52–0.67)	(0.56–0.67)	(0.55–0.66)						

Levene's test was used for the equality of variances. When data was normally distributed, one-way ANOVA was performed for among device comparisons and bilateral comparison of the devices was utilized by Tukey's HSD test including 0.2 PU ($F = 10.694$, $p = 0.0001$*), 0.5 PU ($F = 18.601$, $p = 0.0001$*), 0.5 µl ($F = 6.28$, $p = 0.0001$*) and 0.6 PU ($F = 36.777$, $p = 0.0001$*). When data was not normally distributed including 0.1 PU ($\chi^2 = 51.522$, $p = 0.0001$*), 0.1 µl ($\chi^2 = 24.107$, $p = 0.0001$*), 0.2 µl ($\chi^2 = 15.030$, $p = 0.0101$*), 0.3 PU ($\chi^2 = 72.641$, $p = 0.0001$*), 0.3 µl ($\chi^2 = 23.146$, $p = 0.0001$*), 0.4 PU ($\chi^2 = 49.847$, $p = 0.0001$*), 0.4 µl ($\chi^2 = 38.882$, $p = 0.0001$*) and 0.6 µl ($\chi^2 = 19.993$, $p = 0.001$*), Kruskal–Wallis analysis was used for among device comparisons and Mann–Whitney *U*-test [with Bonferroni correction (α/iteration number = 0.00333)] was used for bilateral comparison of the devices.

Table 5. Statistical data regarding comparison of three devices (Periotron 8000®-1, -2, -3) at 20°C and 25°C (n = 20)

Volume		20°C vs. 25°C				Periotron-1		Periotron-2		Periotron-3	
		F	*p*	χ^2	*p*	*U*	*p*	*U*	*p*	*U*	*p*
0.1	PU			51.522	**0.0001***	0.0001	**0.0001***	172.500	0.461	75.000	**0.0001***
	µl			24.107	**0.0001***	68.000	**0.0001***	111.000	0.015	193.000	0.862
0.2	PU	10.694	**0.0001***				1.000		1.000		**0.0001***
	µl			15.030	**0.0001***	118.000	0.026	134.000	0.076	185.500	0.698
0.3	PU			72.641	**0.0001***	37.500	**0.0001***	95.500	0.004	7.500	**0.0001***
	µl			23.146	**0.0001***	57.000	**0.0001***	164.500	0.341	137.000	0.091
0.4	PU			49.847	**0.0001***	67.500	**0.0001***	111.500	0.015	32.500	**0.0001***
	µl			38.882	**0.0001***	128.500	0.052	63.000	**0.0001***	134.500	0.076
0.5	PU	18.601	**0.0001***				**0.001***		0.442		**0.027***
	µl	46.905	**0.0001***				0.054		0.546		0.204
0.6	PU	36.777	**0.0001***				**0.013***		0.968		0.084
	µl			19.993	**0.001***	127.000	0.049	161.000	0.301	65.500	**0.0001***

Levene's test was used for the equality of variances. When data was normally distributed, One-way ANOVA was performed for among device comparisons and bilateral comparison of the devices was utilized by Tukey's HSD test including 0.2 PU ($F = 10.694$, $p = 0.0001$*), 0.5 PU ($F = 18.601$, $p = 0.0001$*), 0.5 µl ($F = 6.28$, $p = 0.0001$*) and 0.6 PU ($F = 36.777$, $p = 0.0001$*). When data was not normally distributed including 0.1 PU ($\chi^2 = 51.522$, $p = 0.0001$*), 0.1 µl ($\chi^2 = 24.107$, $p = 0.0001$*), 0.2 µl ($\chi^2 = 15.030$, $p = 0.0101$*), 0.3 PU ($\chi^2 = 72.641$, $p = 0.0001$*), 0.3 µl ($\chi^2 = 23.146$, $p = 0.0001$*), 0.4 PU ($\chi^2 = 49.847$, $p = 0.0001$*), 0.4 µl ($\chi^2 = 38.882$, $p = 0.0001$*) and 0.6 µl ($\chi^2 = 19.993$, $p = 0.001$*), Kruskal–Wallis analysis was performed for among device comparisons and Mann–Whitney *U*-test [with Bonferroni correction (α/iteration number = 0.00333)] was used for bilateral comparison of the devices.
*Significant.

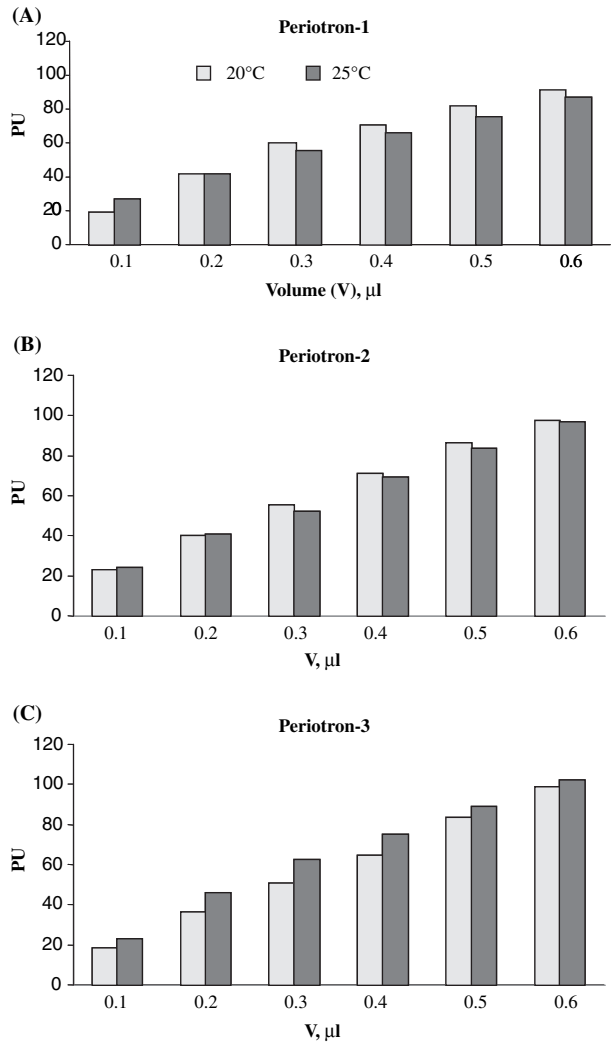

Fig. 4. Diagrammatic presentation of electronic data for 20°C and 25°C for each particular device. (A) Periotron-1; (B) Periotron-2; (C) Periotron-3.

31, 34, 37, 38, 42, 55) and are similar to the study of Suppipat & Suppipat (16), where distilled water provided higher readouts than serum.

When Garnick *et al.* (10) tested the consistency in measurement with different calibration liquids, a 5% to 11% error range in the assessment of the test liquids as a result of fluid evaporation was noted due to room temperature and room humidity. Suppipat & Suppipat (16) revealed increased readout values at higher room temperature, where this effect was attributed to a higher evaporation rate at higher temperature, especially when the volume exceeded 0.2 µl. They also suggested that the difference in readings could be due to a combination of the faster spread as the viscosity of fluid decreased with increasing temperature and a higher evaporation rate at higher temperature (16). Although Ciantar & Caruana (30) did not directly analyse the effect of environmental conditions, they agreed with the suggestions of Suppipat & Suppipat (16) that environmental humidity and temperature should have been kept constant throughout the process so as not to cause variations. In the present study, it was observed that electronic readings could vary between and within individual devices, and there was variability in minimum–maximum and mean PU readouts and microlitre conversions of each calibration volume at different room conditions. When PU scores and corresponding microlitre values were concerned, it was observed that the compatibility of the devices was better for microlitre values than PU readings and also better for 20°C.

In the present study, increased room temperature generally resulted in higher readings, which followed a similar trend to the earlier study of Suppipat & Suppipat (16) and these results at least partially support their findings (16). However, this was not true for all measurements and all volumes because some electronic readings at 25°C were noticed to be lower than 20°C. In fact, each device provided different PU readouts under different room conditions. Periotron-1 and -2, located at relatively higher room humidity, provided increased PU readouts for higher volumes (≥ 0.3 µl) at 25°C compared to 20°C, whereas Periotron-3 at lower room humidity demonstrated higher PU readouts for all calibration volumes at 25°C compared to 20°C. This may indicate that both the increased room temperature and altering humidity levels could result in different readings and these findings may be interpreted as uniqueness of each device under certain local circumstances. Readout values for known amounts of fluids were shown to differ widely between device models (HAR-600 and HAR-6000) in the ultra low volume ranges (< 0.2 µl), which make direct comparison of different devices invalid (54). Thus, we confirm the study of van der Bijl *et al.* (54) suggesting that under any circumstances, each device (the latest version of Periotron) should be considered as unique and self-specific, and we also support the comment of Griffiths (2), 'Machines differ markedly in their range and each machine needs its own calibration'.

As local room conditions may vary, they are likely to play a pivotal role in the process of volume quantification (53) and study designs need to be well thought-out for minimizing such volumetric distortions. Because a 5°C increase in room temperature with different humidity levels may interfere with the readings, not only the room temperature but also the humidity may need further concern and standardization (whenever possible). However,

Table 6. Periotron unit (PU) scores for 3 × and 5 × replications at 20°C and 25°C [mean ± SEM and range (min–max)]

		20°C			25°C		
		Periotron 8000®			Periotron 8000®		
*n**	(μl)	1	2	3	1	2	3
3	0.1	18.66 (17–21)	23.66 (22–26)	21.66 (15–26)	25.33 (23–27)	25.33 (22–32)	26.00 (25–28)
	0.2	41.33 (37–46)	39.66 (31–48)	42.00 (39–47)	41.33 (38–45)	50.00 (48–53)	41.00 (30–48)
	0.3	62.00 (61–63)	56.00 (53–58)	52.66 (51–56)	57.33 (54–61)	51.33 (48–56)	68.33 (64–73)
	0.4	69.66 (66–76)	72.00 (70–75)	69.00 (66–72)	66.33 (66–67)	65.33 (63–67)	76.66 (74–80)
	0.5	81.00 (80–82)	82.33 (82–83)	82.33 (80–85)	75.00 (68–81)	83.66 (80–88)	88.00 (85–93)
	0.6	88.33 (84–91)	98.33 (94–101)	99.66 (94–105)	88.33 (85–91)	99.00 (96–105)	101.66 (99–105)
5	0.1	18.8 (17–21)	23.6 (21–26)	21.6 (15–26)	27.0 (23–30)	26.6 (22–30)	25.8 (25–28)
	0.2	42.4 (37–46)	37.4 (31–48)	41.6 (38–47)	42.4 (38–45)	46.4 (40–53)	42.8 (30–48)
	0.3	61.8 (60–63)	56.2 (53–58)	53.8 (51–57)	55.8 (53–61)	51.4 (48–56)	65.2 (59–73)
	0.4	69.6 (63–76)	73.0 (70–75)	66.0 (58–72)	65.8 (62–68)	67.4 (63–71)	74.8 (69–80)
	0.5	82.0 (80–84)	83.6 (82–87)	82.0 (80–85)	77.2 (68–85)	85.0 (80–89)	88.2 (84–93)
	0.6	90.0 (84–93)	97.6 (94–101)	101.0 (94–105)	88.0 (85–91)	96.8 (90–105)	100.4 (95–105)

*Number of replications.

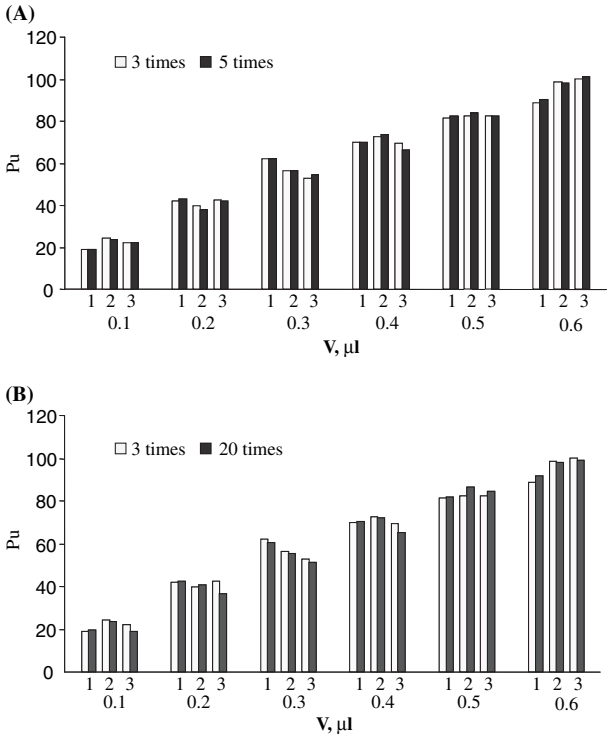

Fig. 5. Mean Periotron unit (PU) scores regarding number of replications tested for 20°C for three devices. Numbers 1, 2 and 3 represent each particular device. (A) 3 × vs. 5 ×; (B) 3 × vs. 20 ×.

even in climate-controlled areas, as ours, it should be acknowledged that achieving a stable room humidity may be more problematic compared to room temperature.

Besides generating a precise calibration data, maintaining the reliability of this data (10, 30–32, 34, 37, 38, 54, 56) is important to avoid volumetric errors (3, 4, 15, 16, 54). For this purpose various protocols including a daily or a weekly calibration or repetition of the previous calibration curve based on a 5 PU change is all available (20, 28, 30, 35, 41). The differences in 20°C and 25°C readings observed in the present study are in agreement with the previous studies addressing the impact of local conditions on electronic readouts and support the recommendation of attempts to minimize temperature and humidity variations in the examination room (31), such as calibration of the device immediately prior to sample collection and subsequent analysis (31), checking of the reading accuracy after including the variations in room climate and in working efficiency of the device itself, everyday (16), rapid transfer of sample strips to the device (34), placement of the device closer to the working area enabling immediate transfer of the clinical samples and immediate placement of the sample strips into sealed containers after measurement (40).

Regarding the appropriate number of repetitions for the generation of a reliable calibration curve, suggestions and clinical applications seem to vary. Preshaw *et al*. (34) recommended that each calibration volume should be repeated at least in triplicates for Periotron 6000®, whereas Deinzer *et al*. (20) suggested repetition of five times. However, Griffiths (2) suggested that duplicate volumes were sufficient for Periotron 8000®. Although statistical analysis was not performed, the mean, minimum and maximum PU values for additional 5 × or 20 × replicates did not seem to be far different than the

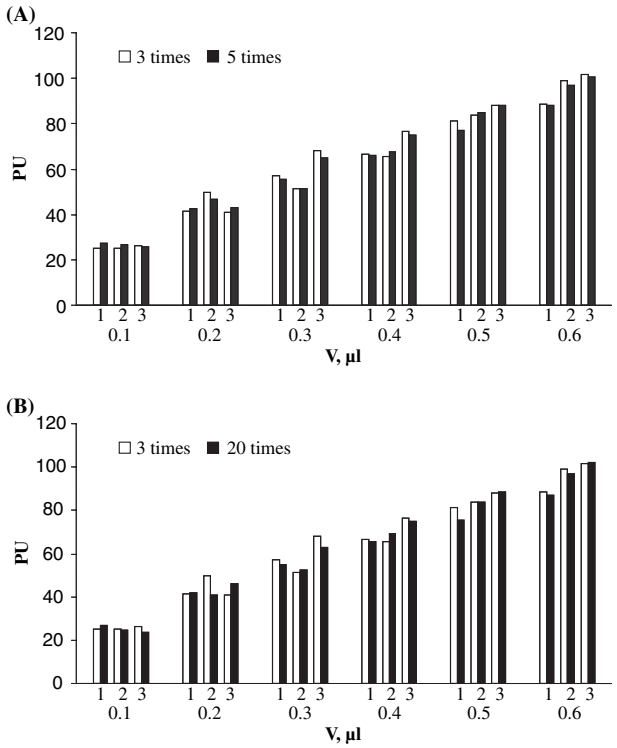

Fig. 6. Mean Periotron unit (PU) scores regarding number of replications tested for 25°C for three devices. Numbers 1, 2 and 3 represent each particular device. (A) 3 × vs. 5 ×; (B) 3 × vs. 20 ×.

values of 3 ×, and additional replicates did not seem to add further benefit. Thus, our findings may support the reliability of triplicate readings for Periotron 8000®, as similar to Periotron 6000® (34).

GCF is a fluid with unique volumetric features that do not resemble other biological fluids (2, 4, 5, 57), and study designs need to comply with these unique features (2, 3). Following a successful clinical GCF sampling, volume quantification is the next critical step where precise determination of the actual volume is the main goal. Considering the limited quantity of GCF at most sites, in order not to cause volumetric distortions, study designs need to take into account the potential factors that operate during volume quantification. Based on the findings of the present study, minimizing the risk of evaporation by chairside localization of the electronic device, transferring GCF-containing strips to the device immediately, attempting to regain the retained fluid within the device, generating a precise calibration curve with triplicate readings, checking and maintaining the reliability of the calibration data with regard to the local environmental conditions and keeping in mind the uniqueness of each device can be recommended for precise volume quantification. Thus, for GCF-related studies, the clinicians need to be familiar with the clear impact of the methodological preferences and standardization attempts starting with the *in vivo* GCF sampling and continuing with volume quantification processes (2, 3, 5, 16, 30, 35).

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