Validation of substances used for calibrating the Periotron 8000® instrument and conversion equations: an in vitro study

M. Jordán-López, P. J. Almiñana-Pastor, F. M. Alpiste-Illueca, A. López-Roldán

Department of Stomatology, Faculty of Medicine and Dentistry, University of Valencia, Valencia, Spain

ABSTRACT — OBJECTIVE: *Periotron 8000® is an electronic instrument that quantifies the volume of gingival crevicular fluid and saliva. Previous literature has proposed use of different fluids for calibration of the instrument, including human serum. The objectives were to compare different fluids used for instrument calibration, determine the correlations, and the most appropriate conversion equation for the model.*

MATERIALS AND METHODS: *The fluids evaluated were physiological saline, human serum, fetal bovine serum, and saliva. The Periotron 8000® instrument was calibrated with each fluid, and the correlation between these substances was analyzed. The calibration data were adjusted to a straight line, a second-, third-, and fourth-degree polynomial. R^2 (goodness-of-fit) values and the root mean square error (RMSE) were calculated for each regression model.*

RESULTS: *All the correlations were significant. However, saliva correlated more strongly with physiological saline solution. The fourth-degree polynomial was the most accurate as a conversion equation because it presented higher R^2 and lower RMSE.*

CONCLUSIONS: *The four fluids evaluated are useful to calibrate the Periotron 8000® instrument because they produce accurate regression models. Using saliva as a reference, the best fluid for calibration is physiological saline solution.*

KEYWORDS
Periotron 8000, Calibration, Gingival crevicular fluid, Saliva, Periodontal diagnosis.

INTRODUCTION

Gingival crevicular fluid (GCF) can behave as transudate under physiological conditions or inflammatory exudate under pathological conditions. This fluid is released in the gingival sulcus from the connective tissue due to increased permeability of blood capillaries present under the epithelium of the sulcus[1]. GCF is a critical factor in the ecology of the periodontal pocket because it serves as a protective barrier and allows measurement of the rate of growth of subgingival microorganisms. Moreover, GCF is a potential marker for periodontal diseases[1]. The composition of GCF reflects the state of inflammation of gingival and periodontal tissues, and analysis of this fluid can provide information about the pathogenesis of periodontal disease[2].

Corresponding Author
Andrés López Roldán, MD; e-mail: andreslopezroldan@gmail.com

DOI: 10.32113/ijmdat_202110_364

Absorbent paper strips are used for collecting and analyzing GCF, which is absorbed by capillarity. This fluid can be collected intra- or extracrevicularly[3]. The collection is quick, easy, and non-invasive, can be applied to individual sites, and allows quantification of the volume of GCF[3]. Volume of GCF is measured by introducing the absorbent paper strips into the Periotron®, an electronic device designed to quantify this fluid[4].

GCF is collected by placing the white portion of a Periopaper® strip in the gingival sulcus of the patient[3].

Periotron® measures the electrical capacitance of the sample. The electric field created by opposite charges between the plates of the instrument induces molecular polarity, reducing the difference in electrical potential between the plates and increasing electrical capacitance. The analysis is rapid and has no detectable effect on the GCF sample[5]. Volume of the sample volume is measured after calibrating the instrument with different fluids[5]. To the best of our knowledge, no studies have compared the utility of different fluids in calibration of the Periotron® instrument. Chapple et al[6] recommended performing calibrations with human serum owing to similar density and viscosity as that of GCF. Nonetheless, no well-designed studies have analyzed the effectiveness of this biological fluid in calibration. The difficulty in obtaining human serum and the nature of this biological fluid justify comparison between these substances in the present study. The primary objective of this study was to compare the different fluids used to calibrate the Periotron 8000® instrument. The analyzed substances were human serum, animal serum (fetal bovine serum), physiological saline solution, and saliva. The secondary objectives were to determine the correlation between the analyzed fluids and compare different regression methods to assess the most appropriate equation for conversion of Periotron units to microliters. Although the most suitable calibration fluid is GCF, saliva was used as the reference fluid due to the complexity in obtaining sufficient volumes of GCF.

MATERIALS AND METHODS

The analyzed fluids were 0.9% physiological saline solution, serum obtained from the researchers' blood, fetal bovine serum, and saliva collected at rest.

As per the calibration protocol, measurements were made in the range of 0.1 to 0.9 µL in increments of 0.1 µL, and calibration was measured five times for each increment and each fluid, totaling 180 measurements. A Periopaper® strip was moistened with each solution and placed between the plates of the instrument. The results were obtained in Periotron units and transferred to the calibration table. This procedure was performed for all samples.

STATISTICAL ANALYSIS

Based on the results obtained, a table with Pearson's r value was created to determine the correlation between the three calibration fluids and saliva and different regression models were built using the computer program "Excel" to determine the most accurate conversion equation. The data were adjusted to a straight line, a second-, third-, and fourth-degree polynomial. Polynomials are algebraic expressions with two or more variables and constants. These expressions were obtained from the calibration data and provided equations that allowed conversion of Periotron units to microliters. The R^2 value, which indicates goodness-of-fit, and the root mean square error (RMSE) were calculated for each model. R^2 values range from 0 to 1. Values closer to 1 indicate a better fit. RMSE measures the amount of error between two datasets, i.e., compares a predicted value with an observed or known value.

RESULTS

The calibration results shown as mean Periotron units for all samples are presented in Tables 1 to 4.

A table with Pearson's r values (Table 5) based on calibration data was built to determine the correlations between the evaluated fluids. All relationships were significant (*p*-values under 0.05) and the best matched correlation was between physiological saline solution an saliva (Pearson´s coefficient=0.998).

In the regression models, calibration data obtained from each fluid were adjusted to a straight line, a second-, third-, and fourth-degree polynomial. R^2 was calculated for each polynomial (Figure 1 to 4).

In order to apply to our observations to clinical practice, we used the corresponding linear regression model constructed with the calibration values obtained with physiological serum.
The equation for this model was:
Volume (physiological serum, uL) =
Periotron units/169,59

With this equation we were able to interpolate the volume of GCF on the Periotron paper by using physiological serum as calibration fluid.

DISCUSSION

With respect to the correlation between fluids, Pearson's r value allowed for assessment of the association between two quantitative variables

Increments in µl	1	2	3	4	5	Average
0.1 µl	17	11	13	17	13	14.2
0.2 µl	41	35	43	45	50	42.8
0.3 µl	67	51	69	72	70	65.8
0.4 µl	76	75	74	76	80	76.2
0.5 µl	103	91	96	92	96	95.6
0.6 µl	103	107	104	106	109	105.8
0.7 µl	128	122	117	115	118	120
0.8 µl	123	129	135	133	121	128.2
0.9 µl	136	139	138	139	144	139.2

Table 1. Calibration with physiological saline solution.

Increments in µl	1	2	3	4	5	Average
0.1 µl	24	24	21	18	23	22
0.2 µl	41	42	42	39	44	41.6
0.3 µl	62	63	66	62	65	63.6
0.4 µl	88	86	75	79	80	81.6
0.5 µl	87	92	92	95	94	92
0.6 µl	113	107	111	108	112	110.2
0.7 µl	113	124	116	114	115	116.4
0.8 µl	125	130	131	120	118	124.8
0.9 µl	128	136	135	126	125	130

Table 2. Calibration with human serum.

Increments in µl	1	2	3	4	5	Average
0.1 µl	14	15	16	21	23	17.8
0.2 µl	34	47	39	43	36	39.8
0.3 µl	54	66	62	57	58	59.4
0.4 µl	74	76	79	73	73	75
0.5 µl	88	90	87	98	98	92.2
0.6 µl	106	105	112	110	113	109.2
0.7 µl	125	121	122	126	117	122.2
0.8 µl	132	129	143	143	134	136.2
0.9 µl	157	160	167	163	158	161

Table 3. Calibration with fetal bovine serum.

Increments in µl	1	2	3	4	5	Average
0.1 µl	16	22	21	21	24	20.8
0.2 µl	54	61	57	51	44	53.4
0.3 µl	72	74	86	84	68	76.8
0.4 µl	93	91	96	93	84	91.4
0.5 µl	108	106	102	109	108	106.6
0.6 µl	126	120	112	121	114	118.6
0.7 µl	133	127	128	128	126	128.4
0.8 µl	135	146	149	143	150	144.6
0.9 µl	153	145	155	146	152	150.2

Table 4. Calibration with saliva.

Table 5. Correlations between four calibration fluids.

Correlations between calibration fluids

		Physiological saline solution	Saliva	Human serum	Fetal bovine serum
Physiological saline solution	Pearson's correlation	1	.998**	.996**	.992**
	Two-tailed t-test		.000	.000	.000
	N	10	10	10	10
Saliva	Pearson's correlation	.998**	1	.997**	.986**
	Two-tailed t-test	.000		.000	.000
	N	10	10	10	10
Human serum	Pearson's correlation	.996**	.997**	1	.985**
	Two-tailed t-test	.000	.000		.000
	N	10	10	10	10
Fetal bovine serum	Pearson's correlation	.992**	.986**	.985**	1
	Two-tailed t-test	.000	.000	.000	
	N	10	10	10	10

*** Correlation was significant at p<0.01 (two-tailed)*

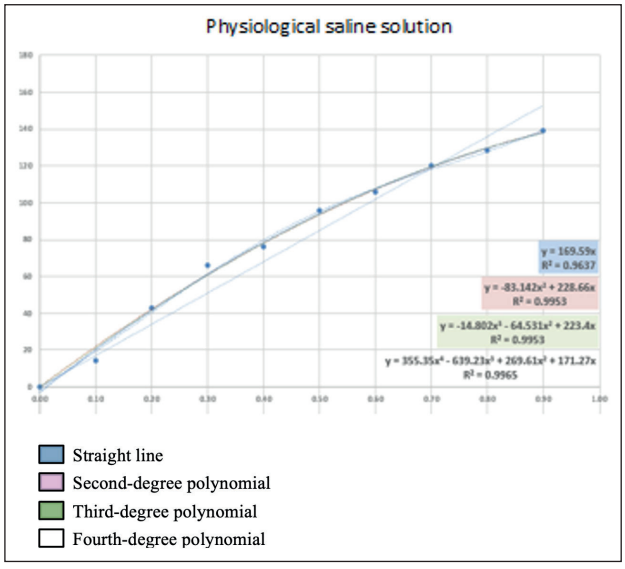

Figure 1. Goodness-of-fit of calibration data using physiological saline solution.

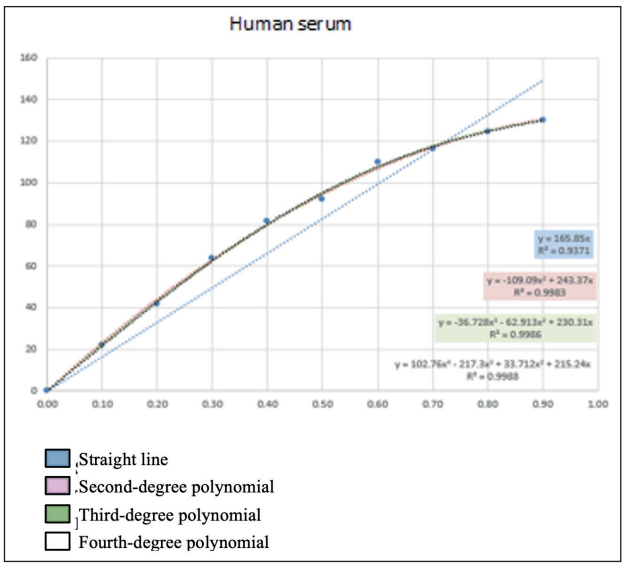

Figure 2. Goodness-of-fit of calibration data using human serum.

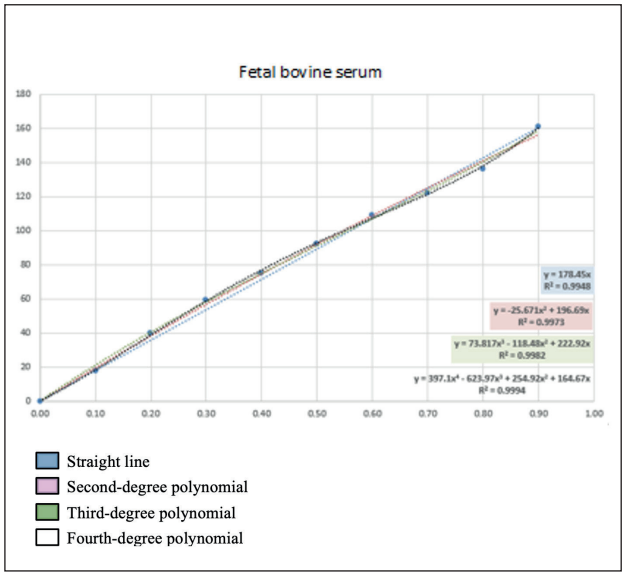

Figure 3. Goodness-of -fit of calibration data using fetal bovine serum.

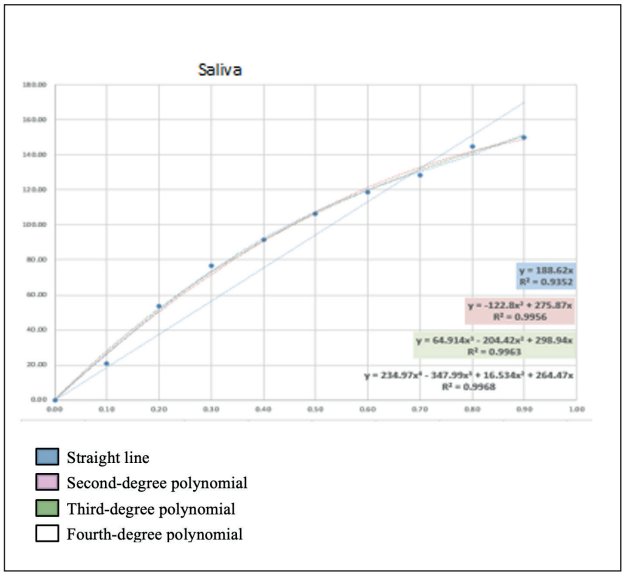

Figure 4. Goodness-of-fit of calibration data using saliva.

Pearson's r value 0 indicates absence of correlation between two variables, whereas values closer to 1 indicate a higher correlation between the variables. All correlations were significant, with values higher than 0.9.

Physiological saline presented the most significant correlation with saliva (r = 0.998), followed by human serum and fetal bovine serum.

The R^2 and RMSE values of the regression models for the calibration fluids are shown in Table 6. All analyzed fluids produced accurate and robust models in all cases (R^2 was higher than 0.9). The fourth-degree polynomial had the best fit to the model (R^2 was close to 1 and RMSE was low).

CONCLUSIONS

The four fluids analyzed were useful to calibrate the Periotron 8000® instrument because they produced accurate regression models. Using saliva as a reference, the substance most suitable for calibration was physiological saline, which is an advantage because saline is more accessible than human serum. Although human serum is more commonly used, the results using physiological saline are more reproducible.

The most suitable equation to convert Periotron units to microliters was the fourth-degree polynomial because of its high accuracy.

These results can serve as the basis for future studies using other substances.

Adjustment equation	R2	RMSE	Fluido
Linear	0.9637	9.13	Physiological saline solution
Second-degree	0.9953	3.28	Physiological saline solution
Third-degree	0.9953	3.27	Physiological saline solution
Fourth-degree	0.9965	2.84	Physiological saline solution
Linear	0.9371	11.46	Human serum
Second-degree	0.9983	1.84	Human serum
Third-degree	0.9986	1.66	Human serum
Fourth-degree	0.9988	1.59	Human serum
Linear	0.9948	3.78	Fetal bovine serum
Second-degree	0.9973	2.71	Fetal bovine serum
Third-degree	0.9982	2.2	Fetal bovine serum
Fourth-degree	0.9994	1.25	Fetal bovine serum
Linear	0.9352	13.03	Saliva
Second-degree	0.9956	3.93	Saliva
Third-degree	0.9963	3.1	Saliva
Fourth-degree	0.9968	2.91	Saliva

Table 6. R2 and root mean square error values of regression models for calibration fluids.

Conflict of Interest:
The authors declare that they have no conflict of interest.

References

1. Goodson JM. Gingival crevice fluid flow. Periodontology 2000 2003; 31: 43-54.
2. Wassall RR, Preshaw PM. Clinical and technical considerations in the analysis of gingival crevicular fluid. Periodontology 2000 2016; 70: 65-79.
3. Griffiths GS. Formation, collection and significance of gingival crevice fluid. Periodontology 2000 2003; 31: 32-42.
4. Chapple ILC, Landini G, Griffiths GS, Patel NC, Ward RS. Calibration of the Periotron 8000 and 6000 by polynomial regression. J Periodont Res 1999; 34: 79-86.
5. Ciantar M, Caruana DJ. Periotron 8000: calibration characteristics and reliability. J Periodont Res 1998; 33: 259-264.
6. Chapple IL, Cross IA, Glenwright HD, Matthews JB. Calibration and reliability of the Periotron 6000 for individual gingival crevicular fluid samples. J Periodont Res 1995; 30: 73-79.