

Development of a Dried Blood Collection, Transport, and Test System for C-Reactive Protein for Use in Assessing Inflammatory Status in a Dental Setting

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ABSTRACT

Background: Much press has been given to the “oral-systemic connection”, and the importance of good oral health to maintaining good overall health. One of the evident markers of oral inflammation is the acute phase reactant, C-Reactive Protein (CRP), which becomes elevated in the bloodstream during periodontal maladies. CRP may thus be used as both a marker of oral inflammation, and a tool to assess the effectiveness of periodontal therapies. Dental professionals have looked to the clinical laboratory community to provide a simple, accurate, and sensitive means to determine CRP in a dental office setting. Since dental offices are not staffed to perform moderately complex laboratory testing, and venipuncture is not practical in dental offices, our objective was to develop a CRP laboratory method for a fingernick blood sample which could be collected chairside in a dental setting.

Methods: The Roche Integra hs-CRP method was adapted for the Roche Diagnostics Integra 400 System to measure CRP in extracted capillary dried blood spot (DBS) samples collected onto Whatman 903 filter paper. Blood extractions were made by punching four 3-millimeter spots from a dried blood sample and eluting into a buffered saline solution. Assay modifications included increasing the analytical sample volume, decreasing the antibody reagent volume, adapting the calibration from a spline curve to a linear model, and decreasing the lowest measurable set point of the calibration curve. Test method validation included assessment of precision, analytical accuracy, sensitivity, linearity, and hemoglobin interference. The accuracy of the system was assessed by comparing CRP results from DBS samples as determined by the modified Roche Integra method, to CRP in paired plasma samples as determined using the standard Roche Integra hs-CRP assay.

Results: Within-run precision coefficients of variation (CV's) (n=20) at CRP levels of 1.0 and 3.9 mg/L were 7.7% and 5.8%, respectively. Between-run CV's (n=20) at the same CRP levels were 11.8% and 9.5%, respectively. The minimum detectable concentration of DBS CRP was determined to be 0.40 mg/L, (n = 20). Assay linearity was demonstrated between 0.6 and 20.0 mg/L). Least-squares regression analysis comparing plasma and DBS levels of CRP (n=55, range 0.4 – 19.8 mg/L) yielded a correlation coefficient (R²) of 0.98, y = 0.9995x + 0.008. No influence from any degree of hemolysis was apparent across the analytical range for CRP.

Conclusions: The Roche Integra hs-CRP assay modified by Healthy Life Laboratories for DBS analysis was shown to provide a strong correlation with the Roche plasma method, with suitable precision and sensitivity for use in a dental setting to assess oral inflammation. Dental professionals have begun incorporating CRP testing as part of their periodontal assessment and treatment protocols, and have successfully demonstrated that the collection of a fingernick sample using the provided single-use, self retracting, and minimally invasive lancet is within their accepted scope of patient care services.

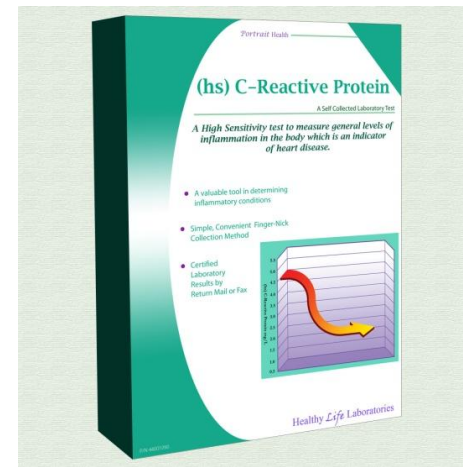
INTRODUCTION

Bleeding gums is a symptom that many adults dismiss, since it can be caused by simply brushing too hard. But there may be nothing simple about bleeding gums. As a symptom of gum disease, bleeding gums can be an indicator of an underlying metabolic disorder such as diabetes, or an early predictor of heart attack or stroke.

Dentists, and now physicians, are aware that bacteria from inflamed gums can enter the bloodstream and trigger the liver to produce proteins such as C-Reactive Protein (CRP). Elevated CRP levels caused by gum disease can make teeth-cleaning a life-threatening procedure. Often the dental professional tears gum tissue during the teeth-cleaning process, releasing disease-causing bacteria. As these bacteria circulate throughout the body, they may incorporate into blood clots, purportedly fostered by CRP. Depending on where the clot occurs, it can cause a heart attack, stroke, deep-vein thrombosis, or even a pulmonary embolus.

Dentists sought a convenient, reliable and accurate screening tool to identify patients suspected of having an elevated CRP level. A dried blood spot CRP test kit was developed at Healthy Life Laboratories, Inc. (HLL) to fulfill this requirement. The patient deposits three drops of capillary blood onto a filter paper card. The card is mailed to HLL, where it is analyzed for high-sensitive CRP. The result is relayed to the dentist as well as to the patient. If elevated CRP levels are discovered, the dentist can postpone invasive oral procedures until the CRP level is lower, which often includes the use of anti-inflammatory agents. The Healthy Life Laboratories' dried blood spot CRP test has been shown to be easy-to-use by patients and dental office personnel while still providing substantially equivalent results to traditional laboratory CRP testing.

METHODS



Healthy Life Laboratories' hs-CRP Test Kit



- The patient or healthcare professional completes a Laboratory Authorization Form (LAF) and uses the provided lancet to perform a finger nick.
- Three drops of capillary blood are spotted onto a Whatman 903 filter paper card, air-dried for 15 minutes and sealed in a desiccated pouch.
- The pouch containing the blood spot card and the LAF are placed into the provided return postage envelope and mailed to Healthy Life Laboratories for hs-CRP analysis.
- Healthy Life Laboratories analyzes the patient sample for hs-CRP using a modification of the standard Roche hs-CRP assay.
 - Four 3-mm dried blood spots are punched from each standard, control and patient sample, placed into a borosilicate glass tube and extracted using 200µL of a buffered saline solution.
 - The tubes are rotated for 2 hours at ambient temperature.
 - Extracted samples are analyzed on the Roche Integra 400 analyzer using modified Roche CRPHS reagents.
 - A dried blood standard curve is plotted and the CRP values for the controls and patients are determined from the calibration curve.

RESULTS

Within-Run Precision

Within-run precision was determined by assaying capillary dried blood containing two concentrations of CRP. Each of the samples was assayed in replicates of two in ten different runs (n=20). The data are presented below.

Mean CRP (mg/L)	Standard Deviation	% Coefficient of Variation
1.02	0.031	7.72%
3.91	0.071	5.78%

Between-Run Precision

Between-run precision was determined by a series of duplicate measurements of two different capillary dried blood samples over ten different analytical runs (n=20). The data are presented below.

Mean CRP (mg/L)	Standard Deviation	% Coefficient of Variation
1.02	0.047	11.78%
3.91	0.116	9.47%

Analytical Specificity

HLL Dried Blood CRP Result	Plasma CRP Results	
	Plasma Negative (< 3.0 mg/L)	Plasma Positive (≥ 3.0 mg/L)
Negative (<3.0 mg/L)	23 TN	3 FN
Positive (≥3.0 mg/L)	0 FP	32 TP
Total	23	35

FN=False Negative, FP=False Positive, TN=True Negative, TP=True Positive

The **Diagnostic Sensitivity** [TP/(TP+FN)] x 100 is **91.4%**.

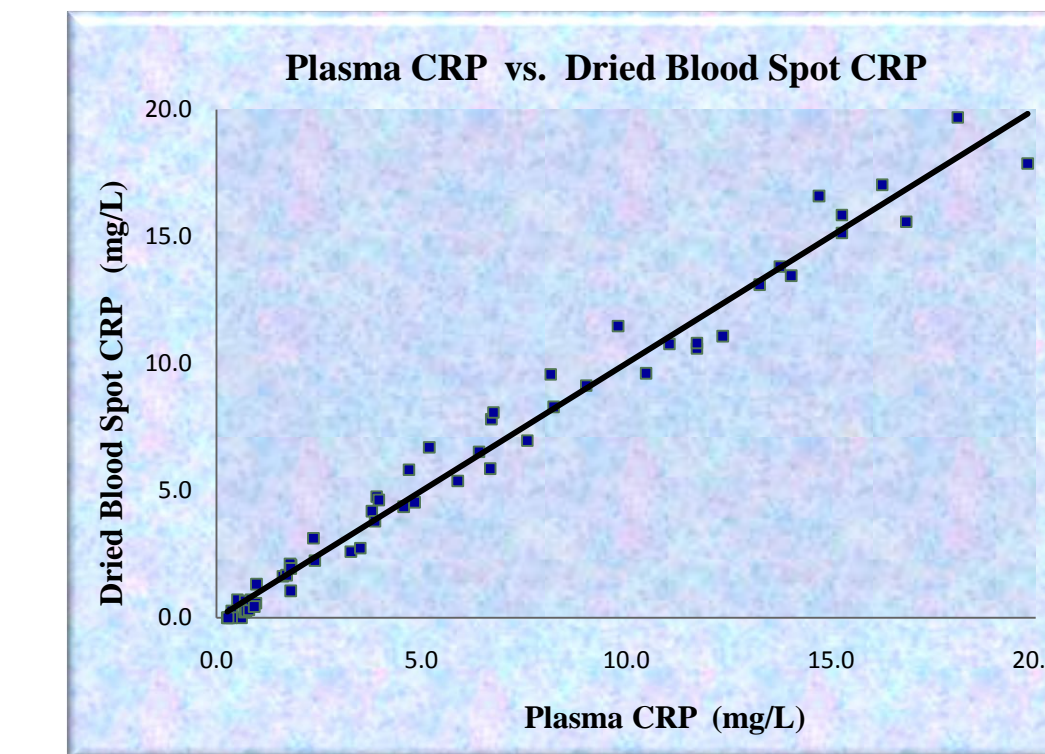
The **Diagnostic Specificity** [TN/(TN+FP)] x 100 is **100%**.

The **Diagnostic Accuracy** [(TP+TN)/(TP+TN+FP+FN)] x 100 is **94.8%**.

Accuracy

Fifty-eight (58) paired samples of plasma and dried whole blood were collected and analyzed for CRP using the Roche Diagnostics CRPHS assay for serum/plasma, and the Healthy Life Laboratories modified CRPHS assay for dried blood. Regression data are summarized below.

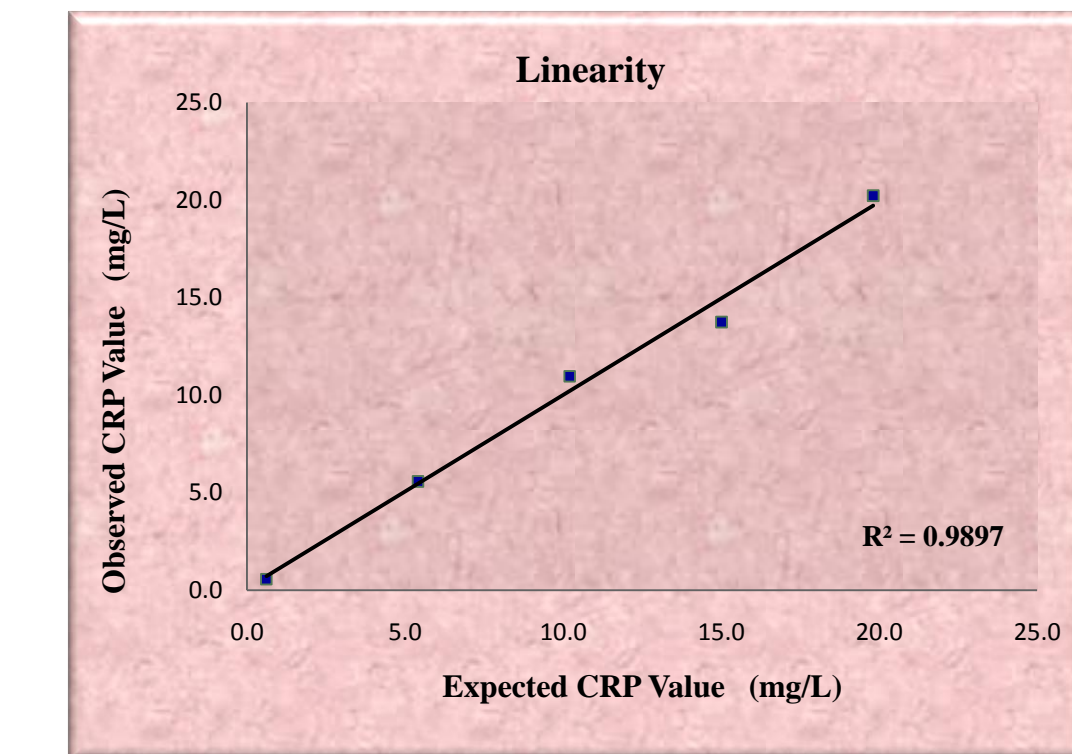
N = 58			
Correlation Coefficient (R ²)		0.98	
Slope		0.99	
Intercept		0.03	
CRP (mg/L)	Plasma	Dried Blood Spots	
Mean	6.14	6.13	
Std. Dev.	5.587	5.656	
Range	0.4 – 19.8	0.3 – 19.7	



Linearity and Recovery

Dried whole blood spots were made from 2 samples of known low and high plasma CRP values. Admixtures of these spots were prepared to determine assay linearity and recovery. The data are presented below.

Observed CRP (mg/L)	Expected CRP (mg/L)	% Recovery
20.22	19.81	91.4%
13.74	15.01	102.9%
10.96	10.21	107.3%
5.57	5.41	91.6%
0.56	0.61	102.1%



CONCLUSION and DISCUSSION

- Oral examinations of more than 5000 adults in four U.S. communities determined that the two largest causes of increased levels of CRP in healthy adults were periodontal disease and being overweight.
- According to the National Institute of Dental and Craniofacial Research, about 80% of U.S. adults currently have some form of gum disease, ranging from simple inflammation, called gingivitis, to a serious disease called periodontitis, which results in damage to the bone.
- Periodontal cleaning procedures alone can cause the spread of many of the 400 types of microorganisms commonly indigenous to the mouth, which studies have shown can elevate a person's baseline CRP reading by as much as 300%.
- Patients suspected of having an elevated CRP level can be tested at the dental office using Healthy Life Laboratories' high-sensitive C-Reactive Protein Test prior to undergoing an invasive procedure.
- The small sample volume requirement of only three drops of fingernick capillary blood, allows a convenient way for the dental office to assess gum disease and obtain an accurate and reliable CRP result.
- A patient with an elevated CRP level can take anti-inflammatory agents to reduce the level of CRP and then retake the Healthy Life Laboratories' CRP test in the convenience of their home or at the dental office to verify that their CRP level has been reduced and a periodontal procedure can now be safely performed.
- If the CRP level remains elevated, the patient is referred to his/her physician, since an elevated CRP level can be an indicator of risk for underlying cardiovascular diseases and cerebrovascular diseases.
- Recent studies have shown that elevated CRP levels are also strongly associated with damage to areas of the brain responsible for critical thinking and mental function, specifically perception, memory, judgment, and reasoning.
- Just three drops of blood and a Healthy Life Laboratories' CRP test kit could facilitate the identification of individuals who are unknowingly at a higher risk for vascular disease or diminished mental function.
- The Healthy Life Laboratories' modification to the Roche CRPHS assay permits analysis of dried whole blood spots with results and performance characteristics that are substantially equivalent to that of serum or plasma.
- The Healthy Life Laboratories' CRP test kit has been met with favorable response by patients and clinicians due to its sensitivity, accuracy, convenience, easy-to-follow instructions and presentation of results in a personalized and educational report.
- Healthy Life Laboratories also offers CRP in combination with other tests, such as Hemoglobin A1c, which has become increasingly popular as a tool to detect metabolic syndrome and assess diabetes risk.