



## Determination of the Correlation of Whole Blood Lead and Saliva Lead Levels in Humans

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### Introduction and Background

Lead has been shown to be toxic to most living things. It has been shown that body concentrations of lead, even at low levels, can be very detrimental to human beings. In addition, it has been shown that lead concentrations today are three to five hundred times greater than that of preindustrial background concentrations. In adults, elevated blood pressure is the most common effect of lead toxicity at blood levels less than 10µg/dL. At doses higher than 40µg/dL, kidney problems and infertility result. Moreover in children, lead has been shown to interfere with normal neurodevelopment. Profound IQ changes have been shown with lead levels even as low as 5µg/dL. Furthermore, children who have blood lead levels as low as 4µg/dL have been shown to be significantly associated with clinically diagnosed attention-deficit/hyperactivity disorder (ADHD).

Whole blood is the accepted method for testing lead content in humans which requires specialized personnel and equipment. Studies have shown significant correlations between whole blood lead content and saliva lead content ( $r=0.72$ ,  $p < 0.01$ ). Another study suggests that approximately one percent of the lead found in the body is in the plasma unbound. This unbound plasma lead is quite significant since it reflects the internal lead level that can exert effects on human organs. Moreover, there is a significant correlation ( $r^2=0.922$ ) of the lead in plasma with saliva.

In 2005, Western Slope Laboratory began its research study to determine if lead can be detected in saliva using our method and if there is a correlation between saliva lead levels and blood lead. At the time, 95 subjects gave blood and saliva samples that were tested for the presence of lead. The study clearly demonstrated that lead can be detected in the saliva at very low levels using our method. Moreover, these levels were not consistently tested for using an ICP-MS previously. In addition, the study suggests that there is a relationship between the level of lead in the saliva and the level of lead in the blood. Additional studies were undertaken to determine what that relationship is and define the statistical significance of that relationship. Whole blood and salivary lead levels are able to demonstrate detrimental levels of lead as a screen. This finding is significant because it will also allow for additional screening at reduce costs for many communities.

### Methods and Materials

Blood samples were collected using accepted Center for Disease Control and Prevention (CDC) protocols for whole blood lead. All samples were collected, prepared, and run according to standard operating procedures by contract facilities and therefore only the data will be discussed further.

Saliva samples were collected using either a modified Saliva-Sampler™ (StatSure) device or modified OraSure® device (OraSure Technologies). Each has an indicator when the appropriate volume of saliva is collected. The samples are mixed with a buffer upon collection in the tube. The pad and liquid are removed from the collection container and placed into a Salivette® (Sarstaedt) that does not contain a

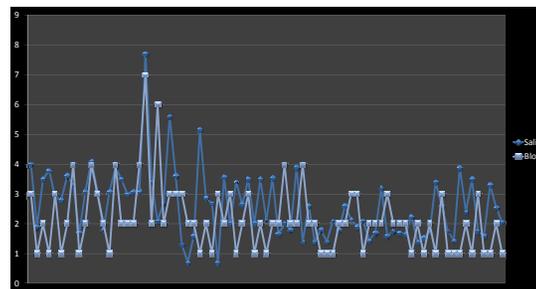
### Methods and Materials

pad. The tube is centrifuged. The resulting liquid is acid digested and prepped according to the patented method by David R. Schneider, Ph.D. (US 8, 049, 165 B2). All lead and indium were purchased from VWR. Samples were run on a PerkinElmer Elan 6000 ICP-MS with a type-II nebulizer. The method was validated for precision, accuracy, linearity, and lower limits of detection and quantification. All samples were collected from children in both North Carolina and Texas. Each collection site gained approval from their respective Institutional Review Board prior to participation in this research. Moreover, informed consent and assent, where applicable, was used in all cases.

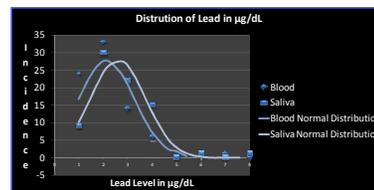
No adverse effects were seen in either the 2005 and 2010-2011 studies.

### Results

Using this methodology, we compared whole blood lead samples with saliva lead samples. Paired sets of the data shown below in Graph One. The data trends the same and in many instances the levels of lead are the same. In fact, As demonstrated in Graph Two, the distribution of concentration was the same and the mean values were the same. This result was statistically significant.



Graph One: All Samples (paired blood and saliva values) in µg/dL



Graph Two: Distribution of All samples

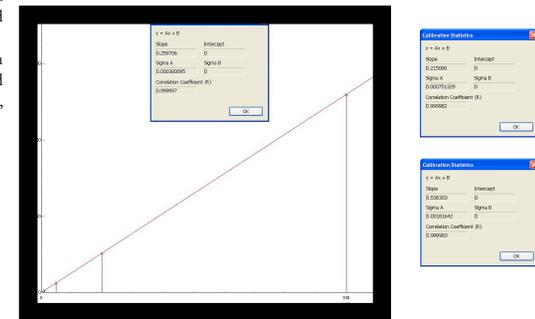
Distribution	T-value	% Confidence
1-Tail	1.65	95%
2-Tail	1.98	97.5%

	Blood	Saliva
Mean	2.14	2.61
Variance	1.30	1.28

### Results

Graph Three shows a composite of the three lead isotopes while Table One notes some of the method validation information.



Graph Three: Linearity Information and Graph for Lead 206, 207, and 208 respectively

COMPOUND NAME	Isotope(s)	Repeatability %RSD	Counts (cps) @ 100ng/mL	Uncertainty (95% CI)	Linearity	Accuracy %	LLOD ng/mL	LLOQ ng/mL
Lead	205.975	6.94434	836854.29	0.46		196.57474	0.5	0.5
	206.976	7.48465	727966.87	1		195.59843	0.5	0.5
	207.977	6.46411	1757088	0.02		194.16247	0.5	0.5

Table One: Lead Method Validation Data

### Discussion and Conclusion

Using the Schneider methodology, we are able to strongly correlate salivary lead levels with the CDC whole blood lead levels. This correlation has been demonstrated at lead levels below the 10 µg/dL action level. Future studies will include lead levels in the low, median, and high levels as well as a larger number of samples. In addition, samples from many regions will be solicited.

The impact of a salivary lead screening test will allow for more testing and the non-invasiveness of the test will lead to faster adaptation of the method.

#### References:

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