

Validation of the Gingival Crevicular Fluid sample collection technique

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Objectives: Biomarker assessments using crevicular fluids are becoming increasingly important in research. Current methods used for collection, quantification and extraction vary, with difficulties in assessing samples with small fluid volumes and protein amounts. The objective of this study is to validate methodology for the collection, recovery and resolution of proteins from crevicular fluids collected using methyl cellulose strips.

Methods: Fluid saturation of the strips was measured linearly (mm) using known increments of absorbed water in triplicate for 3 masked examiners. Linear equations were established to determine fluid volumes absorbed and correlation analysis evaluated results for examiners. Protein extraction was assessed using different concentrations of BSA dissolved in PBS with tween 20. Protein solutions (5 ul), at 1.0, 0.5, 0.25, 0.12, or 0.06 ug/ul, were placed onto the paper strip for 30s using a micropipettor. The papers were then soaked in 100ul of extraction buffer made up of PBS with 0.1% Triton-X-100 overnight at 4°C with constant shaking. The next day the strips were removed and then were washed (3X) in the extraction buffer. All extraction and wash samples were collected, dried, and reconstituted in 200ul extraction buffer. To determine the elution efficiency of BSA extraction, triplicate 80ul of the aliquots were analyzed using Bradford assay. The absorbance was read in a micro plate spectrophotometer at 590nm and the protein concentrations were calculated from the standard curve.

Results: Visual assessment of saturation was significantly correlated with fluid amounts (0.91; $p < 0.001$) and correlations between examiners were significant (0.93; $p < 0.003$). Protein extraction demonstrated 55% yield at concentrations over 0.5 ug/ml. **Conclusion:** Linear measurement of visible saturation along the GCF strips provided a reliable method of absorbed fluid volume determination, however protein levels collected in minute amounts may not be recovered with high yields using this extraction protocol. [study supported by NIDCR R01DE017882].