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Reproducible Saliva Collection as Basis for Cotinine Testing in Oral Fluid

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Introduction: When collecting saliva for routine diagnostic purposes a number of basic problems should be prevented: analytes should not be unspecifically absorbed in or on the collection device, analyte stability should be assured and the sample volume should be known.

Objective: A newly commercially available saliva collection system was evaluated for the measurement of cotinine in saliva. In oral fluid obtained from volunteers, total sample volume, amount of extracted oral fluid, pH value and α -amylase concentration as sample parameters were determined to prove the reproducibility of the sampling process. As a practical application salivary cotinine levels were measured as indicator to distinguish smoking from non-smoking individuals.

Method: 176 healthy volunteers rinsed their mouth for 2 min. with a Saliva Extraction Solution (SES) from the Greiner Saliva Collection System (Greiner Bio-One). The total amount of sample was determined by weighing, the proportion of harvested oral fluid was determined by photometric measurement of an internal standard in the collection system. The pH value was determined photometrically with a pH-reagent (Microgenics) on an Olympus AU640 Clinical Analyzer. α -Amylase concentration was measured with a modified urine protocol using the Olympus α -amylase reagent. Salivary cotinine was quantified using the modified urine DRI Cotinine Assay (Microgenics) on the Olympus AU640 Clinical Analyzer.

Results: Under the described collection conditions, the individual harvested volume of oral rinsing fluid ranged from 4.4 mL to 9.8 mL (median 6.6 mL) containing a fraction in oral fluid of 34%–93% (median 62%). The pH value in the collected samples ranged from 4.7 to 5.6 (median: 4.9). Saliva amylase activity showed a broad inter-individual range between 4,000 U/L and 600,000 U/L (median: 78,000 U/L). Cotinine concentrations were found from 0 ng/mL to 300 ng/mL with a defined cut-off of 40 ng/mL to discriminate self declared smoker versus non-smokers.

Conclusion: Saliva collection using a liquid matrix allows a simple and reliable oral fluid collection. This is the basis for reproducible quantitative determination of analytes in oral fluid, which could be demonstrated by the evaluated parameters.