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Introduction

In routine saliva collection for diagnostic purposes the amount of collected sample should not be subject to variability and analytes should not be unspecifically absorbed on or in a collection device during the collection process. A novel saliva collection system utilizing an extraction fluid as carrier system was tested for its routine use in the detection of cotinine in saliva to be able to discriminate smoking from non-smoking individuals.



Methods

For saliva collection a new, commercially available Saliva Collection System® (SCS) by Greiner Bio-One was used: It consists of a Rinsing Solution, to clean the oral cavity, a Saliva Extraction Solution (SES) with specified pH value (pH 4.2), a Saliva Collection Beaker and two evacuated Saliva Transfer Tubes to store the oral fluid sample.

176 healthy control individuals rinsed their oral cavity with the Saliva Extraction Solution for two minutes according to the manufacturer's manual. In the collected samples, the amount of collected fluid was determined volumetrically and by weighing. The proportion of harvested saliva (to calculate the dilution factor) was determined by photometric measurement of the containing yellow dye, using the Greiner Bio-One Saliva Quantification Kit (SQK). The pH value was determined by direct measurements using a pH-electrode and with a photometric pH-reagent (Olympus) applied on an Olympus AU640 Clinical Analyzer.

Amylase in oral fluid was determined with the Olympus urine amylase reagent and cotinine with the immunological DRI cotinine urine test from Microgenics, after modification of the measuring protocol to obtain higher sensitivity [Fig.1].

Cotinine in oral fluid with DRI cotinine assay: Application for Olympus AU640 (modified protocol)		
Sample volume:	35 µl	
Reagent volume:	R1:	87 µl
	R2:	65 µl
Wavelength:	Primär:	340 nm
	Sekundär:	410 nm
Method:	Rate1	
Reaktion slope:	+	
Measuring point 1:	First:	13
	Last:	17
Calibration with spiked oral fluid samples (50 - 1000 ng/mL)		

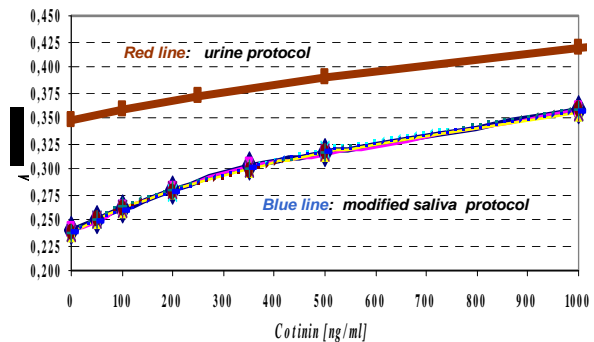


Fig.1: Calibration curves of the DRI cotinin assay

Results

Male and female healthy control persons (median of age: 28 years), self-declaring as smokers or non-smokers, provided a saliva sample using the Greiner Bio-One Saliva Collection System.

The total volume of harvested oral fluid ranged between 4.4mL - 9.8mL (median: 6.6mL), with a saliva content ranging between of 34% - 93% (median 62%). For analytical precision the actual amount of collected saliva must be known accurately: Using the Greiner Bio-One Saliva Quantification Kit the measurement of saliva controls (low: 30% saliva fraction) regularly gave a CV of <1.9% compared to a CV of <0.7% for the high control (with 70% saliva fraction).

The pH value of the buffered Saliva Extration Solution (pH 4.2) in the collected saliva samples shifted to pH 4.7 - 5.6 (median at pH: 4.9) [Fig.2].

The amylase activity in the oral fluid samples determined with a modified urine assay run on an OLYMPUS 640 Clinical Analyzer, displayed a broad inter-individual range of enzyme activity between 4,000 U/L and 600,000 U/L (median at 78,000 U/L). No changes of the enzyme activity could be detected so far after repeated freeze and thaw cycles of the collected oral fluid. This parameter could be utilized as a specific marker for oral fluid.

After minor modifications of the assay protocol the Microgenics DRI urine cotinine the assay showed much higher sensitivity [Fig.1], allowing the detection of cotinine in oral fluid below 10ng/ml (LOD: 10ng/ml). For cotinine concentrations in the oral fluid samples, values from 0 ng/mL to 300 ng/mL were found. A cut-off at 40ng/ml cotinine was chosen to differentiate (self-declared) smoker from non-smokers [Fig.3].

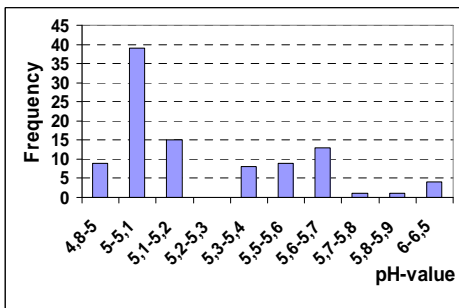


Fig.2: Ranges of pH-values in the collected oral fluid of individuals

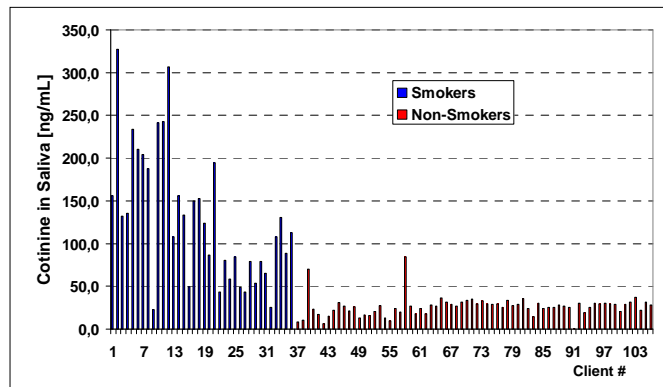


Fig.3: Cotinine levels in oral fluid of (self-declared) smokers and non-smokers

Conclusions

The Greiner Bio-One Saliva Collection System® based on a liquid matrix for saliva extraction allows reliable and reproducible oral fluid collection under very controlled conditions: Saliva is collected at a defined pH and sufficient sample material is always available for further analysis. This is a reliable basis for reproducible and quantitative determination of different parameters in saliva. In case of the determination of cotinine in oral fluid it is shown that this parameter allows a clear differentiation of smokers from non-smoking individuals under routine conditions.