Evaluation of the Greiner Bio-One saliva collection device for the analysis of cortisol with UPLC-MS/MS

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Introduction
Salivary cortisol (C) reflects unbound (free) serum C concentration and has therefore become a valuable diagnostic tool in endocrinology. However, collecting oral fluid (OF) from xerostomic individuals with absorption-based systems like the Salivette Cortisol (S, Sarstedt) can be cumbersome. In this study we compared the stimulating liquid-based Saliva Collection System (G, Greiner Bio-One) to the S applying a sensitive UPLC-MS/MS method for C quantification.

Methods
Salivettes: 20 volunteers took part in 2 series of 10 collection series A, 10 volunteers each collected 3 consecutive OF samples using the same collection device in 45 samples series B in which volunteers collected a different device in random order in 45 samples. In both series total saliva volume and C were measured.

Sample collection: OF samples were collected using the G and S device according to the manufacturer. OF concentrations were determined using the UPLC-MS/MS method. The method is based on the extraction of cortisol with polyethylene glycol (PEG) and its derivatization with an oxygen-bonded Column using 300 μm i.d. columns packed with 75 μm C8 stationary phase. The derivatized cortisol was determined using a QTRAP 4500 LC-MS/MS system equipped with a Turbo ion sprayer. The mobile phase was a mixture of 0.1% formic acid and methanol (75:25). The OF were measured with 20 μl injections. The limit of detection was 0.05 ng/ml. Linear range of 100 pg/ml was used.

Results
A. Consecutive sampling with the same collection devices

Fig. 1 Cortisol: Chromatogram and calibration

Fig. 2 Cortisol: Quality control low

Fig. 3 Cortisol: Quality control high

Fig. 4 3 OF samples collected with Greiner

Fig. 5 3 OF samples collected with Salivette

B. Consecutive sampling with the two collection devices

Fig. 6 Greiner and Salivette: 4 samples / 2 devices

Fig. 7 Mean C values of Greiner and Salivette

Fig. 8 Sampling order

Fig. 9 Saliva Collection System - Greiner Bio-One

Fig. 10 Salivette for cortisol analysis - Sarstedt

1. stopper
2. synthetic swap
3. suspended insert
4. centrifuge vessel

(A) open the Salivette
(B) put the swab in the oral cavity
(C) chew the swab gently for 1 minute at least
(D) return the saturated swab to the insert
(E) close the insert with the stopper