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**“Peri-analytics” reference ranges for drug screening in saliva using the Greiner-Bio-One collection device**

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**Introduction:** Saliva attracts increasing attention in drugs of abuse testing because of ease of collection and less risk for adulteration/substitution. Still “peri-analytics” tests for validation of specimens are needed.

**Aims:** To find suitable “peri-analytics” and establish reference ranges for sample volume, saliva concentration, amylase and cortisol.

**Methods:** Saliva samples were collected using the Greiner-Bio-One SCS pH 4.2 device. Saliva concentration of the saliva/buffer mixture was quantified spectrophotometrically on an Olympus AU640 using the saliva quantification kit. Amylase was determined with Olympus urine reagent after 1:100 sample dilution on the AU640. SRM multi-target drug screening including cortisol quantification was performed after alkaline LLE of 1 mL saliva/buffer on an UPLC/MS-MS operating in ESI+. One patient group (n=1017 patients, 3390 samples) and two reference groups were studied (n=455).

**Results:** All four parameters were independent within the groups and could not be correlated. Mean recovered volume in all groups was about 4 mL but 1.8% of the samples in patient group were suspicious of manipulation (<1 mL). Amylase concentration in saliva showed no gender, age or group specific difference. Amylase normal range for patients was defined from 23000 to 433000 U/L. Fortyone samples (1.2%) from patients were suspicious of substitution (amylase <10000 U/L). Cortisol values in controls ranged from 1.03-5.93 ng/mL (mean 2.51, median 2.27) and were much lower in patients with a skewed distribution (0.12-5.94 ng/mL, mean 1.76, median 1.04). Among ten samples having cortisol levels below 0.12 ng/mL eight had normal amylase concentrations. Saliva concentration was normally distributed in controls but in patients a skewed distribution (mean 56%, median 60%) was observed, suggesting that two minutes collection time for substituted patients may not be sufficient.

**Conclusion:** All four parameters investigated are promising to prove the authenticity of a saliva sample. Xerostomia in opiate addicts demands separate normal ranges for Cortisol.

**Key Words :** saliva concentration, amylase, cortisol, multi-target drug screen, LC-MS/MS