



# "Peri-analytics" reference ranges for drug screening in oral fluid using the Greiner-Bio-One collection device

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## Introduction

Oral fluid (OF) attracts increasing attention in drugs of abuse testing of methadone maintenance patients and within other settings. The ease of non-invasive sampling under close supervision decreases the chances for adulteration or substitution of the sample by the patient. Nevertheless "peri-analytics" tests for validation of specimens are needed. The term "peri-analytics" in our lab defines all kind of analysis and observations around a sample to exclude adulteration. There are a lot of different collection devices available with sometimes remarkable differences in 1. influence on the collection process itself, 2. drug recovery, 3. sample volume, 4. collection time and 5. interferences in chromatographic methods. We decided for a liquid based device buffered at acidic pH to assure that sufficient sample volume is collected in a reasonable time when xerostomic patients are tested. The absence of detergents which could interfere in our chromatographic method or in sample preparation procedures is another advantage of this collection system. **Aims of this study:** to verify suitability as "peri-analytics" marker and establish reference ranges for 1. %OF concentration, 2. sample volume, 3. amylase and 4. Cortisol. Including of Cortisol quantification in our LC-MS/MS screening method.

## Methods

**Subjects / patient samples:** 1 patient and 2 reference groups were studied; see results section.  
**Sample collection:** OF samples were collected using the Greiner-Bio-One (GBO) SCS pH 4.2 device according to the manufacturer. Collection time was 2 min. or determined by gravity. %OF concentration of the OF/buffer mixture was quantified spectrophotometrically on an Olympus AU540 using the GBO saliva quantification kit. Salivary Amylase was determined with Olympus urine reagent after 1:100 sample dilution with saline.  
**Multi-target drug screening including Cortisol quantification** was conducted after alkaline LLE (ToxTube A) of 1 mL saliva/buffer fortified with 22 deuterated standards (0.5-2.5 ng; Fig 5) on a Waters Acquity/Nevo UPLC-MS/MS. Separation of 5 µL extract was performed by 13 min gradient elution (MopA = 5 mM formate pH 3, MopB = ACN + 0.1% formic acid) on a HSS C18 1.8 µm, 2.1 x 150 mm column kept at 50°C. System operated in ESI+ and MRM mode with 2 transitions monitored per analyte.



## Conclusion

- The four peri-analytics markers were independent and seem not to be correlated. Parallel reduced values may therefore be indicative for adulteration, shortened collection time etc.
- All four parameters investigated are promising to prove the authenticity of an OF sample.
- Opiates addicts demand separate reference ranges for Cortisol.
- Distribution of %OF concentration in PG suggests that prolonged collection time for xerostomic patients like opiates addicts must be considered.

## UPLC-MS/MS method

Fig.5 MS method: time windows and analytes

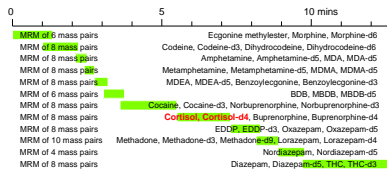


Fig.6 OF/SES calibration Cortisol

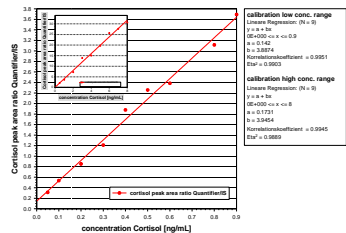
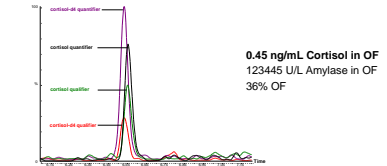


Fig.7 Patient sample with low Cortisol concentration



## subjects / patients population

Fig.8 Age distribution methadone patients group (PG)

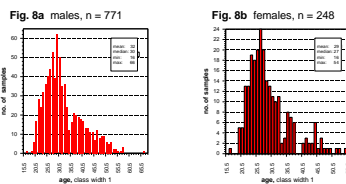


Fig. 9 Age distribution "normal group" (NG)

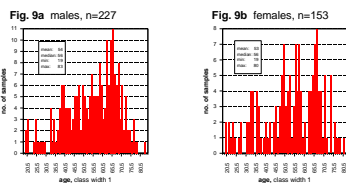
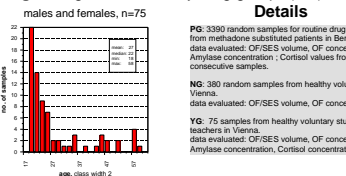


Fig. 10 Age distribution "young group" (YG) males and females, n=75



**PG:** 3390 random samples for routine drug screening from methadone substituted patients in Berlin. data evaluated: OF/SES volume, OF concentration, Amylase concentration, Cortisol values from 200 consecutive samples.

**NG:** 380 random samples from healthy volunteers in Vienna.

**YG:** 75 samples from healthy voluntary students and teachers in Vienna. data evaluated: OF/SES volume, OF concentration, Amylase concentration, Cortisol concentration.

## Results

### 1. %OF concentration

Fig.1a males PG, n=2257

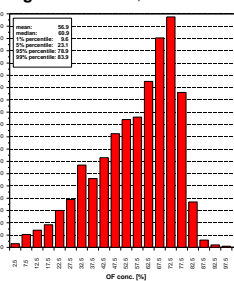


Fig.1b females PG, n=833

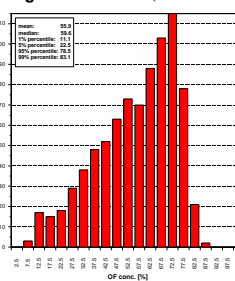


Fig.1c males NG, n=227

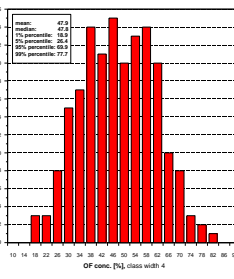
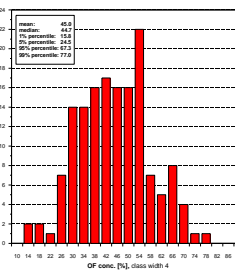


Fig.1d females NG, n=153



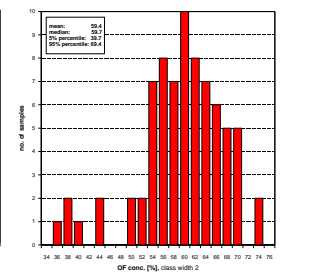
### 2. OF volume [mL]

Fig.2 descriptive statistics

	NG males	NG females	NG total	YG total
mean	4.2	4.0	4.1	6.8
median	4	4	4	6.6
1% percentile	2.5	2.3	2.5	-
5% percentile	3	3	3	4.7
95% percentile	5.5	5.9	5.5	9.6
99% percentile	8	7	7.3	-
min	2.5	2	2	4.4
max	9	7	9	9.8
n	227	153	380	75

%OF concentrations in the reference groups were normally distributed at about 59% (NG) or 48% (YG) while the PG revealed a skewed distribution (mean 56%) suggesting that collection time for this population may not have been sufficient. The reference range for %OF was derived from NG and gave values from 26% to 74% (5% to 95% percentile). The reference groups showed OF volumes around 4 mL (NG) and 6.8 mL (YG) resp. For the PG the OF volumes could not be evaluated, because the whole sample volume was not always available. However, 1.8% of the samples for PG were suspicious of manipulation (<1mL). The reference range for OF volume was also derived from NG and gave values from 3 mL to 5.5 mL (5% to 95% percentile).

Fig.1e total YG, n=75



### 3. Amylase concentration in OF

Fig.3a males PG, n=2557, no. of samples <10000: 36

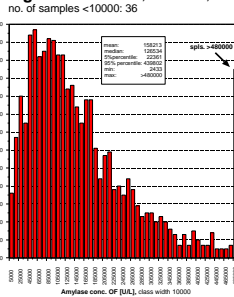


Fig.3b females PG, n=833, no. of samples <10000: 5

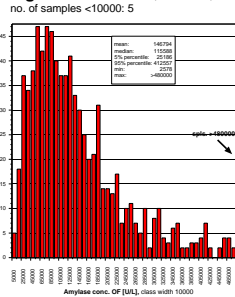
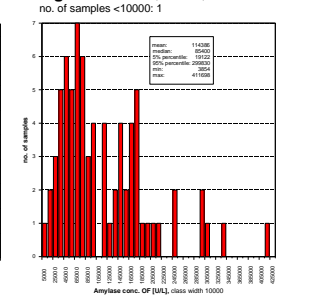


Fig.3c total YG, n=75, no. of samples <10000: 1



### 4. Cortisol concentration in OF

Fig.4a total PG, n=200

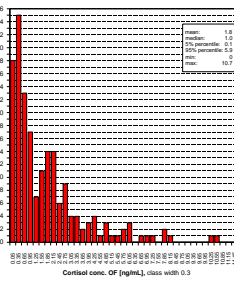
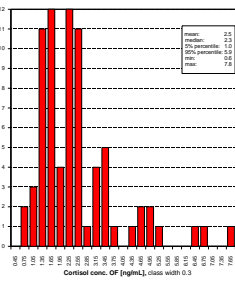


Fig.4b total YG, n=75



OF Amylase concentration in PG showed no gender or age dependent differences. Even though YG database is small we assume no difference between PG and "normal population". Reference range OF Amylase (5% to 95%) taken from PG: 23000 - 43300 U/L.

167 (41) samples from PG were suspicious of substitution (4.9% (1.2%), Amylase <23000 U/L (<10000)). From these spits, 23 (13.8%) revealed low %OF concentration.

Cortisol values in YG ranged from 1.03-5.83 ng/mL (5% to 95% percentile) and were much lower in PG with a skewed distribution (0.12 - 5.84 ng/mL, 5% to 95% percentile).

Ten samples from PG had Cortisol levels below 0.12 ng/mL, but eight had normal Amylase concentrations.