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## Collecting Saliva

### Different methods

Depending on which specific hormones which are being quantified in salivary diagnostic assays, the collection device itself may have an influence on accuracy of testing. With respect to the various steroid hormones, there exists different degrees of affinity (adsorption) for the plastic that may be used in the collection device. The amount of adsorption depends on the solubility of the steroid in non-polar, organic solutions. Regarding common steroid hormones, cortisol is least likely to bind with plastic, whereas progesterone is best soluble in non-polar fluids, and is therefore more likely to bind to the walls of a plastic collection container.

Care must be taken to select a collection device for salivary diagnostics which is appropriate for the assessment of each kind of steroid hormone.

The most appropriate collection device would be glass (including the stopper) which creates serious handling disadvantages. Therefore, IBL-Hamburg has developed a special plastic tube material with nearly the same adsorption characteristics. For convenience, the saliva should be expressed through a piece of straw into the collection device. This type of collection device is absolutely necessary for accurate assessment of progesterone in saliva. For the measurement of other steroid hormones in saliva collection devices from a variety of different materials may be acceptable, although there are not the best ones (see figures below). However, it is highly recommended that only one "standard" collection device be used for all salivary samples to eliminate confusion at the point of sample collection.

In order to assess the level of adsorption, IBL-Hamburg performed studies with  $^3\text{H}$  labeled steroid hormones:

An overnight incubation of  $^3\text{H}$  labeled progesterone in saliva, put in upright stored collection devices, leads to a significant decrease of the progesterone concentration in the fluid, dependent on the kind of collection device material (figure 7). In contrast to this, no influence of the collection device material can be detected when  $^3\text{H}$  labeled testosterone (as well as DHEA, estradiol and cortisol) in saliva is stored in the same way overnight (figures 8, 9, 10).

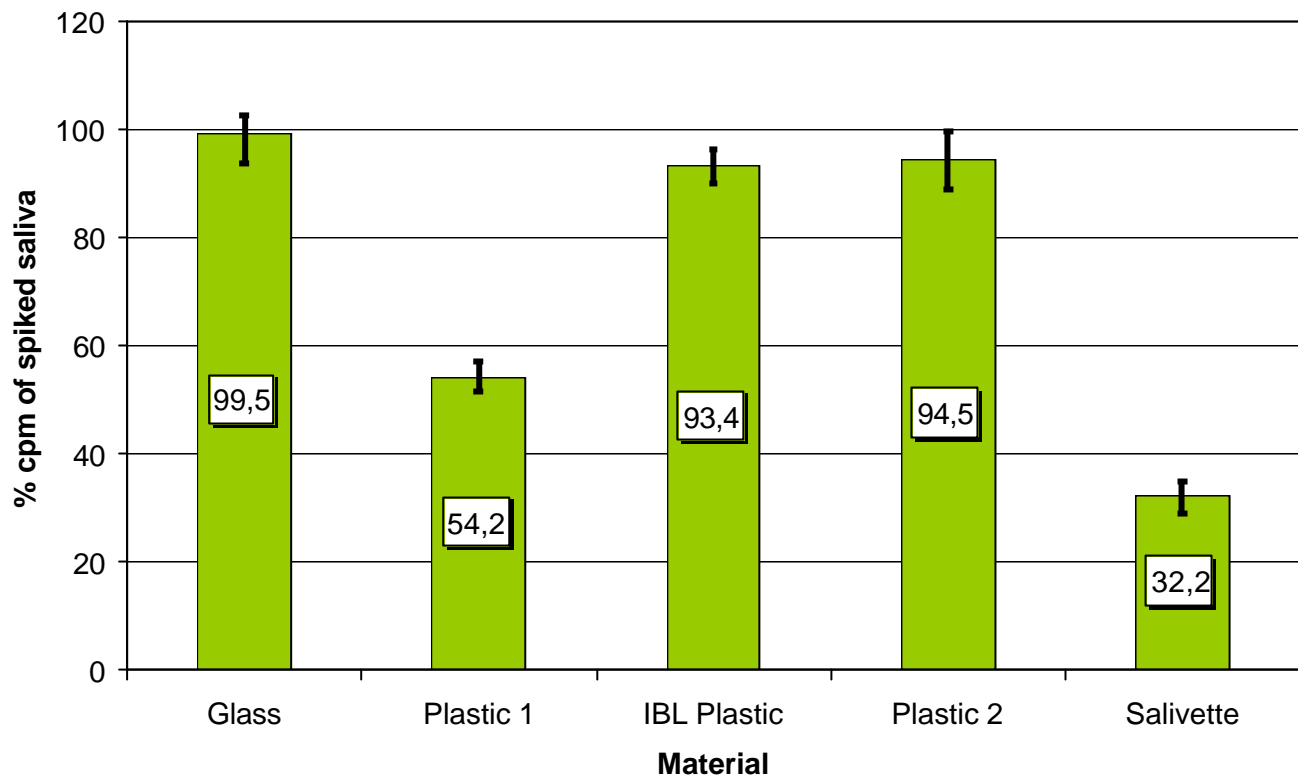


Fig. 7: Influence of the tube material on the salivary progesterone values.

A pooled saliva sample of males was spiked with  $^3\text{H}$  labeled progesterone (concentration of  $^3\text{H}$  progesterone about 440 pg/ml). 2 ml of saliva were pipetted into collection devices of different material with a diameter of 12 mm and a tube length of 75 mm. Tubes were stored overnight upright in a rack at 22 °C. The next day the cpm of 100  $\mu\text{l}$  sample of each tube were measured and compared with the cpm of the spiked saliva (11633 cpm = 100 %). For the Salivettes 2 ml of saliva were pipetted on the cotton roll, incubated for 1 hour, centrifuged and then stored overnight.

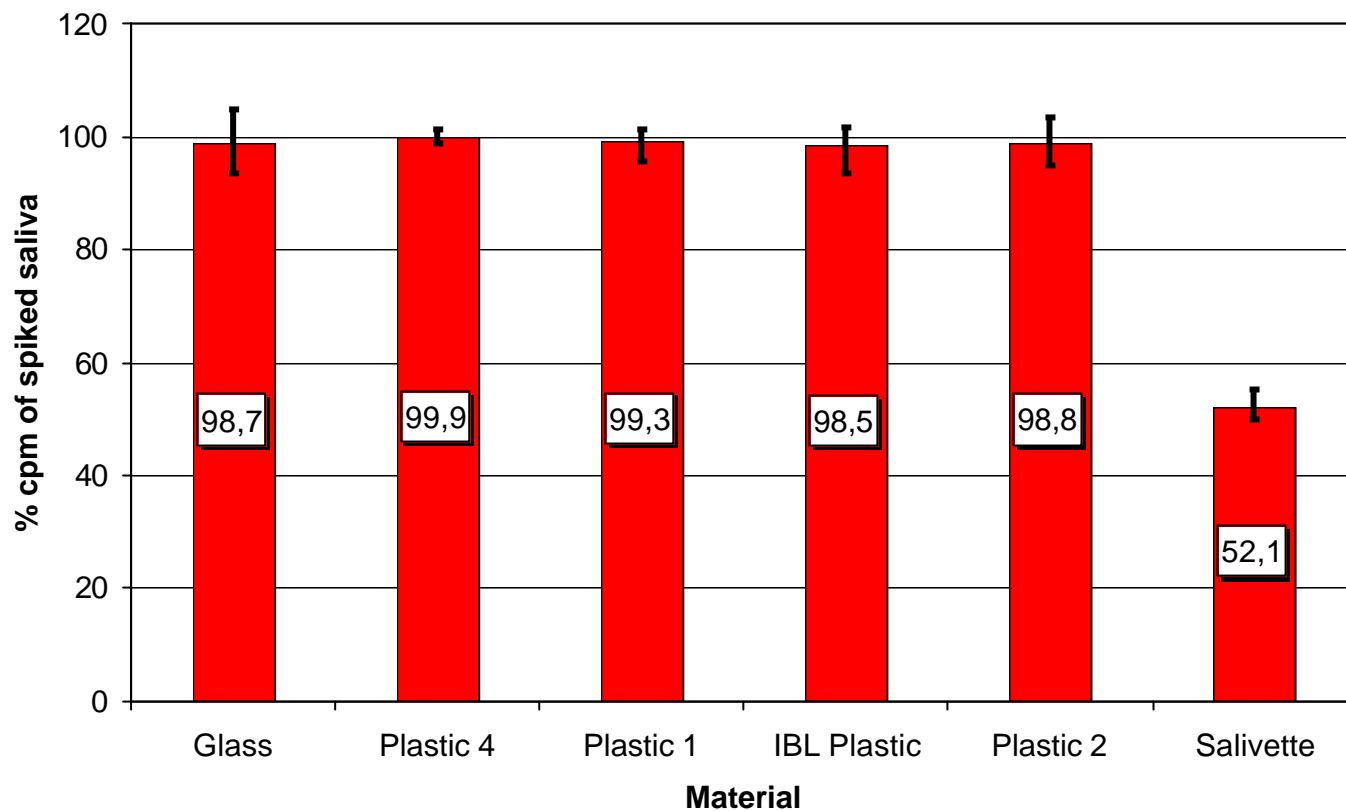


Fig. 8: Influence of the tube material on the salivary testosterone values.

A pooled saliva sample of females was spiked with  $^3\text{H}$  labeled testosterone (concentration of  $^3\text{H}$  testosterone about 460 pg/ml). 2 ml of saliva were pipetted into collection tubes of different material with a diameter of 12 mm and a tube length of 75 mm. Tubes were stored overnight upright in a rack at 22 °C. The next day the cpm of 100  $\mu\text{l}$  sample of each tube were measured and compared with the cpm of the spiked saliva (11839 cpm = 100 %). For the Salivettes 2 ml of saliva were pipetted on the cotton roll, incubated for 1 hour, centrifuged and then stored overnight.

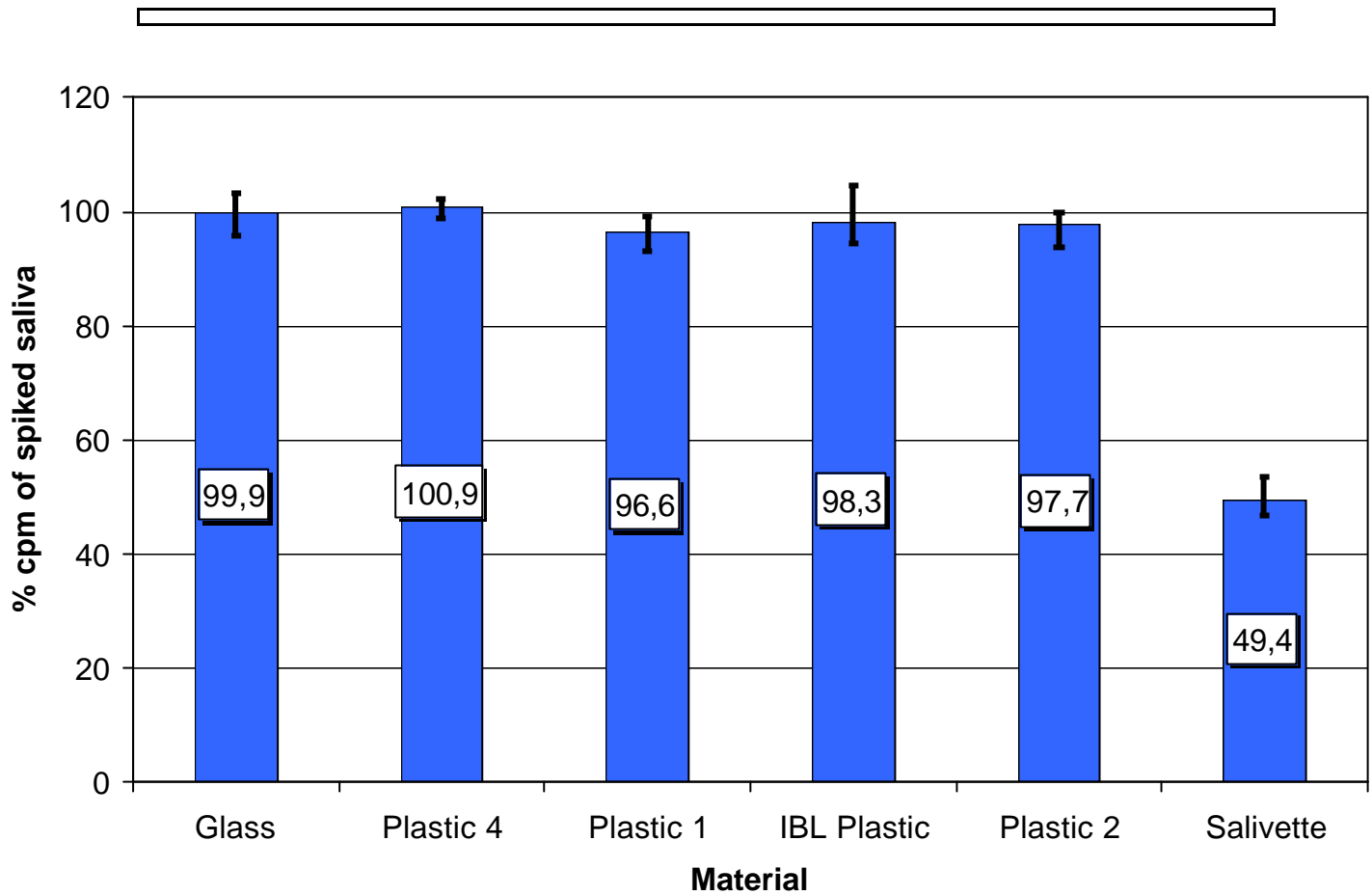


Fig. 9: Influence of the tube material on the salivary estradiol values.

A pooled saliva sample of males was spiked with  $^3\text{H}$  labeled estradiol (concentration of  $^3\text{H}$  estradiol about 490 pg/ml). 2 ml of saliva were pipetted into collection devices of different material with a diameter of 12 mm and a tube length of 75 mm. Tubes were stored overnight upright in a rack at 22 °C. The next day the cpm of 100  $\mu\text{l}$  sample of each tube were measured and compared with the cpm of the spiked saliva (9450 cpm = 100 %). For the Salivettes 2 ml of saliva were pipetted on the cotton roll, incubated for 1 hour, centrifuged and then stored overnight.

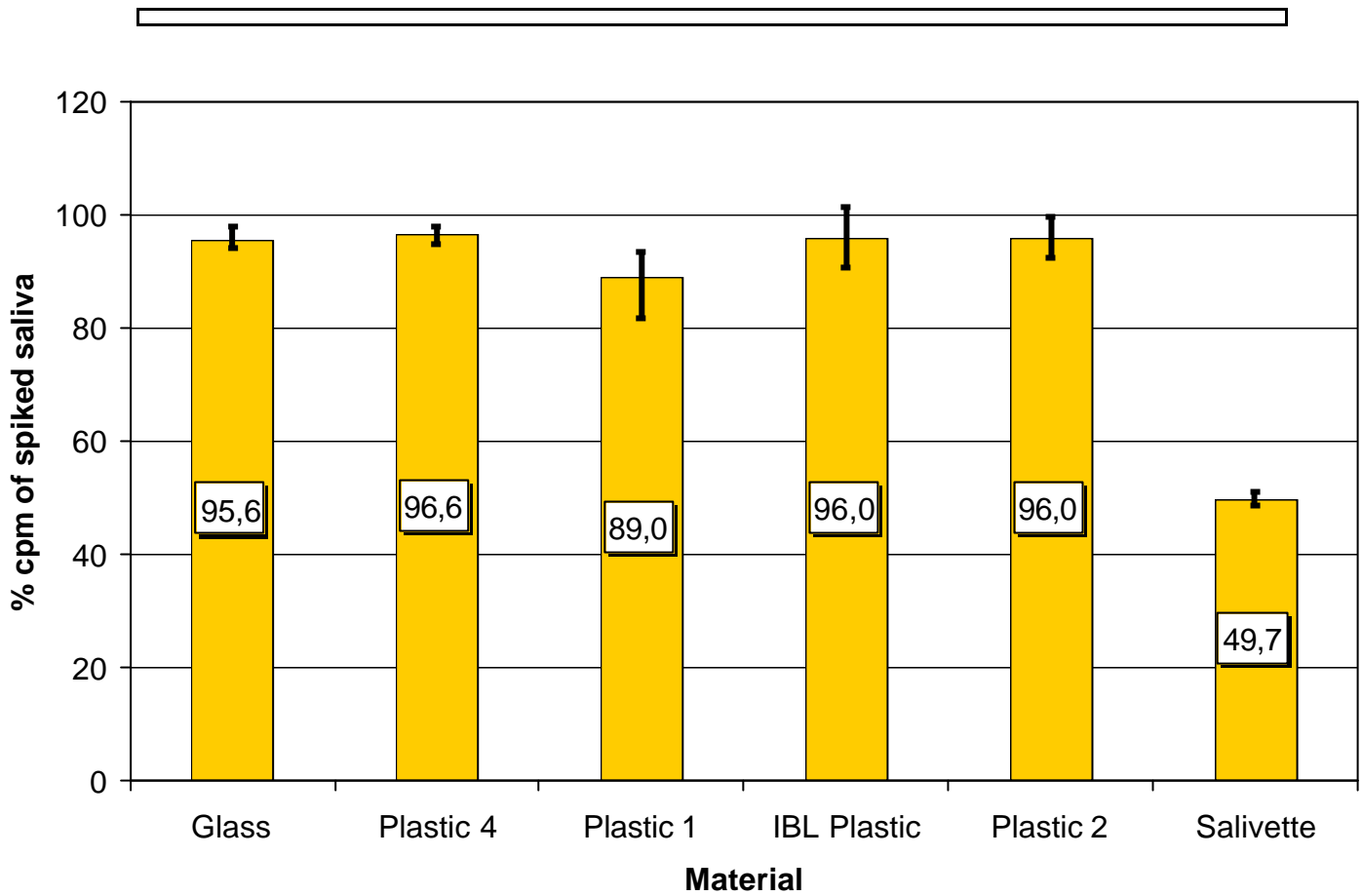


Fig. 10: Influence of the tube material on the salivary DHEA values.

A pooled saliva sample of males was spiked with  $^3\text{H}$  labeled progesterone (concentration of  $^3\text{H}$  DHEA about 370 pg/ml). 2 ml of saliva were pipetted into collection tubes of different material with a diameter of 12 mm and a tube length of 75 mm. Tubes were stored overnight upright in a rack at 22 °C. The next day the cpm of 100  $\mu\text{l}$  sample of each tube were measured and compared with the cpm of the spiked saliva (12289 cpm = 100 %). For the Salivettes 2 ml of saliva were pipetted on the cotton roll, incubated for 1 hour, centrifuged and then stored overnight.

The overnight incubation of  $^3\text{H}$  labeled progesterone solutions in different collection tubes on a roller reveals, that the material of the stopper also influences the level of analyte adsorption (figure 11).

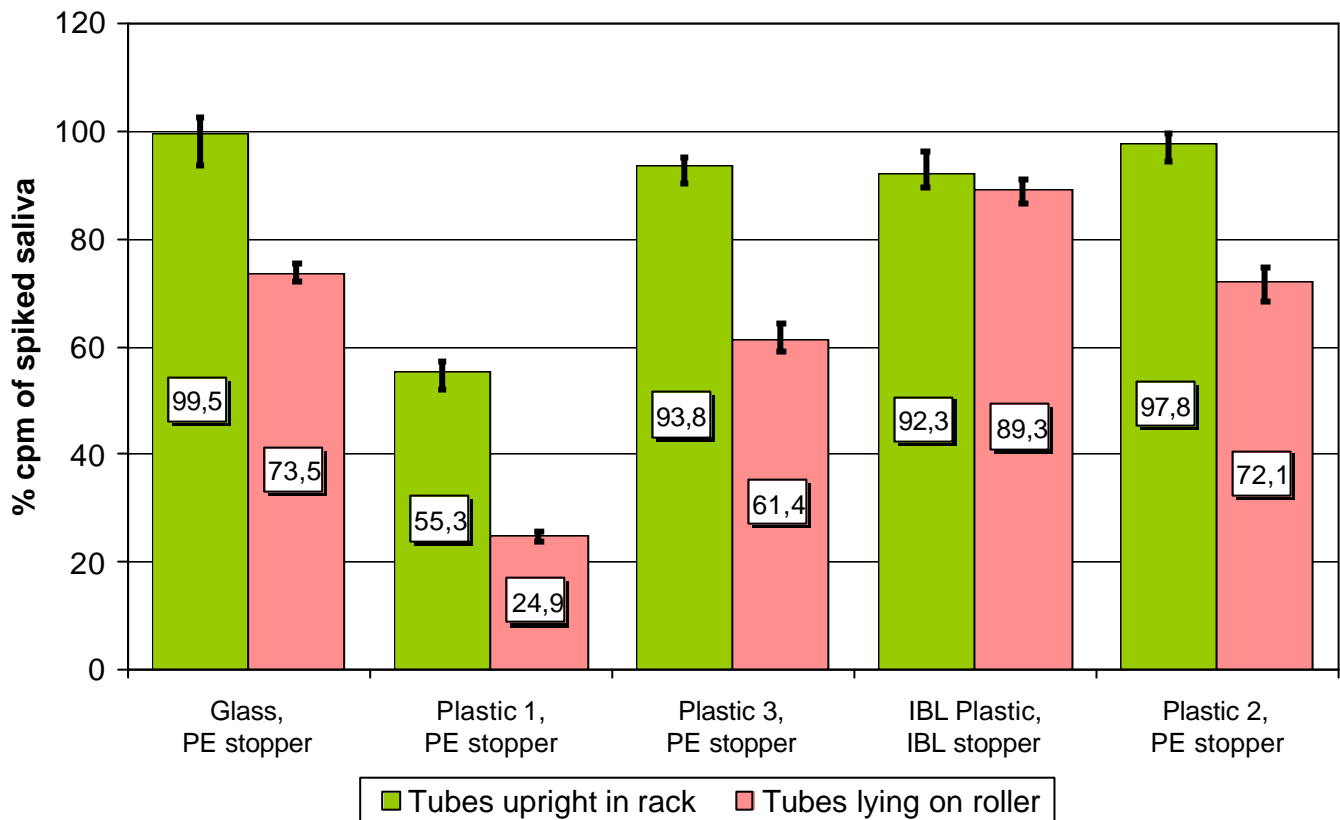


Fig. 11: Influence of the collection tube and the stopper material on the salivary progesterone values.

The same study design as described in figure 7 (with an additional tube; plastic 3). The tubes were incubated a second night on a roller at 22 °C. The next day the cpm of 100  $\mu\text{l}$  saliva were measured and again compared with the cpm of the spiked saliva (11633 cpm = 100 %).

Different concentrations of  $^3\text{H}$  labeled progesterone in saliva stored in plastic collection tubes seem not to have any influence on the amount of adsorption, whereas small volumes ( $< 200 \mu\text{l}$ ) of saliva in the tubes do have a greater amount of adsorption even in the IBL-Hamburg tubes (figures 12, 13).

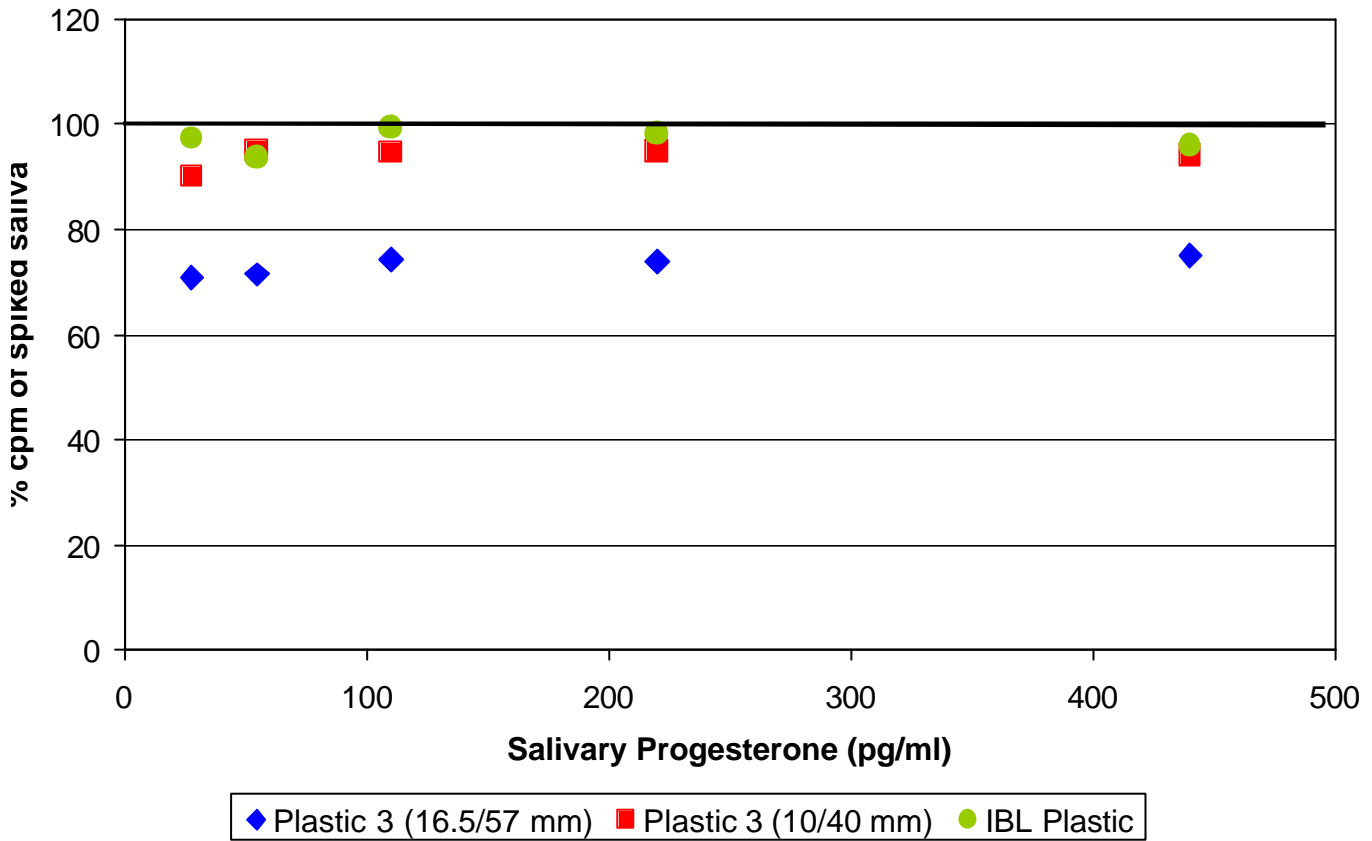


Fig. 12: Influence of the salivary progesterone level on the amount of adsorption. A pooled saliva sample of males was spiked with  $^3\text{H}$  labeled progesterone (concentration of progesterone in the saliva 440 pg/ml). Then a two fold dilution with saliva of males was made up to a  $^3\text{H}$  labeled progesterone concentration of 28 pg/ml. 1.5 ml saliva of each dilution was pipetted in two tubes of each kind and incubated on a roller overnight at 22 °C. The next day the cpm of 500  $\mu\text{l}$  of each sample were measured and compared to the cpm of the spiked saliva of each dilution step.

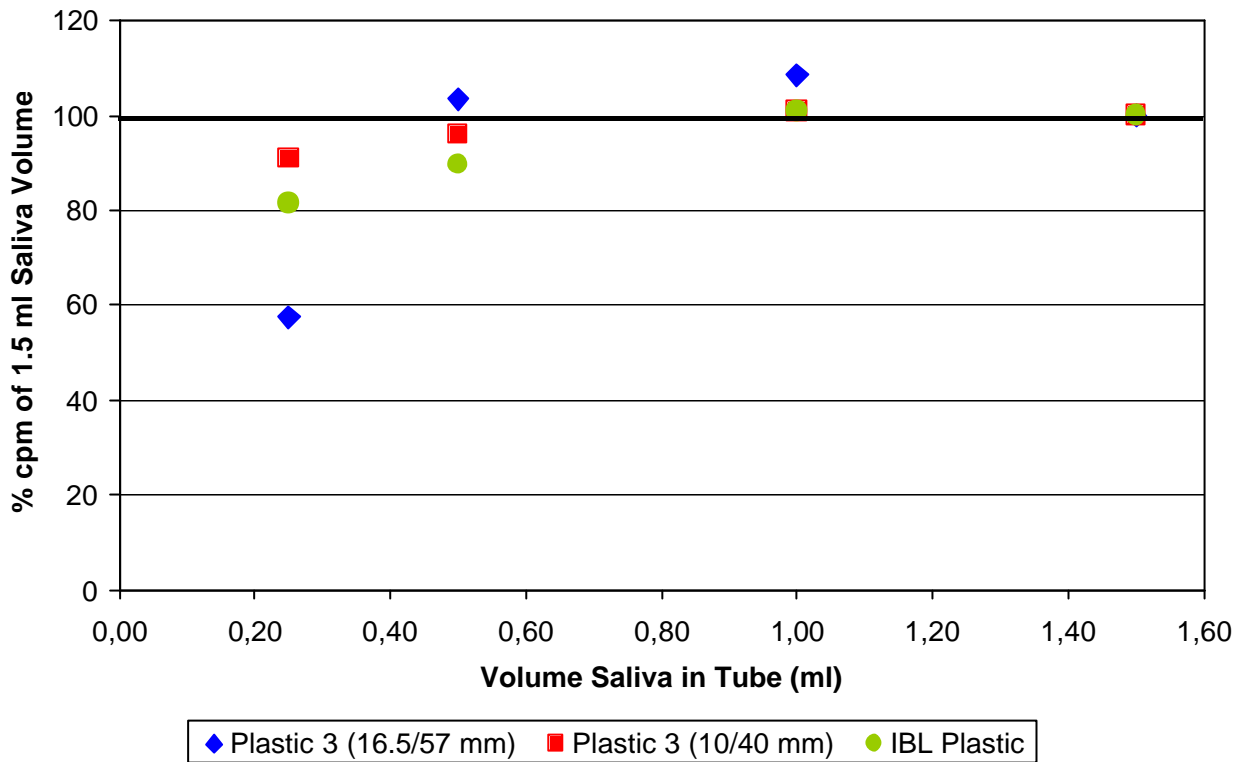


Fig. 13: Influence of the volume of the collected saliva sample on the progesterone values. A pooled saliva sample of males was spiked with <sup>3</sup>H labeled progesterone concentration of progesterone in the saliva 440 pg/ml). Then 1.5, 1.0, 0.5 and 0.25 ml of the spiked saliva were pipetted each in two tubes of each kind and incubated on a roller overnight at 22 °C. The next day the cpm of 100 µl of each sample were measured and compared to the cpm of the 1.5 ml saliva (= 100%).

Collection of saliva using a cotton or a polyester roll (Salivette, Sarstedt) may seem attractive in some instances because of convenience and ease of use. However, this collection device is only appropriate for the assessment of cortisol, and is not suitable as a collection device for the other steroid hormones. The cotton material (and even the polyester material) contains components (e.g. phytoestrogens) which interfere with the immunoassays for the hormones (false high values; figures 14, 15). On the other hand, the above mentioned <sup>3</sup>H labeled steroid hormone studies revealed that a significant amount of the hormones was retained in the cotton (and polyester; false too low values; figures 7, 8, 9, 10). Therefore, use of the Sarstedt Salivette collection device for quantifying steroid hormones other than cortisol in saliva is likely to yield arbitrary and unreliable results.

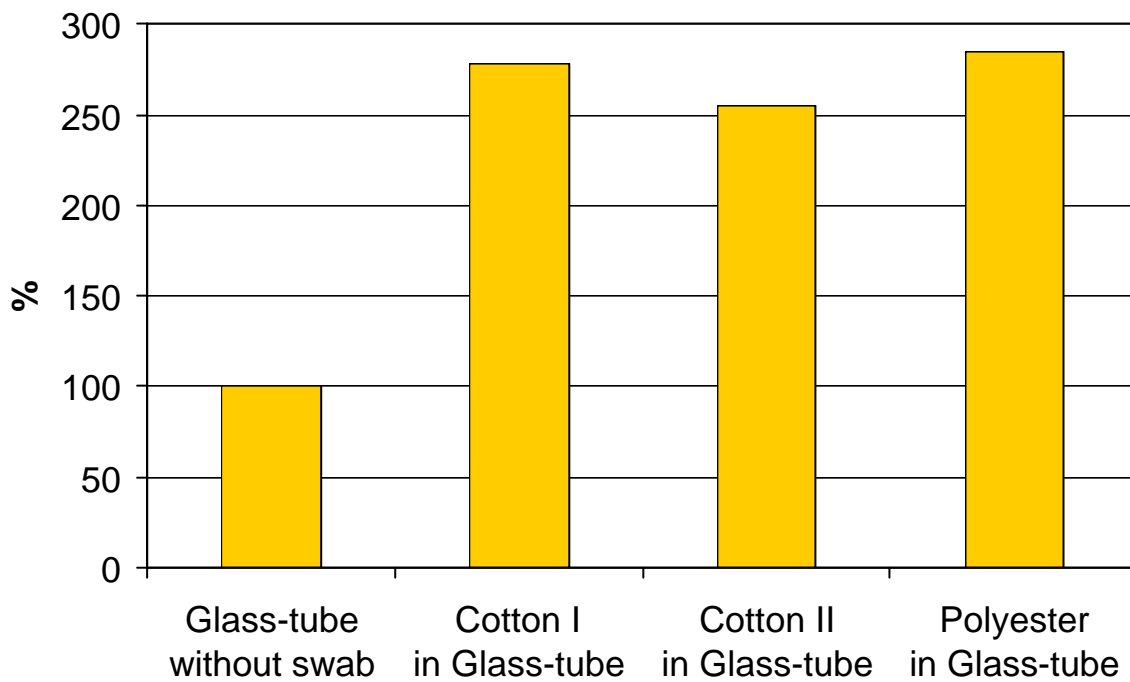


Fig.14: Influence of the collection device swab material on the measured saliva progesterone concentration. Different swabs are soaked with the same saliva sample. The progesterone concentration of the saliva collected in the glass tube without a swab is set at 100 %.

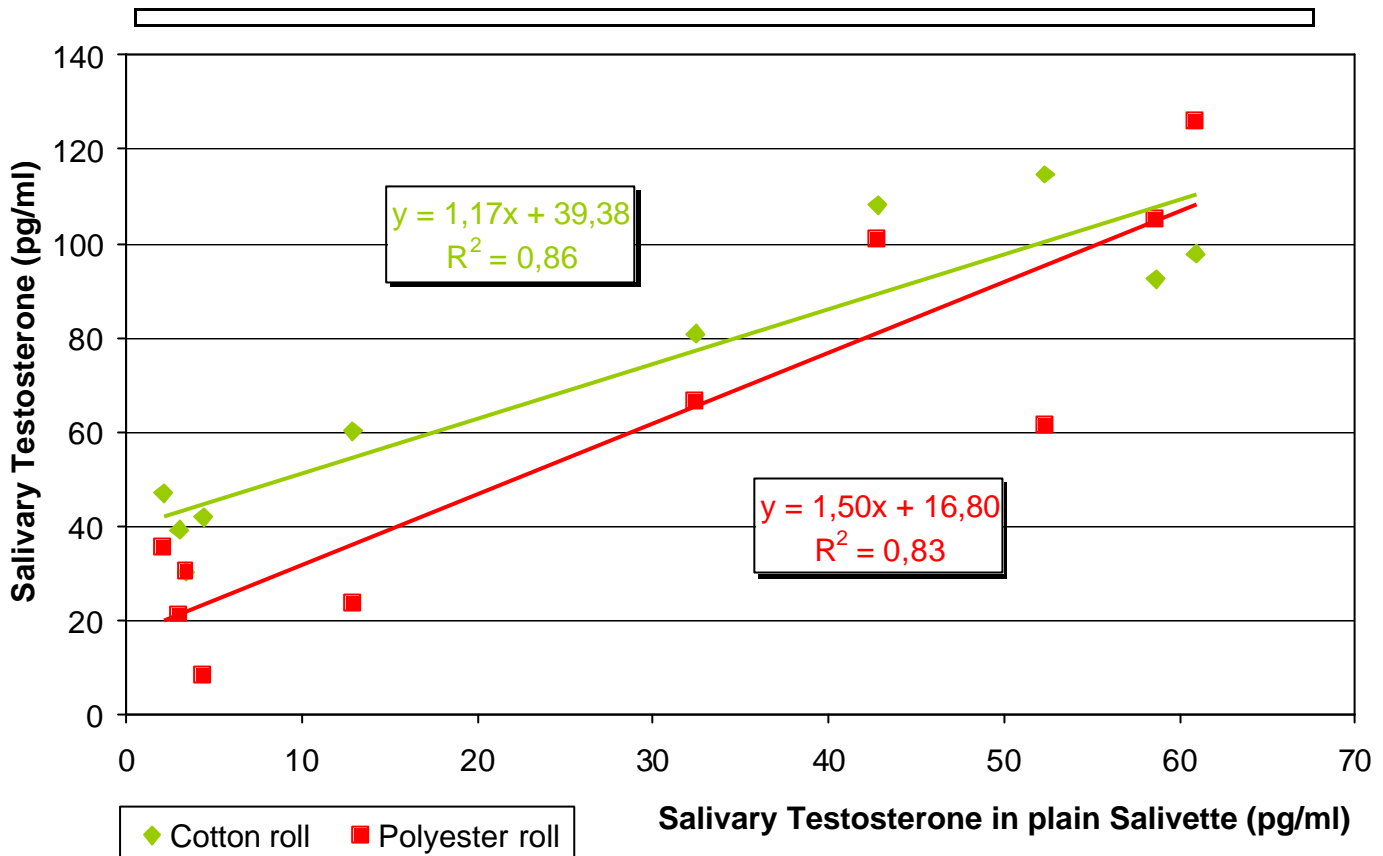


Fig. 15: Influence of the collection device swab material on the salivary testosterone values. 2 ml of saliva from 5 females and 5 males (salivary testosterone range 2 – 60 pg/ml) were pipetted in duplicates in Salivettes with a cotton roll, with a polyester roll and without any roll inside. The samples were incubated 20 hours at 8 °C and then centrifuged (3000 rpm, 5 minutes). Finally the testosterone level of the samples of each kind of device are assessed and compared to each other.

Some commercial collection devices may contain citric acid within the swab, in order to stimulate the saliva flow by chewing on the swab. The physiological range of the pH value of saliva (about 6.4 – 7.4) has no effect on the steroid assays, but concentrated acids will interfere with immunoassays.

The following chart represents a list of various saliva collection devices, **most of them are not appropriate for the assessment of steroid hormones in saliva.**

Name	Material	Manufacturer
Glass tube	pay attention to the stopper material!	--
IBL-Hamburg tube	special plastic material	IBL-Hamburg
Salivette	cotton (polyester) roll + centrifugation tube	Sarstedt
Ora-Sure	pad on „lollipop“ stick	Epitope
Omni-Sal	Collector with absorbing pad	Saliva Dignostic Systems
Saliva-Sac	ultrafiltration device	BioQuant
Oral Diffusion Sink	diffusion into a $\beta$ -cyclodextrin filled tube	not available anymore
Abusa-Stick	Saliva swab	Chem-Elec
Alcoscan	Saliva swab	Lifescan
Saliva collection device	rayon ball + expulsion by screwing a piston	Trinity Biotech
Quick Absorber	triangular paper strip	Inami Co. Ltd.

Table 3: Saliva collection devices (P. Haeckel and P. Hänecke, 1996, altered)

To summarize the above mentioned subject, the most convenient method for collecting saliva is to express the saliva through a piece of straw into a **special plastic tube (IBL-Hamburg)** which can be used for centrifugation.

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## Hints

In the following list there are summarized some hints for the collection of saliva.

- It is highly recommended to use **special plastic collection tubes of IBL-Hamburg** for the assessment of all steroid hormones in saliva
- Except for Cortisol, do **not use cotton or polyester rolls** for saliva collection (arbitrary values)
- Do not use swabs containing citric acid
- Do collect Saliva before **brushing of teeth** or at least 30 minutes after
- Do collect Saliva at least **30 minutes after eating** or drinking
- For saliva flow stimulation, chewing on an inert material like Parafilm<sup>®</sup> is recommended
- Do **not use even the slightest red saliva samples** (because of blood contamination)
- Saliva samples for steroid measurement may be stored for up to
  - 5 days at room temperature
  - 10 days at 2 - 8 °C
  - longer periods at -20 °C
- Freezing of the saliva samples and centrifugation just before testing is recommended

Table 4: Hints for saliva sampling

**NOTE:** Contamination of saliva sample with trace amounts of blood can create a risk for false elevated steroid hormone levels. Therefore, if a slight reddish color is noted in the saliva sample, it should be discarded and another sample should be collected at a time when oral bleeding or trauma is not evident. For cortisol assessment, visual observation is sufficient for the evaluation of blood contamination in the sample, but for other steroids additional tests for detection of non-visible traces of blood are recommended.

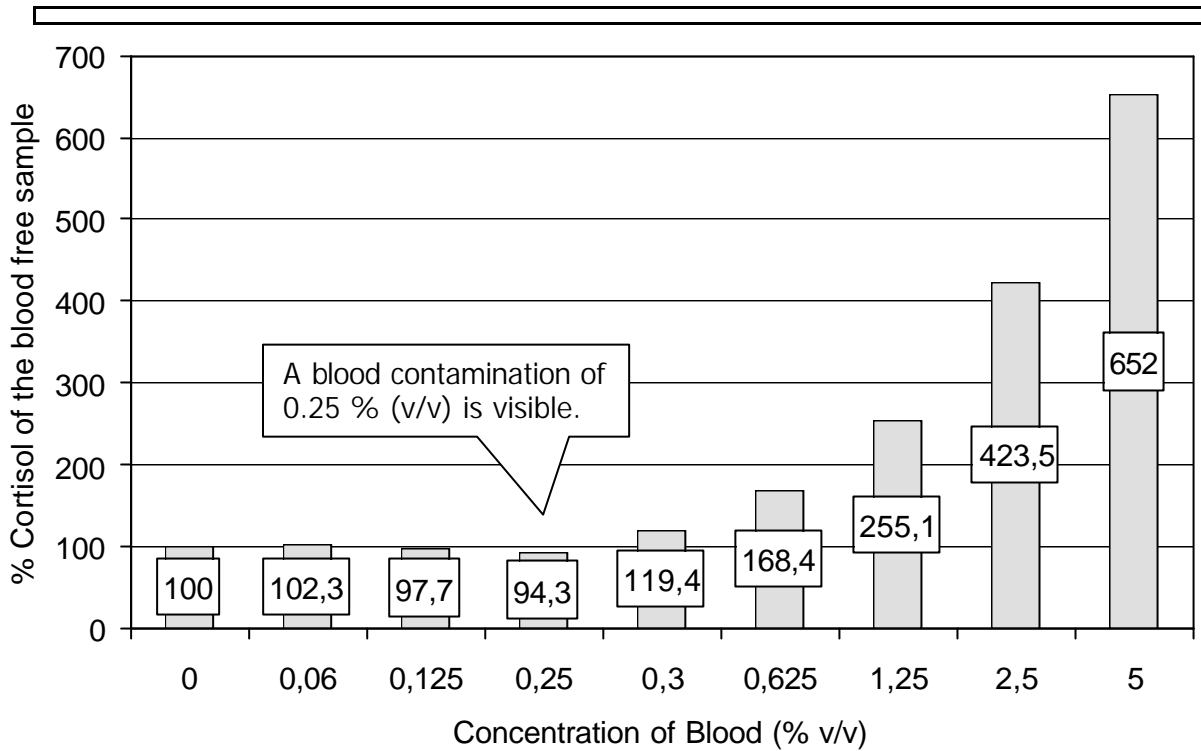


Fig. 16: Influence of blood contamination on the salivary cortisol level. Blood with a cortisol concentration of 21.9 µg/dl was added to a saliva sample with a cortisol level of 0.86 ng/ml, and the measured cortisol level was compared with the cortisol concentration of the blood free saliva sample.

At this time, the most convenient method for detecting the presence of blood in a sample is the use of a dipstick designed for the detection of blood in urine. For the assessment of blood contamination in saliva, a sample aliquot is diluted with distilled water. Enzymes such as Pseudoperoxidase which may have an influence on immunoassays are also detected by this dipstick.

Other methods to detect blood are very sensitive but on the other hand the test procedure is very time consuming and relatively expensive.