Testosterone Saliva ELISA

- Reliable detection of free testosterone in saliva
- Minimal cross reactivity with other steroids
- Automated processing on open systems possible

**Technical data**

- **Coating**: Monoclonal anti-testosterone antibody
- **Calibration**: Quantitative, in picogrammes per millilitre (pg/ml), 6 calibrators
- **Sample material**: Human saliva, passively collected (without auxiliary means)
- **Reagents**: Ready for use, with the exception of the wash buffer (10x); colour-coded solutions
- **Test procedure**: 90 min/30 min/15 min (sample/conjugate/substrate incubation); room temperature, fully automatable
- **Measurement**: 450 nm, reference wavelength between 620 nm and 650 nm
- **Test kit format**: 96 break-off wells; kit includes all necessary reagents
- **Order no.**: EQ 6151-9601 S

**Clinical significance**

The steroid hormone testosterone belongs to the group of male sex hormones (androgens). It is regulated by the hypothalamus and pituitary gland and synthesised, via several intermediate steps, from cholesterol predominantly in the Leydig cells of testis and ovaries. Together with follicle-stimulating hormone, testosterone activates spermatogenesis in men. The hormone is also responsible for protein biosynthesis, muscle growth and preservation of bone density. It further regulates sexual behaviour in men. In the serum, the majority of the testosterone is bound with a high affinity to the sex hormone-binding globulin (SHBG) and with a low affinity to albumin. Up to 2% of the testosterone remains unbound in the serum and is thus biologically active. The unbound steroid hormone is able to diffuse through cells. Thus, independent of salivation, only biologically active testosterone reaches the saliva. The Testosterone Saliva ELISA is designed for the detection of biologically active testosterone in saliva.

Investigation of testosterone levels in male individuals is indicated in patients presenting with underdeveloped or diminished primary or secondary sexual characteristics, which generally result from hypogonadism. This endocrine functional disorder can occur in the testis (primary) or hypothalamus/pituitary gland (secondary). It manifests itself by drastically decreased testosterone levels in serum and saliva. Testosterone levels should be determined in female individuals showing signs of virilisation, such as an increase in body or facial hair or a deepened voice. The cause could be a tumour in the ovaries or adrenal cortex, polycystic ovarian syndrome or congenital adrenal hyperplasia. In athletes, testosterone and cortisol concentrations are gaining importance in monitoring of the fitness level. The proportion of biologically active testosterone increases shortly after the onset of physical stress and drops again during recovery. During continuous severe physical stress the testosterone concentration falls below its original level.

**Diagnostic application**

The Testosterone Saliva ELISA is based on a sensitive and specific monoclonal antibody. This antibody shows low cross reactivity with other steroids (e.g. dihydrotestosterone, dehydroepiandrosterone, cortisol) which can occur in much higher concentrations than testosterone in the body and could therefore interfere with the measurement result. The Testosterone Saliva ELISA shows a high analytical sensitivity and is, at the same time, not susceptible to interference.
Detection limit

The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest clearly detectable testosterone concentration. The lower detection limit for the Testosterone Saliva ELISA is 1.6 pg/ml.

Linearity

The linearity of the Testosterone Saliva ELISA was determined by performing at least 4 serial dilutions of 5 saliva samples. The linear regression $R^2$ was >0.95 for all sera. The Testosterone Saliva ELISA is linear in the tested concentration range of 4–250 pg/ml.

Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on triplicate determinations performed in ten different runs.

Reference range

Ten saliva samples were taken throughout the day from each of 120 apparently healthy blood donors between 19 and 49 years of age (80 women and 40 men). The diurnal testosterone profile is characterised by a circadian rhythm. In the first two hours after getting up in the morning the concentration decreases significantly. Therefore, saliva samples should not be taken earlier than two hours after getting up.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number (n)</th>
<th>Median</th>
<th>Range (5-95th percentile)</th>
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<th>Median</th>
<th>Range (5-95th percentile)</th>
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<tr>
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<td>23</td>
<td>12-46</td>
<td>15</td>
<td>79</td>
<td>51-129</td>
</tr>
</tbody>
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Mean values of two saliva samples taken in the morning at an interval of two hours

These reference ranges refer to samples taken in the morning. Since the testosterone concentration decreases even further during the day, reference ranges for certain periods of time should be determined (morning, afternoon, etc.). Each laboratory should use their own normal values, established under specific ambient conditions, for defined points in time, age and gender groups.

Correlation

The EUROIMMUN Testosterone Saliva ELISA was compared to a commercial ELISA from Salimetrics (sample range 9–138 pg/ml). The correlation was $R^2=0.902$.