and reproducible data generated by reliable genotyping assays are available. The LightCycler assay for the detection of CAPNI0 polymorphisms offers a rapid and reliable method for association studies.

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References


Automated Measurement of Salivary Cortisol, Maarten O. van Aken,1* Johannes A. Romijn,1 Johannes A. Miltenburg,2 and Eef G.W.M. Lentjes3 1Department of Endocrinology and Metabolic Diseases and 2Laboratory of Clinical Chemistry, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands; 3Endocrinology Laboratory, University Medical Center Utrecht, Postbox 85090, 3508 AB Utrecht, The Netherlands; *address correspondence to this author at: Department of Endocrinology and Metabolic Diseases, C4R, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands; fax 00-31-715248136, e-mail m.o.van_aken@lumc.nl

A new serum cortisol assay was introduced on the Elecsys (Roche), a random access analyzer, with reportedly good performance in the low (nmol/L) concentration range (preliminary data provided by the manufacturer) and with low cross-reactivity with cortisone [0.3% at 2.7 μmol/L cortisone (package insert)]. This prompted us to evaluate the performance of this new assay for the measurement of salivary cortisol.

For this study, saliva samples were collected with a Salivette® (Sarstedt), with an insert containing a sterile polyester swab for collection of the saliva, yielding a clear and particle-free sample. The salivettes were used according to the instructions provided by the manufacturer. Samples collected this way are stable at room temperature for at least a week and, therefore, offer the opportunity to collect samples at home (1, 2). Salivettes containing saliva were centrifuged at 2000g for 10 min, and the filtrates were stored frozen (–20 °C). Before analysis, the samples were thawed, mixed, and placed on the Elecsys analyzer without pretreatment.

The Roche cortisol assay is a competitive electrochemiluminescence immunoassay (ECLIA) that uses a sheep polyclonal antibody. Endogenous cortisol contained in the sample is liberated from the binding proteins by danazol and, subsequently, competes with a cortisol derivative (a cortisol–peptide–Tris bipyridyl ruthenium complex) for the binding sites on the biotinylated antibody. After the addition of streptavidin-coated paramagnetic particles, the biotin on the antibody can bind to the streptavidin of the microparticle and form a complex. This complex is then captured on the surface of the magnetic electrode. Electrical stimulation of the ruthenium complex induces chemiluminescent emission, which is measured by a photomultiplier. The assay was calibrated against Enzymun-Test-Cortisol, which in turn was calibrated via isotope dilution-mass spectrometry. The cortisol assay was used as instructed by the manufacturer without modifications. The sample volume for the assay was 20 μL. Processing time is 18 min.

The assay linearity of the cortisol determinations in saliva was studied according to the NCCLS EP-6 protocol.
in a saliva sample with a low cortisol concentration with added cortisol (Sigma Chemical Co) at a concentration of 240 nmol/L (3). This sample was diluted with the untreated sample. The correlation between added and measured cortisol was linear with slope = 0.99, intercept = −2.9 nmol/L, \( r^2 = 0.998 \).

A precision profile was established based on saliva samples with or without cortisol additions. Each sample was aliquoted 10 times and stored frozen. An analysis of the samples was performed on 10 different days over a time span of 4 months (Fig. 1). The functional detection limit (20% interassay CV) as determined from these measurements is 2.0 nmol/L. The performance of the Elecsys cortisol assay in the low concentration range renders it suitable for the measurement of cortisol concentrations in saliva.

Interassay precision was evaluated according to the NCCLS EP-5 protocol (4). Two pools of saliva with different cortisol concentration were aliquoted, and cortisol was measured on 20 separate days, twice in each sample. Interassay CV was 12% at 11 nmol/L and 5.0% at 50 nmol/L. The somewhat higher CVs, compared with those found in the former experiment, reflected a shift in the concentrations after a calibration.

Cross-reactivity by other steroids was investigated by adding increasing amounts of the interfering compound to a saliva sample with a cortisol concentration of 5 nmol/L. Apparent cortisol concentration at 10, 100, and 1000 nmol/L of the cross-reactant cortisone and 11-deoxycortisol never exceeded, respectively, 0.6% and 1.8% of the added amount. For 6β-hydroxycortisol, however, there was an increasing interference, i.e., apparent cortisol at 10 nmol/L 30%, at 100 nmol/L 40%, and at 1000 nmol/L 50% of the added amount. For 21-deoxycortisol at a concentration of 10, 100, and 1000 nmol/L, apparent cortisol concentrations were 44%, 24%, and 14%, respectively. Importantly, interference by cortisone, an abundant steroid metabolite in saliva, is negligible. By contrast, the presence of 6β-hydroxycortisol or 21-deoxycortisol in saliva has not been reported. These steroids are easily excreted in urine (6β-hydroxycortisol) or are present only at low concentrations in serum, which makes it unlikely that the concentrations in saliva will interfere with the cortisol assay.

We compared the Elecsys cortisol assay with an in-house RIA (5) in salivary samples. Correlation of salivary cortisol measured by ECLI (Elecsys) vs RIA was: Elecsys = 0.92 × RIA − 1.6 nmol/L [n = 34; r = 0.84; SD of slope = 0.1; SD intercept = 1.9; \( S_{y-x} = 4.3 \) nmol/L (Deming regression analysis)].

Reference intervals were estimated from an unselected group of healthy individuals, 26 male and 32 female (20–80 years old). Saliva samples were obtained between 07:00 and 08:00 h and between 23:00 and 24:00 h (late night). The mean (SD) for morning salivary cortisol was 13.4 (3.2) nmol/L and for late night cortisol was 3.55 (0.94) nmol/L. No sex differences were observed. Although these values are similar to previously reported reference intervals, they should be established in each laboratory because different assays produce different results (6).

This is the first report of a fully automated nons isotopic assay for the measurement of cortisol in saliva. Detection limit and reproducibility in the low nanomolar concentration range suggest it will be a useful tool in the assessment of the activity of the hypothalamic-pituitary-adrenal axis. This method offers several advantages over isotopic assays and commercially available enzyme immunoassays (7, 8). It is automated, samples need no pretreatment, results can be obtained within 20 min, and collecting a specific number of samples is not needed for efficient use. This makes it a suitable test for daily laboratory and clinical use, as recently advocated for the diagnosis of Cushing syndrome (9).

References