bance/μg) was determined using the concentration of a sample that gave an absorbance value within the linear range of the assay and was expressed relative to the absorbance/μg of BSA (corresponding to the gradient of the calibration curve):

$$\text{Interference} = \frac{\text{absorbance}/\mu g \text{ of aminoglycoside}}{\text{absorbance}/\mu g \text{ of BSA}} \times 100\%$$

Neomycin, gentamicin, tobramycin, and paromomycin gave higher responses (442%, 304%, 259%, and 135%, respectively) than BSA (100%), whereas geneticin, kanamycin, streptomycin, and dihydrostreptomycin gave lower responses (26%, 21%, 7%, and 3%, respectively; n = 5; CV <5%). The aminoglycosides gave negligible interference in the CBB assay (<0.2% relative to BSA) and failed to generate turbidity in the BEC assay.

Aminoglycoside interference in urine was demonstrated with urine control containing the aminoglycosides at 0.2 g/L. Although the data are based on model experiments, they reflect aminoglycoside concentrations that could reasonably be present in urine from patients (1). The PRM control value (0.36 ± 0.01 g/L protein) increased 244% with neomycin (1.24 ± 0.01 g/L), 142% with gentamicin (0.87 ± 0.02 g/L), 111% with tobramycin (0.76 ± 0.02 g/L), 53% with paromomycin (0.55 ± 0.02 g/L), 11% with geneticin (0.40 ± 0.01 g/L), 8% with kanamycin (0.39 ± 0.01 g/L), 3% with streptomycin (0.37 ± 0.01 g/L), and 3% with dihydrostreptomycin (0.37 ± 0.02 g/L; n = 5; CV <5.5%). The interference was significant (P <0.01) with neomycin, gentamicin, tobramycin, paromomycin, geneticin, and kanamycin but not significant (P >0.1) with streptomycin or dihydrostreptomycin. In contrast, neither the CBB control value (0.21 ± 0.01 g/L protein) nor the BEC control value (0.28 ± 0.01 g/L) were affected by aminoglycosides at 0.2 g/L.

Thus, in contrast to the Roche and Cobas Fara PRM assays, which are resistant to aminoglycoside interference, the Sigma PRM assay resembles the Dade Behring PRM assay in its susceptibility to interference (1). This may reflect the formulation of the reagents or the volume ratio of sample to reagent. The clinical importance of this interference is evident for tobramycin [at urinary concentrations >0.2 g/L (1)] but more difficult to assess for the other aminoglycosides, whose urinary concentrations do not appear to have been reported.

In conclusion, we have confirmed interference in the PRM assay by gentamicin, neomycin, and tobramycin (1–3); reported interference from additional aminoglycosides; and demonstrated a susceptibility of the Sigma PRM assay to interference from aminoglycosides in urine at 0.2 g/L. In contrast to the PRM assay, the CBB and BEC assays are resistant to aminoglycoside interference.

References

1. Koerbin G, Taylor L, Dutton J, Marshall K, Low P, Potter JM. Aminoglycoside interference with the Dade Behring pyrogallol red-molybdate method for the measurement of total urine protein [Letter]. Clin Chem 2001;47:2183–4.
2. Fujita Y, Mori I, Kitano S. Color reaction between pyrogallol red-molybdenum(VI) complex and protein. Bunseki Kagaku 1983;32:E379–86.
3. Watanabe N, Kamel S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K, et al. Urinary protein as measured with a pyrogallol-red-molybdate complex manually and in a Hitachi 726 automated analyzer. Clin Chem 1986;32:1551–4.
4. Lott JA, Stephan VA, Pritchard KA. Evaluation of the Coomassie Brilliant Blue G250 method for urinary protein. Clin Chem 1983;29:1946–50.
5. Marshall T, Williams KM. Total protein determination in urine: elimination of a differential response between the Coomassie Blue and Pyrogallol Red protein dye-binding assays. Clin Chem 2000;46:392–8.
6. Iwata J, Nishikaze O. New micro-turbidimetric method for determination of protein in cerebrospinal fluid and urine. Clin Chem 1979;25:1317–9.

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New Enzyme Immunoassay for Salivary Cortisol

To the Editor:
The measurement of salivary cortisol is emerging as the simplest approach in the diagnosis of Cushing syndrome (1–3). One of the problems with previous methods is the lack of a Food and Drug Administration (FDA)-cleared method. We have evaluated an enzyme immunoassay (EIA) for salivary cortisol marketed by Salimetrics (State College, PA) and recently cleared by the FDA for in vitro diagnostic use.

We compared the new EIA with our modified RIA (2) in 147 samples. The first set of samples (n = 44) was collected at 2300 and 0700 from a group of apparently healthy adult individuals (n = 22; age range, 25–60 years; 11 females and 11 males). The second set of samples (n = 30) was collected at 2300 by patients (n = 30; age range, 12–84 years; 22 females and 8 males) to screen for Cushing syndrome. The third set of samples (n = 73) was collected between 0600 and 1000 from participants (n = 42; age range, 6–14 years; 21 females and 21 males) enrolled in a study of allergic rhinitis. The study was approved by the appropriate Institutional Review Boards, and consent was obtained. Saliva was sampled as described previously (2) with a collecting device (Salivettes with no preservative; Sarstedt).

Salivary cortisol was measured by two methods. The serum cortisol RIA [Coat-a-Count TKCO; Diagnostic Products (DPC)] was used as commonly modified for the measurement of salivary cortisol (2). The salivary cortisol EIA (product no. 1-1102; Salimetrics) was used as instructed without modification. The sample volumes were 200 and 25 μL

for the modified RIA and EIA, respectively, and the incubation times were 3 h for the RIA and a total of 80 min for the EIA. The EIA calibrators ranged from 0.2 to 49.7 nmol/L and were provided in a saliva-like matrix with a nonmercury preservative.

The lower detection limit of the EIA was 0.3 nmol/L. The intraassay imprecision (CV) was 5.2% at 3.1 (SD, 0.2) nmol/L (n = 10) and 2.6% at 10.4 (0.3) nmol/L (n = 10). Interassay (total) imprecision (CV) was 11% at 2.8 (0.3) nmol/L (n = 10), 11% at 10.1 (1.1) nmol/L (n = 10), and 6.9% at 25.0 (1.7) nmol/L (n = 10).

The correlation of the salivary cortisol measured by RIA (x axis) vs EIA (y axis) yielded a slope of 0.84 (SE, 0.01) and a y-intercept of 1.2 (SE, 0.3) nmol/L ($r^2 = 0.96$; $S_{y|x} = 2.5$ nmol/L; n = 147) with results spanning the range 0.3–130 nmol/L. The slope was significantly less than unity, and the y-intercept was significantly >0. Because the critical range for a salivary cortisol assay for screening for Cushing syndrome is 0.3–10.0 nmol/L (1–3), Fig. 1 shows only those data pairs with DPC results ≤10 nmol/L. The slope was not significantly different from unity, and the y-intercept was not significantly different from 0.

On the basis of the correlation shown in Fig. 1, the reference interval for the Salimetrics EIA calculated from a large group of verified healthy individuals (2) at 2300 (the appropriate

Fig. 1. Correlation of salivary cortisol measured by RIA (x axis) vs EIA (y axis) in samples with RIA results ≤10 nmol/L.

Regression statistics: slope = 1.2 (SE, 0.1); y-intercept = −0.3 (SE, 0.2) nmol/L; $r^2 = 0.93$; $F_{(1,75)} = 1004.5$; $P <0.001$; $S_{y|x} =1.0$ nmol/L; n = 77.

time for the diagnosis of Cushing syndrome) was <0.3 to 4.3 nmol/L. We then evaluated salivary cortisol at 2300 in samples from 14 patients (4 males and 10 females; age range, 20–78 years) with Cushing syndrome confirmed by subsequent measurement of additional increased salivary cortisol, increased urinary free cortisol, and/or abnormal low-dose dexamethasone suppression testing using previously described criteria (3). The mean (SD) salivary cortisol as measured by the Salimetrics EIA was 20.4 (13.4) nmol/L (range, 5.3–46.8; 95% confidence interval, 12.7–28.1 nmol/L). Salivary cortisol concentrations at 2300 in these patients with confirmed Cushing syndrome were all clearly increased.

We have previously shown that the DPC RIA yields accurate results for samples enriched with known concentrations of hydrocortisone (4). The Salimetrics EIA yielded results very close to those obtained with the DPC RIA, particularly in the critical diagnostic range of 0.3–10.0 nmol/L. This is in contrast to the results that we recently obtained with the EIA from Diagnostic Systems Laboratories (DSL), which were ~1.7-fold higher than those obtained with the DPC RIA (4). The only relevant disadvantage of the Salimetrics EIA is that the highest calibrator concentration (49.7 nmol/L) is considerably lower than those of the DSL EIA (276 nmol/L) and the DPC RIA (138 nmol/L). This will require more frequent dilution of high-concentration samples from patients with Cushing syndrome, which can have cortisol concentrations >50 nmol/L (1, 2). The advantages of the Salimetrics EIA compared with the DSL EIA are that it produces results not different from those obtained with the DPC RIA in the clinically important range and that it is FDA-cleared for in vitro diagnostic use.

We have previously demonstrated that urinary free cortisol does not correlate with salivary cortisol in healthy individuals primarily because of the episodic and circadian nature of salivary cortisol measurement (2). However, there is good correlation in patients with Cushing syndrome, pri-

marily because of their increased cortisol concentrations throughout the circadian period (2). The standard approach to the evaluation of Cushing syndrome, therefore, has been the measurement of 24-h urinary free cortisol (3). However, a recent study has demonstrated in a large study population that several increased nighttime salivary cortisol measurements are superior to the measurement of urinary free cortisol in establishing the diagnosis of Cushing syndrome (5). Because of its accuracy, simplicity, and cost-effectiveness, we believe that measurement of nighttime salivary cortisol will become the principal method to screen for Cushing syndrome.

References

1. Raff H. Salivary cortisol: a useful measurement in the diagnosis of Cushing's syndrome and the evaluation of the hypothalamic-pituitary-adrenal axis. Endocrinologist 2000;10:9–17.
2. Raff H, Raff JL, Findling JW. Late-night salivary cortisol as a screening test for Cushing's syndrome. J Clin Endocrinol Metab 1998;83:2681–6.
3. Findling JW, Raff H. Diagnosis and differential diagnosis of Cushing's syndrome. Endocrinol Metab Clin North Am 2001;30:729–47.
4. Raff H, Homar JP, Burns EA. Comparison of two methods for measuring salivary cortisol. Clin Chem 2002;48:207–8.
5. Papanicolaou DA, Mullen N, Kyrou I, Nieman LK. Nighttime salivary cortisol: a useful test for the diagnosis of Cushing's syndrome. J Clin Endocrinol Metab 2002;87:4515–21.

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*Address correspondence to this author at: Endocrinology, St. Luke's Physician's Office Bldg., 2801 W KK River Pkwy., Suite 245, Milwaukee, WI 53215. Fax 414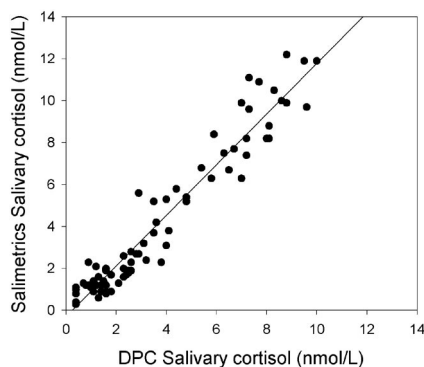-649-5747; e-mail hraff@mcw.edu.