Multicenter Evaluation of Assays for Estradiol and Progesterone in Saliva


Blood samples can be difficult to obtain in studies involving serial sampling, especially in developing countries where there may also be logistic, ethical, and cultural constraints that make frequent blood collection impractical. Assays for steroids in saliva may avoid some of these difficulties. A multicenter study involving laboratories in five countries was carried out to compare the results of assays for salivary estradiol and progesterone performed with centrally provided reagents and assay protocols. Concentrations of salivary steroid as obtained by all but one center were comparable with those reported in the literature. We conclude that assays of hormones in saliva are useful adjuncts to those performed on other body fluids.

Additional Keyphrases: steroid hormones • interlaboratory performance • fertility assessment

Studies on the efficacy and safety of fertility-regulating agents on populations in developing countries can sometimes be hindered by lack of access to clinical laboratories capable of hormone analysis. Partly for this reason has The Special Programme of Research in Human Reproduction of the World Health Organisation established programs for strengthening the capabilities of institutions in such countries to perform research; these programs include providing laboratories with well-characterized immunoassay reagents and protocols (1, 2). However, little is yet known about the endocrinological status of subjects in developing countries, particularly those from rural populations of low economic status. In part this may be attributed to problems associated with conventional sampling regimes—access to staff able to perform venepuncture and a supply of sterile needles are required—and cultural and ethical problems associated with blood collection might also restrict sampling. Because assays for steroids in saliva have been useful for monitoring ovarian function (3–5) and because saliva donation is socially acceptable in many cultures, it was decided to assess whether simple, rapid assays for estradiol and progesterone in saliva (6) would be of value in multicenter clinical studies, especially those involving developing countries.

The following scientists and institutions took part in this study: Dr. C. Romero, University of Chile, Santiago, Chile; Dr. Wang Han Zheng, Shanghai Institute of Planned Parenthood Research, Shanghai, China; Dr. D. Hazra, S.N. Medical College, Agra, India; Dr. H. Goh, University of Singapore, Singapore; and Dr. S. Chearskul, Mahidol Hospital Medical College, Bangkok, Thailand. Four other centers agreed to participate but failed to return data before the study had been concluded.

Materials and Methods

Samples. Detailed protocols on subject selection and sample collection were sent to all participants. Each center was asked to collect daily samples of saliva, beginning with the second day of menstrual bleeding, from six subjects who were not receiving medication and who had regular menstrual cycles. Subjects were asked to collect 3- to 4-mL specimens of saliva approximately 5 min after rinsing their mouths with water. The time of sample collection, recorded by each subject, was always between 15:00 and 17:00 hours. Specimens for cycles were collected at the volunteer’s home and stored in domestic refrigerators before transfer to the laboratory. At the laboratory saliva samples were liquefied by freezing and thawing, and particulate matter was removed by centrifugation before storage at −20°C or assay.

Assays. The assays used (6) were rapid, did not involve extraction, and required 0.5-mL aliquots of saliva for analysis; [125]labeled circulating derivatives were used as tracers. Reagents for this study, obtained from the Swiss Federal Reactor Institute, Wurenlingen, were sent to each participant, together with detailed assay protocols. Quality-control samples were centrally provided to facilitate the comparison of data obtained from different centers as well as for assessment of within- and between-batch quality control of assays performed at each center. At only one center had a staff member had previous experience with these assays.

Data analysis. Raw data were reported by the participants, but data analysis was centrally performed with use of a standardized RIA data analysis and quality control program (7). Assays for which original data were not available were excluded from analysis.

Menstrual cycles were normalized such that day 0 was set as being one day after the concentration of salivary estradiol peaked. Data from the different stages of the cycle were then combined by calculating means and standard errors of the means for follicular, periovulatory, and luteal phases for each center. The quality-control results for the different centers were compared, and the within-laboratory, between-batch and between laboratory, between-batch coefficients of variation (CVs) were calculated.

Results

Acceptability of saliva sampling. None of the laboratories reported problems in recruiting volunteers for this study. Sample collection was considered to be simple, and presented no practical or ethical problems. Specimens were frequently collected and stored at home before being taken to the laboratory. Two centers reported that some saliva samples had apparently deteriorated; they had an unpleasant odor and laboratory staff found them distasteful to handle.

Logistic problems. Shipping reagents to geographically scattered parts of the world presents problems. Several centers did not receive the reagents, and two received them at erratic intervals, making it difficult for them to complete the study.

Assay performance. All but one center found the assays simple to perform. The between-batch reproducibility of results from the four centers that assayed the centrally provided quality control samples is shown in Table 1.

Between-center comparability is shown in Table 2. In both cases no correction has been made for the fact that replicate
Table 1. Between-Batch Reproducibility of Quality-Control Data for Individual Centers

<table>
<thead>
<tr>
<th>Center</th>
<th>Mean (pmol/L)</th>
<th>%CV</th>
<th>n</th>
<th>Mean (pmol/L)</th>
<th>%CV</th>
<th>n</th>
<th>Mean (pmol/L)</th>
<th>%CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>1.2</td>
<td>20</td>
<td>4</td>
<td>3.5</td>
<td>15</td>
<td>10</td>
<td>10.8</td>
<td>9.6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.7</td>
<td>—</td>
<td>3.0</td>
<td>23</td>
<td>9</td>
<td>8.1</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.4</td>
<td>20</td>
<td>5</td>
<td>5.3</td>
<td>17</td>
<td>6</td>
<td>9.0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10.6</td>
<td>11</td>
<td>3</td>
<td>25.5</td>
<td>20</td>
<td>9</td>
<td>42.8</td>
<td>10</td>
</tr>
<tr>
<td>Progesterone</td>
<td>1</td>
<td>414</td>
<td>24</td>
<td>10</td>
<td>705</td>
<td>21</td>
<td>11</td>
<td>1402</td>
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<td>578</td>
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<td>1389</td>
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<tr>
<td></td>
<td>3</td>
<td>512</td>
<td>18</td>
<td>8</td>
<td>852</td>
<td>14</td>
<td>8</td>
<td>1491</td>
<td>9.8</td>
</tr>
<tr>
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<td>49</td>
<td>6</td>
<td>832</td>
<td>12</td>
<td>12</td>
<td>1469</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 2. Summary of Quality-Control Data for All Centers

<table>
<thead>
<tr>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (pmol/L)</td>
<td>%CV (n)</td>
<td>Mean (pmol/L)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1.1</td>
<td>17 (3)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>364</td>
<td>27 (4)</td>
</tr>
</tbody>
</table>

determinations were made for all quality-control pools. Three centers did not provide data on quality-control samples.

The concentrations of estradiol and progesterone in saliva during the follicular, periovulatory, and luteal phases of the menstrual cycle, as measured by each center, are shown in Figure 1. Center 4 measured higher concentrations of estradiol and equivalently higher results for quality-control specimens than did the other centers. Because data for the assay standard curves from this center also indicated that an analytical error had been made, we multiplied the results from this center by a factor based on the ratio between their mean result for the quality-control samples and the consensus value for that quality-control pool.

Discussion

All participants considered that assays on an easily sampled body fluid such as saliva had many advantages over measurements in blood; however, even though many cultures consider saliva collection to be socially acceptable (8), laboratory staff sometimes objected to handling specimens, particularly those not stored frozen.

Between-batch and between-laboratory comparabilities were poorer than those generally found for equivalent assays in serum but were comparable with those reported for salivary assays performed in other centers (5, 9). Assay performance was considered acceptable in view of the very low concentrations of analyte being measured and the fact that the laboratories had not performed these assays before the start of the study.

The concentrations of estradiol in saliva throughout the menstrual cycle were similar to those previously reported (3, 5, 6) and were similar among all centers except center 3. Likewise progesterone concentrations were also similar to those reported in the literature (5, 6, 9), except for centers 3 and 4. Correspondence with laboratory 4 revealed that four of the six subjects in the study were volunteers from an infertility clinic and had originally been recruited for a study on luteal-phase dysfunction. The grossly increased concentrations of both estradiol and progesterone in subjects from center 3 are more difficult to explain. The concentrations of steroids in saliva are 0.5–2% as great as those found in plasma, and small abrasions in the mouth could contaminate saliva with microliter amounts of blood or gingival fluid, which would be enough to significantly increase the measured estradiol and progesterone concentrations. Dietary habits or oral infections could also possibly affect concentrations of salivary steroids, although studies (11) indicate that chronic gingival inflammation is unlikely to result in metabolism of salivary estradiol or progesterone.

Despite the fact that the cause of this apparent population difference has not yet been identified, estradiol and progesterone assays in saliva are clearly useful analytical tools, particularly for studies involving examination of endocrine status over a period of time. Assays for these steroids in saliva have already been shown to be useful in monitoring subjects with unexplained infertility, and for subjects being considered for in vitro fertilization or artificial insemination (5, 6, 11). This study confirms that these assays could also be effective in studying endocrinological changes in population groups from whom regular blood samples would be difficult or impossible to obtain, as well as in projects concerned with the safety and efficacy of fertility-regulating agents in developing countries.

This study received financial support from the Special Programme for Research in Human Reproduction of the World Health Organization. Reagents for estradiol and progesterone assays were generously donated by the Swiss Federal Reactor Institute, EIR, Wurenlingen, Switzerland.

References

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Simple, Rapid Determination of Serum Guanase Activity with the Hitachi 736 Automated Discrete Analyzer

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In this new method for determining serum guanase activity by use of the Hitachi 736-40 automated analyzer, serum is incubated with a mixture of xanthine oxidase, superoxide dismutase, and catalase; a reagent containing KCN, guanine, nitrotetrazolium blue, and Triton X-100 is added; and the increase in absorbance at 570 and 660 nm is measured for 2.4 min. Only 20 μL of sample is required, and results are linearly related to the activity concentration of guanase up to 30 U/L. Within-run and day-to-day precision (CV) was respectively 2.6 to 4.2% and 3.5 to 5.5% over 0–30 U of guanase activity per liter. The normal reference interval, as calculated from data on 40 healthy persons, is 0.1 to 2.2 U/L. Results correlate well (r = 0.997) with those by a kinetic method (Clin Chem 27: 560, 1981). The guanase activity of 150 samples can be measured within 1 h by this method.

Additional Keyphrases: enzyme activity • enzymic methods • reference interval • assessing shock • liver disease • kidney disease

High activities of guanase (EC 3.5.4.3) are found in the liver, kidneys, and brain, but little or no activity in other organs (1, 2). We previously demonstrated (3) that guanase activity in serum increases markedly in patients suffering from shock and that the mean half-life of guanase activity in survivors from shock is significantly shorter than that in non-survivors. Thus, measuring serum guanase may be a useful indicator of survival in cases of severe shock. Moreover, guanase activity in serum increases in various liver diseases—markedly in patients with acute hepatitis, and moderately in those with chronic hepatitis, liver cirrhosis, and hepatoma (4)—and increases slightly in renal disease (5).

Among the many methods used for assaying guanase, Roush and Norris (6) measured the decrease in absorbance at 245 nm. Other methods are based on the products of guanase action such as NH₃, which can be measured by the reaction of Berthelot (7, 8). Ellis et al. (9) reported an automated method for guanase activity in which NH₃ is measured enzymically with an automated analyzer. We have reported (10) an enzymic, one-step kinetic method for use with a centrifugal analyzer, in which xanthine production from guanine was coupled to production of NADH by use of xanthine oxidase (EC 1.2.3.2) with ethanol, catalase (EC 1.11.1.6), and aldehyde dehydrogenase (EC 1.2.1.3).

Here we report a simple, rapid method and compare it with our previous method (10). The present method is based on measuring the superoxide anion (11) produced by guanase in the xanthine–xanthine oxidase system (Figure 1).

Materials and Methods

Materials

Guanine and human albumin were from Sigma Chemical Co., St. Louis, MO. Catalase (1.3 × 10⁶ kU/L, from beef liver) and xanthine oxidase (4 kU/L, from cows’ milk) were from Boehringer, Mannheim, F.R.G. Superoxide dismutase (EC 1.15.1.1) was from Toyobo Co., Ltd., Osaka. Triton X-100, KCN, KH₂PO₄, and K₂HPO₄ were from Wako Pure Chemical Industries, Ltd., Osaka. Nitrotetrazolium blue was from Dojindo Laboratories, Kumamoto; triethylaminoethyl cellulose ("Cellex T") was from Bio-Rad Laboratories.