those reported by other researchers using enzymatic methods, e.g., a within-run CV (n = 11) of 4.4% at 80 µmol/L (10) and a between-run CV (n = 12) of 10.4% at 75 µmol/L (7).

Analytical recovery was examined using plasma specimens (160 µL each) from a reference pool containing 30.6 µmol/L mannose. Samples were supplemented with 40 µL of water, 500 µmol/L mannose, or 1 mmol/L mannose. Each supplementation was performed in triplicate and yielded recoveries of 104.0% for the lower-concentration mannose samples and 102.6% for the higher-concentration samples.

We studied potential interference from endogenous substances using a plasma pool (mannose concentration, 79 µmol/L) obtained from healthy volunteers. A one-tenth volume of the solution containing a possible interfering was added to the plasma. Glucose at 50 mmol/L, fructose at 200 µmol/L, free plus conjugated bilirubin at 340 µmol/L, hemoglobin at 5.0 g/L, uric acid at 600 µmol/L, and ascorbic acid at 20 mmol/L did not affect the measured mannose values.

The mean plasma mannose concentration of 15 specimens from healthy individuals (10 from females and 5 from males), collected in the morning after fasting, was 35.6 ± 12.6 µmol/L. The mean glucose concentration of the same plasma samples was 4.64 ± 0.87 mmol/L. The observed mannose value was comparable to those reported by other investigators: e.g., 39 ± 12 µmol/L (n = 14) (6); 18.5 ± 5.5 µmol/L (n = 6) (7); 53.8 ± 9.3 µmol/L (n = 23) (9); 54.1 ± 11.9 µmol/L (n = 11) (10).

Both mannose and glucose concentrations in plasma samples (n = 21) from nondiabetic and diabetic patients were measured. A close positive correlation (r = 0.779; P < 0.01) was observed between mannose and glucose concentrations. Our data are consistent with those reported previously (6, 7). We also measured the mannose and glucose concentrations in plasma samples collected at 0, 30, 60, and 120 min after oral administration of 75 g of glucose to healthy, 14-h fasted volunteers (two females and three males). The plasma mannose concentration gradually decreased until at least 120 min after glucose ingestion, whereas the glucose concentration increased transiently, as is widely known (Table 1). To the best of our knowledge, this is the first report of the time course of changes in the plasma mannose concentration under physiologic conditions. We are now studying the mechanism of the change in the plasma mannose concentration induced by glucose load.

This study was supported by a grant-in-aid (to I.M.) for the High-Tech Research Center Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by a grant-in-aid (to I.M. and T.T.) for the Scientific Frontier Research Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

### Table 1. Plasma concentrations of glucose and mannose in healthy adults after oral ingestion of 75 g of glucose.

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>4.69 ± 0.38</td>
<td>5.72 ± 0.32a</td>
<td>3.98 ± 0.55</td>
<td>3.54 ± 0.47</td>
</tr>
<tr>
<td>Mannose, µmol/L</td>
<td>48.0 ± 7.5</td>
<td>37.1 ± 4.2a</td>
<td>26.0 ± 6.3a</td>
<td>21.8 ± 4.4b</td>
</tr>
</tbody>
</table>

*Significantly different from mean at time 0: a P < 0.05; b P < 0.005.

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**References**


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**Use of the Architect-i2000 Estradiol Immunoassay during In Vitro Fertilization, Joëlle Taieb, Clarisse Benattar, Rokhaya Diop, Anne Sophie Birr, and Albert Lindenbaum (Hôpital Antoine Béclère, Clamart, France, Department of Biochemistry and Hormonology, 157 rue de la porte de Trivaux, 92141 Clamart cedex, France; *author for correspondence: fax 33-1-45374745, e-mail joelle.taieb@abc.ap-hop-paris.fr)**

In women undergoing in vitro fertilization and embryo transfer (IVF-ET), serial measurements of 17ß-estradiol (E2) can be used to monitor follicular growth, and serum
E₂ has been shown to be correlated with the number and diameter of preovulatory follicles observed on transvaginal ultrasound scan (1–6). The serum E₂ concentration is therefore an essential variable for evaluating the progression of stimulation, adjusting daily gonadotropin therapy, predicting the optimal day for induction of ovulation [administration of human chorionic gonadotropin (hCG)] (7), preventing ovarian hyperstimulation syndrome (8, 9), and ensuring that pituitary function is adequately suppressed if a long-acting gonadotropin-releasing hormone agonist (GnRHa) is used before stimulation (10). Another major use of E₂ measurements is to evaluate ovarian function at day 3 of the menstrual cycle to determine the prognosis of IVF-ET (11).

Because results must be available within a few hours, rapid and automated assays are required. In this study, we measured E₂ with a new automated assay that could be used in random, continuous access mode and assessed its usefulness for monitoring ovulation stimulation for IVF-ET.

The Architect-i2000 E₂ (Abbott Laboratories) assay is a competitive two-step immunoassay based on chemiluminescent microparticle immunoassay technology and can be performed in 29 min. The ACS-180 E₂ assay (Bayer Diagnostics) is a competitive one-step immunoassay based on solid-phase antigen-linked technology and chemiluminescence detection and can be completed in 15 min. The luminescence reaction and the calibration procedure are identical for the two E₂ immunoassays. The two methods are linear up to 3670 pmol/L and use monoclonal antibody derived by coupling the E₂ molecule at the specificity-enhancing sixth position.

The within- and between-run imprecision, detection limits, and functional sensitivities of these assays have been reported previously (12–15). The two methods were compared for 190 serum samples, and their agreement was assessed by the method of Bland and Altman (16). The specificity of each assay was evaluated by assaying 14 and 10 sera from patients receiving 2 mg of micronized 17β-E₂ or E₂ valerate, respectively. E₂ concentrations were determined for 166 serum samples from 25 patients undergoing ovarian stimulation. All patients were treated with a single injection of GnRHa to abolish the activity of gonadotropin hormones during the 2 weeks preceding exogenous gonadotropin administration. In all cases, ovulation was stimulated with recombinant follicle-stimulating hormone (FSHr). All patients received an initial dose of 225 IU FSHr/day during the first 5 days. Subsequent doses of FSHr and the timing of hCG administration were determined according to the usual criteria for follicular maturation (serum E₂ concentrations and transvaginal ultrasound). All sera were assayed simultaneously by the two methods, with single determinations as recommended by the manufacturers. Investigating physicians were blind to the results of Architect-i2000 for monitoring stimulation of ovulation. We also retrospectively analyzed and compared the results obtained with the two methods for 80 sera obtained on day 3 of the menstrual cycle from women selected for the oocyte donation program at our IVF center (11).

The Architect-i2000 E₂ assay was linear within the calibration range. The regression equation was (SD given in parentheses): \( y = 0.988(0.019)x \) (expected) − 11.3(22.6) pmol/L; \( r = 0.999 \).

Linear regression analysis for 190 serum samples with E₂ concentrations of 0–16 500 pmol/L yielded: Architect-i2000 = 1.16(0.013) ACS-180 − 29(92) pmol/L; \( r = 0.988 \). For concentrations of 0–367 pmol/L (n = 58) and 367–16 500 pmol/L (n = 132), we obtained the following equations, respectively: Architect-i2000 = 0.94(0.072) ACS-180 + 62 (3) pmol/L (r = 0.870); and Architect-i2000 = 1.17 (0.018) ACS-180 − 79 (97) pmol/L (r = 0.985). The Architect-i2000 E₂ assay gave higher estimates of E₂ concentration than did ACS-180 over this concentration range (slopes >1 and intercept at +62 pmol/L for high and low E₂ concentrations, respectively). The differences between the results obtained with the two methods were statistically significant (\( P < 0.001 \)) in the nonparametric Wilcoxon matched-pairs signed-rank test. Because the ACS-180 method is not a reference method, we analyzed the results by the method of Bland and Altman (16), taking into account the imprecision of the two methods. The results of this analysis are shown in Fig. 1. A marked positive difference was observed overall for this range of concentrations. This difference was of consequence only in the low concentration range.

The values obtained for serum E₂ depend on the method used (17–20). Various factors may account for the differences between the two methods (21). These factors include differences in calibration curves, antibody specificity, the matrix effect (22–24), and the mathematical relationship permitting the conversion of the signal obtained into E₂ concentration in the system used.

The results obtained with the two E₂ immunoassays for specimens from patients receiving micronized 17β-E₂ or E₂ valerate (25) did not differ significantly (\( P = 0.55 \) and 0.06, respectively, in the nonparametric Wilcoxon test).

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**Fig. 1.** Plot of differences (E₂ Architect-i2000 − E₂ ACS-180) vs the mean concentration measured by the two methods for each sample.
However, all of the values obtained with Architect-i2000 for patients treated with E2 valerate were ~10% higher than those obtained with ACS-180, which corresponded to the cross-reaction of 6% obtained in vitro by the manufacturer.

Before beginning ovarian stimulation, it is necessary to check that down-regulation has been successful to prevent the stimulation of apoptotic follicles. The E2 concentration must be <150 pmol/L by RIA (26) and <184 pmol/L with the ACS-180 system (unpublished data). In 25 samples from women treated with GnRHa for 2 weeks, E2 concentrations obtained with the ACS-180 method were in all cases <184 pmol/L. The mean E2 concentration obtained with the Architect-i2000 assay was 132 pmol/L (range, <169–224 pmol/L). Nineteen samples gave values <169 pmol/L, corresponding to the cutoff for functional sensitivity. Six samples gave E2 concentrations slightly higher than 184 pmol/L (187–224 pmol/L). Thus, the cutoff point for ovarian down-regulation with the Architect-i2000 assay should be set at 225 pmol/L.

For 128 sera from 25 patients undergoing ovarian stimulation (mean of 5.1 measurements per patient), the mean increase in E2 concentration, mean final E2 concentration before hCG administration, mean number of mature follicles (with a diameter >15 mm, as measured by transvaginal ultrasound), and the relationship between E2 concentration and the number of mature follicles are presented in Table 1. The two E2 immunoassays gave similar results. The mean (SD) increase in E2 was 1601 (828) pmol/L for the ACS-180 and 1680 (833) pmol/L for the Architect-i2000 during the first 5 days of stimulation. Between day 6 of stimulation and the day on which hCG was administered, the mean increase in E2 concentration in the ACS-180 assay was 2026 (928) pmol/L from day 6 to day 8, 4030 (2257) pmol/L from day 8 to day 10, and 3997 (2015) pmol/L from day 10 to the day of hCG administration. The mean increase in E2 concentration in the Architect-i2000 assay was 2345 (1119) pmol/L from day 6 to day 8, 4734 (3170) pmol/L from day 8 to day 10, and 4819 (2932) pmol/L from day 10 to the day of hCG administration. The mean final E2 concentrations were 10 335 (2917) pmol/L for the ACS-180 and 12 111 (3853) pmol/L for the Architect-i2000. The number of follicles per patient was 17.2 (range, 10–30), with a mean of 10.7 (range, 5–18) mature follicles. We obtained a value of 965 pmol E2/mature follicle for the ACS-180 and 1132 pmol E2/mature follicle for the Architect-i2000. The results obtained with E2 Architect-i2000 fell within the expected range of values for the amount of E2 per mature follicle.

For 80 sera obtained on day 3 of the menstrual cycle from women donating oocytes at our IVF center, the mean E2 concentration obtained with the ACS-180 was 142 pmol/L (range, <110–337 pmol/L). Eight patients had E2 concentrations >220 pmol/L, corresponding to our cutoff point for the selection of patients for inclusion in the oocyte donation program. The mean E2 concentration obtained with the Architect-i2000 was 334 pmol/L (range, <169–572 pmol/L). The relationship between the results obtained with the two methods may be expressed as: Architect-i2000 = 1.025 ACS-180 + 187 pmol/L. Because of the positive difference, the cutoff point for the day 3 concentration limit (<220 pmol/L) for ACS-180 should be increased to 400 pmol/L for the Architect-i2000. We checked for agreement between the results obtained with the two assays after implementing this adjustment by calculating the $\kappa$ coefficient; a good agreement between the results of the two tests was observed ($\kappa = 0.6875$) (27).

In conclusion, E2 measurements with the automated Architect-i2000 system could be used to monitor ovulation in combination with transvaginal ultrasound. Although the mean E2 values obtained per mature follicle were slightly higher than with the ACS-180, the results obtained with the Architect-i2000 system fell into the range generally expected. As shown previously (28), the functional sensitivity of this method is insufficient for the evaluation of E2 in sera from children, men, or menopausal women. This assay has been optimized for clinical applications in which high concentrations are expected. It could be used for determinations in sera from women at the beginning of the menstrual cycle, to evaluate the functional status of the ovaries, and to evaluate down-regulation before ovarian stimulation. However, such applications require the upward modification of clinical cutoff points. Until assays are better standardized, clinical decision criteria (reference ranges, cutoff points) must be

| Table 1. Mean increase in E2 concentration during 25 cycles of ovarian induction, mean final E2 concentration, mean number of mature follicles, and relationship between E2 concentration and the number of mature follicles for the E2 Architect-i2000 and E2 ACS-180 immunoassays. |
|-----------------|-----------------|
| ACS-180          | Architect-i2000 |
| Mean (SD) increase in E2 concentration, pmol/L* |
| From day 1 to day 6 | 1601 (828)       | 1680 (833)       |
| From day 6 to day 8 | 2026 (928)       | 2345 (1119)      |
| From day 8 to day 10| 4030 (2257)      | 4734 (3170)      |
| From day 10 to day of hCG administration | 3997 (2015) | 4819 (2932) |
| Mean (SD) final E2 concentration, pmol/L | 10 335 (2917) | 12 111 (3853) |
| Mean number of mature follicles (diameter >15 mm) | 10.7 (range, 5–18) |
| Mean amount of E2 (pmol) per mature follicle | 965 | 1132 |

*1 pmol/L = 3.67 ng/mL.
evaluated and, if necessary, modified for each new assay. Collaboration between laboratories and physicians is essential in the setting up of new immunoassays.

Abbott Laboratories provided assay reagent and the assay system without charge.

References
11. Fast Colorimetric Method for Measuring Urinary Iodine, Daniella Gnat,1 Ann D. Dunn,2 Samar Chaker,3 Francois Delange,2,3 Francoise Vertongen, and John T. Dunn2,3* (1 Centre Hospitalier Universitaire St. Pierre, Free University of Brussels, 1000 Brussels, Belgium; 2 Division of Endocrinology, Department of Medicine, University of Virginia, Charlottesville, VA 22908; 3 International Council for the Control of Iodine Deficiency Disorders, Box 801416, University of Virginia Health System, Charlottesville, VA 22908; * author for correspondence: fax 434-243-9195, e-mail jtd@virginia.edu)

International groups recommend the following median urinary iodine concentration as the best single indicator of iodine nutrition in populations: severe deficiency, 0–0.15 \( \mu \text{mol/L} \) (0–19 \( \mu \text{g/L} \)); moderate deficiency, 0.16–0.38 \( \mu \text{mol/L} \) (20–49 \( \mu \text{g/L} \)); mild deficiency, 0.40–0.78 \( \mu \text{mol/L} \) (50–99 \( \mu \text{g/L} \)); optimal iodine nutrition, 0.79–1.56 \( \mu \text{mol/L} \) (100–199 \( \mu \text{g/L} \)); more than adequate iodine intake, 1.57–2.36 \( \mu \text{mol/L} \) (200–299 \( \mu \text{g/L} \)); and excessive iodine intake, \( \geq 2.37 \mu \text{mol/L} \) (\( \geq 300 \mu \text{g/L} \)). The range in which the median falls is more important than the precise number (2, 3).

Many methods for assessing urinary iodine exist (3–8), most based on the Sandell–Kółthoff reaction (9), in which iodide catalyzes the reduction of ceric ammonium sulfate (yellow) to the colorless ceros form in the presence of arsenic acid. Although iodide is the chemical form for both the catalytic reaction and in urine, some preliminary treatment is needed to rid urine of impurities, most commonly by acid digestion (3, 5). We have extended previous approaches (5, 6, 10) with improved conditions and here present a new method (“Fast B”) that is rapid, inexpensive, reliable, and flexible.

The equipment required for the Fast B method includes a heating block, Pyrex test tubes (13 \( \times \) 100 mm), two fixed-volume pipettes (0.5 mL and 1.0 mL), one adjustable pipette (0–200 mL), and a multipipet (Eppendorf) for quick reagent volume additions of 0.125 and 0.1 mL. The basic chemicals used are potassium iodate, arsenic trioxide, ammonium persulfate, ammonium cerium(IV) sulfate dihydrate, sodium chloride, ferroine, and sulfuric acid.

The solutions used in the assay are as follows:

(a) Ammonium persulfate solution: 114.0 g of ammonium persulfate made up to 500 mL with water (stable for at least 1 month at 20–25 °C away from light)
(b) 2.5 mol/L \( \text{H}_2\text{SO}_4 \)