

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.

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Fast Colorimetric Method for Measuring Urinary Iodine, Daniella Gnat,¹ Ann D. Dunn,² Samar Chaker,¹ Francois Delange,^{1,3} Françoise Vertongen,¹ and John T. Dunn^{2,3*} (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, $\geq 2.37 \mu\text{mol/L}$ ($\geq 300 \mu\text{g/L}$) (1). 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–Kolthoff reaction (9), in which iodide catalyzes the reduction of ceric ammonium sulfate (yellow) to the colorless cerous form in the presence of arsenious 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 μL), and a multipipette (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, ferroin, and sulfuric acid.

The solutions used in the assay are as follows:

- 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 $^{\circ}\text{C}$ away from light)
- 2.5 mol/L H_2SO_4

- (c) Arsenious acid solution: 10 g of As_2O_3 , 50 g of NaCl, 400 mL of 2.5 mol/L H_2SO_4 , and 600 mL water; heated gently to dissolve, diluted to a final volume of 2 L, filtered, and stored in a dark bottle away from light at 20–30 °C (stable for at least 6 months)
- (d) Sodium chloride: 40 g in 200 mL of water
- (e) 10.8 mol/L H_2SO_4
- (f) Ceric ammonium sulfate: 16 g in 1 L of 1.35 mol/L H_2SO_4 (stable for more than 6 months in a dark bottle)
- (g) Iodine calibrators: working solutions of 0.40 $\mu\text{mol/L}$ (50 $\mu\text{g/L}$), 0.79 $\mu\text{mol/L}$ (100 $\mu\text{g/L}$), and 2.37 $\mu\text{mol/L}$ (300 $\mu\text{g/L}$), prepared from concentrated iodate solution [788 $\mu\text{mol/L}$ (100 mg/L)], made by dissolving 168.5 mg of potassium iodate in 1 L of water. Working solutions of other concentrations can be prepared as needed
- (h) Ferriorene/arsenious acid solution: prepared shortly before use by mixing 2 mL of 10.8 mol/L H_2SO_4 , 2 mL of arsenious acid solution, 4 mL of 200 g/L sodium chloride, and 2 mL of ferriorene

We obtained fresh samples from healthy individuals and hospitalized patients in Brussels and frozen samples from epidemiologic studies in Europe and Africa. The urine samples were not treated with acid, but thymol crystals had been added to some of the samples in their country of origin before transfer to the laboratory. Results were compared with those obtained with the Technicon AutoAnalyzer II (Bayer/Technicon Instruments) (11) in use in our Brussels laboratory for more than 20 years and periodically subjected to routine external quality control.

We investigated several conditions to improve the previously described method (10), including use of ammonium persulfate in place of the more toxic chloric acid, a longer time for color development, and smaller sample volumes.

The final procedure developed is as follows. Each tube, containing 0.15 mL of urine or of calibrator and 1.0 mL of ammonium persulfate solution, is heated for 1 h in the block at 100 °C. After the solution is cooled at room temperature, 0.5 mL of arsenious acid solution is added to each tube and mixed on a vortex-mixer. At least 15 min later, 0.125 mL of fresh ferriorene–arsenious acid solution is added. Tubes are mixed on a vortex-mixer and ranged in racks as follows: three calibrators [0.40 $\mu\text{mol/L}$ (50 $\mu\text{g/L}$), 0.79 $\mu\text{mol/L}$ (100 $\mu\text{g/L}$), and 2.37 $\mu\text{mol/L}$ (300 $\mu\text{g/L}$)], followed by the urine samples and controls, and at the end, a second set of the same three calibrators. Each batch contains a total of 45–55 tubes, including samples, blanks, and controls. To each tube we rapidly add 0.1 mL of ceric ammonium sulfate solution with the multipipetter, with rapid shaking of each rack, and observe all tubes closely. After an initial blue color, samples first turn purple and then orange/brown. The speed of the color change depends on the iodine concentration. As each sample turns purple, it is placed in another rack in order of color change after addition of the ceric ammonium sulfate. Thus, all tubes, including calibrators and samples, are

now ranked in order of color change. We then count the number of samples falling into each of the four categories [$>2.37 \mu\text{mol/L}$ ($>300 \mu\text{g/L}$), 0.79–2.37 $\mu\text{mol/L}$ (100–300 $\mu\text{g/L}$), 0.40–0.78 $\mu\text{mol/L}$ (50–99 $\mu\text{g/L}$), and $<0.40 \mu\text{mol/L}$ ($<50 \mu\text{g/L}$)] from the position of each tube relative to the positions of the calibrators.

When we compared the results obtained for 286 urine samples by the Fast B method with the results obtained with the AutoAnalyzer II method (Table 1), 275 (96.2%) were placed in the correct category by Fast B. Of the 11 discordant values, all were close to range cutoffs: 6 were false positives (samples with concentrations of 0.63, 0.71, 0.74, and 0.76 $\mu\text{mol/L}$ by the AutoAnalyzer II method were placed in the 0.79–2.37 $\mu\text{mol/L}$ range by the Fast B, and samples with concentrations of 2.22 and 2.24 $\mu\text{mol/L}$ were placed in the >2.37 range), and 5 were false negatives (samples with concentrations of 0.83 and 0.83 $\mu\text{mol/L}$ by the AutoAnalyzer II method were placed in the 0.40–0.78 range by the Fast B, and samples with concentrations of 2.46, 2.52, and 2.39 $\mu\text{mol/L}$ were placed in the 0.79–2.37 range).

Approximately 45 samples, including 39 unknowns, can be handled in each analytical run. The color change is readily recognized visually. Under our conditions, samples with an iodine concentration $>2.37 \mu\text{mol/L}$ ($>300 \mu\text{g/L}$) change color in <2 min, those with a concentration of 2.37 $\mu\text{mol/L}$ (300 $\mu\text{g/L}$) change color at ~ 2 min, those with a concentration of 0.79 $\mu\text{mol/L}$ (100 $\mu\text{g/L}$) change color at ~ 5 min, those with a concentration of 0.40 $\mu\text{mol/L}$ (50 $\mu\text{g/L}$) change color at ~ 10 min, and those with a concentration of 0.08 $\mu\text{mol/L}$ (10 $\mu\text{g/L}$) change color at ~ 40 min. For most purposes, it is satisfactory simply to record the number that have not changed before the 0.40 $\mu\text{mol/L}$ (50 $\mu\text{g/L}$) calibrator and not wait. We have focused on calibrators that bracket the recommended ranges for defining iodine nutrition (1). Other calibrators between 0.40 and 2.37 $\mu\text{mol/L}$ can be used to define other ranges of interest. Our experiments were conducted at a laboratory temperature of 20–25 °C. The speed of the Sandell–Kolthoff reaction is influenced by temperature and may need to be carried out at controlled temperatures in hot or cold climates (12).

From three urine samples with different iodine concentrations [0.30 $\mu\text{mol/L}$ (38 $\mu\text{g/L}$), 0.76 $\mu\text{mol/L}$ (96 $\mu\text{g/L}$), and 2.01 $\mu\text{mol/L}$ (255 $\mu\text{g/L}$), respectively, authenticated by the AutoAnalyzer], we ran 10 aliquots of each sample separately in the same run; all 30 were correctly placed in the three categories: $<0.40 \mu\text{mol/L}$ ($<50 \mu\text{g/L}$), 0.40–0.78

Table 1. Comparison of iodine concentrations in 286 urine samples measured by Fast B and AutoAnalyzer II.

Iodine concentration range, $\mu\text{mol/L}$ ($\mu\text{g/L}$)	No. of samples	
	Fast B	AutoAnalyzer
<0.40 (<50)	39	39
0.40–0.78 (50–99)	74	76
0.79–2.37 (100–300)	112	109
>2.37 (>300)	61	62

$\mu\text{mol/L}$ (50–99 $\mu\text{g/L}$), and 0.79–2.37 $\mu\text{mol/L}$ (100–300 $\mu\text{g/L}$). We also analyzed an aliquot of each of the three samples for 13 consecutive days (a total of 39 samples); 38 of the 39 (97.5%) were placed correctly, and the 39th was placed in the category immediately above the correct category.

We diluted a urine sample containing 6.3 $\mu\text{mol/L}$ iodine to give the following concentrations: 3.15, 2.10, 1.58, 1.05, 0.79, 0.63, and 0.53 $\mu\text{mol/L}$. The Fast B placed each in the correct range except the last, which was classified as $<0.40 \mu\text{mol/L}$. For comparison, the AutoAnalyzer gave respective values of >1.97 , >1.97 , 1.45, 1.03, 0.79, 0.61, and 0.54 $\mu\text{mol/L}$. We added KIO_3 to a low-iodine sample (0.35 $\mu\text{mol/L}$) to produce samples containing 0.67, 0.98, 1.30, and 1.62 $\mu\text{mol/L}$ iodine. Measurement by Fast B placed each in the correct range. For comparison, the AutoAnalyzer gave values of 0.64, 1.03, 1.34, and 1.54 $\mu\text{mol/L}$, respectively.

Ascorbic acid at concentrations of 0, 3.78, 7.96, or 15.92 mmol/L added to a sample containing 1.15 $\mu\text{mol/L}$ (146 $\mu\text{g/L}$) iodine did not change the iodine concentration measured by the AutoAnalyzer (1.14–1.15 $\mu\text{mol/L}$) or by Fast B [remaining in the 0.79–1.18 $\mu\text{mol/L}$ (100–150 $\mu\text{g/L}$) category]; for this experiment, other KIO_3 calibrators were used to create the category of 0.79–1.18 $\mu\text{mol/L}$ (100–150 $\mu\text{g/L}$).

No change in iodine concentration was detected by either the AutoAnalyzer or Fast B after the addition of potassium thiocyanate at concentrations of 0.172, 0.344, or 0.688 mmol/L or of D-glucose up to 56 mmol/L (10.14 g/L).

The placement of values within the ranges described here satisfies most epidemiologic purposes (1) and is more cost-effective than analyzing and reporting individual samples. One technician can easily measure 200 samples in a working day, and depending on salaries, the cost may be less than US \$0.10/sample. One of us (D.G.) trained two technicians from a developing country in African to be proficient in the method after 3 days of instruction and practice. The investment in equipment is low, and except for pipettes, the only instrument is the heating block, which might be replaced by a boiling water bath if necessary.

In conclusion, the Fast B method described here is rapid, simple, reliable, flexible, and inexpensive and provides an attractive means for assessing iodine nutrition in populations, especially in developing countries.

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Effects of Midstream Collection and the Menstrual Cycle on Urine Particles and Dipstick Urinalysis among Healthy Females, Mie Morimoto,^{1*} Hidekatsu Yanai,² Kenichi Shukuya,³ Hitoshi Chiba,² Kunihiko Kobayashi,⁴ and Kazuhiko Matsuno¹ (¹ College of Medical Technology, Hokkaido University, North-12, West-5, Sapporo 060-0812, Japan; Departments of ² Laboratory Medicine and ⁴ Pediatrics, Hokkaido University School of Medicine, North-14, West-5, Sapporo 060-8648, Japan; ³ Department of Laboratory Medicine, Kyorin University School of Medicine, 6-20-2, Shinkawa, Mitaka-shi, Tokyo 181-8611, Japan; * author for correspondence: fax 81-11-706-4916, e-mail mie@cme.hokudai.ac.jp)

For urinalysis, midstream collection is recommended (1–3). Health-associated reference limits for leukocyte and erythrocyte counts in female urine are important for detecting hematuria, pyuria, and urinary tract infection (3). To understand the effects of urinary collection and the menstrual cycle on urinalysis, we examined first-stream and midstream urine samples from healthy female students with use of an automated dipstick reader and the fully automated urine cell analyzer, UF-100.

Specimens were obtained from 64 healthy female students (age range, 18–20 years) at the College of Medical Technology. All were asymptomatic and had no extant urologic disease. They were instructed to collect only first and midstream urine samples in sterile containers at the same time and not to wipe or spread the labia. The volume of the first urine was measured, and the specimen was analyzed within 2 h. The students provided written, informed consent to participate in the study as well as information about their menstrual cycles. Specimens were classified into four groups according to the number of days after menstruation as follows: menstrual (1–7 days