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# Letters to the Editor

## Comparison of Citrate-Fluoride-EDTA with Fluoride-EDTA Additives to Stabilize Plasma Glucose Measurements in Women Being Screened during Pregnancy with an Oral Glucose Tolerance Test: A Prospective Observational Study

### *To the Editor:*

We recently highlighted the importance of implementing recommended preanalytical standards to avoid missing the diagnosis of gestational diabetes mellitus (GDM)[1] *(1)*. The placement of samples on an ice slurry with separation within 30 min, however, is not always practical. We therefore carried out a prospective observational study to compare the incidence of GDM and mean glucose concentrations after stabilizing maternal glucose samples with a citrate-fluoride-EDTA (CFE) tube with the incidence after stabilization with fluoride-EDTA (FE) tubes. Both index tests were benchmarked against the ideal reference *(2)*.

Selectively screened women were recruited with consent when they presented for a routine 24–32 weeks oral glucose tolerance test (OGTT). Exclusion criteria were multiple pregnancy, age <18 years, and inability to understand English.

The primary outcome was any increased glucose concentration diagnostic for GDM at the time of a 75-g OGTT using the IADPG criteria *(3)*. We included 121 women with tripled samples to determine the effect of sample handling on the incidence of GDM. ANOVA and McNemar's test were used to determine statistical significance.

We obtained 3 venous samples at each time point of the OGTT. In the Perinatal Centre, 2.7 mL of venous blood was collected into an FE tube. This reference sample was managed according to the recommended standards to stabilize glucose and placed in an ice water slurry with cell separation and analysis within 30 min *(2)*. The second sample was collected into a tube containing a citrate and fluoride EDTA preparation (CFE). Because the preservative is liquid, the dilution effect of the preparation is 1.16. To avoid incorrect measurements tubes must be filled to the fill line to achieve the correct mixing ratio. The third sample was collected into a second FE tube. Both the second and third samples were handled according to the customary hospital practice (index tests). Timing of sampling and analysis were carefully recorded.

All samples were analyzed using an automated enzymatic (hexokinase) method on the main laboratory analyzer (Beckman Coulter AU640). Quality control was run 3–5 times daily and was acceptable. National Academy of Clinical Biochemistry (NACB) preanalytical standards to prevent glycolysis were implemented under close supervision.

Of 121 women, the mean age was 32 ± 5.7 years, the mean (SD) body mass index (BMI) was 27.1 (5.4) kg/m$^2$, 36.4% (n = 44) were nulliparous, and 27.2% (n = 33) were obese. All women had at least one risk factor for GDM. The incidence of GDM for the reference FE, CFE, and customary FE samples was 41.3% (n = 50), 35.3% (n = 43), and 17.4% (n = 21), respectively ($P < 0.01$ for CFE and $P < 0.001$ customary FE compared with reference FE). The mean fasting and 1- and 2-hour results of the index samples stabilized with CFE and FE tubes under customary conditions are shown in Table 1, compared against the results from samples stabilized with FE and handled under the reference conditions. In 23% (n = 28) of women, there was a positive bias in the citrate sample of 0.1–0.2 mmol/L, without miscategorization of cases. It is possible that the reference sample may have allowed some glycolysis to occur despite strict implementation of the NACB recommendations.

The phlebotomy–analysis interval for the fasting and 1- and 2-hour samples was 20 ± 9, 17 ± 8, and 17 ± 9 min under the reference NACB iced-FE conditions compared with 163 20, 95± 20 and 32 ± 20 min with the index tests (CFE and FE stabilizers) (all $P <$ 0.001). All reference iced-FE conditions samples were centrifuged and analyzed within 30 min.

Assuming the diagnostic sensitivity, specificity, positive and negative predictive values, and accuracy of the reference FE test were all 100%, for the customary FE test they were 42.0%, 100%, 100%, 71%, and 76%, respectively, and for the CFE sample 86%, 100%, 100%, 91.0%, and 94.2% respectively. Most notable was that compared with stabilization with FE alone, stabilization with CFE doubled the diagnostic sensitivity of the test from 42.0% to 86.0% ($P <$ 0.0001), and improved accuracy of the test from 76% to 94.2% ($P <$ 0.0001).

The use of CFE stabilizer is practical in different clinical settings and may reduce underdiagnosis of GDM. We have been unable to identify a larger cohort using the IADPSG diagnostic criteria that evaluated the use of citrated tubes. Misdiagnosis due to a false-negative OGTT may potentially have serious clinical consequences.

Previous studies of glucose stabilization in volunteers have concluded that citrate is a superior glucose sample stabilizer to fluoride *(4, 5)*. In the absence of an ice-slurry and early cell separation and analysis, we recommend that in contemporary obstetrics the use of FE tubes be replaced by CFE



[1] Nonstandard abbreviations: GDM, gestational diabetes mellitus; CFE, citrate-fluoride-EDTA; OGTT, oral glucose tolerance test; IADPSG, International Association of Diabetes and Pregnancy Study Groups; NACB, National Association of Clinical Biochemistry; BMI, body mass index.

**Table 1. Mean (SD) maternal glucose concentrations compared with NACB recommendations as reference.[a]**

| | Reference FE | Customary CFE | Customary FE | *P* value[b] |
|---|---|---|---|---|
| Storage | Ice-water slurry | Room temperature | Room temperature | |
| Fasting | 90 (7.2) [5.0 (0.4)] | 88.2 (9) [4.9 (0.5)] | 81 (9) [4.5 (0.5)] | <0.0001 |
| 1-h | 142.2 (37.8) [7.9 (2.1)] | 140.4 (37.8) [7.8 (2.1)] | 135 (37.8) [7.5 (2.1)] | <0.0001 |
| 2-h | 104.4 (23.4) [5.8 (1.3)] | 100.8 (23.4) [5.6 (1.3)] | 100.8 (23.4) [5.6 (1.3)] | <0.0001 |

[a] Concentrations are presented as mg/dL [mmol/L].
[b] One-way ANOVA.

tubes for the measurement of maternal glucose for the diagnosis GDM. This is particularly important for the fasting sample because in reality the phlebotomy–cell separation interval is longest.

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