

COOH losses in urine from contact with LDPE.

Because of limitations of available leftover patient urine volume, analysis time, and cost, we only performed single experiments, which demonstrated that the THC-COOH concentrations in the inverted, metal lid containers of urine specimens varied by -50% to +9% from their plastic-only counterparts. When averaged, a reduction of 29% was observed. Specimens collected and stored in these containers could potentially yield falsely low THC-COOH results. We caution the adoption of new specimen containers until they are investigated on all subjected analytes.

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Will the Use of Lyophilized Citrate Tubes Lead to the Over-Diagnosis of Diabetes in Pregnancy?

The International Association of Diabetes and Pregnancy Study Group (IADSPG)¹ plasma glucose concentration thresholds used for diagnosing gestational diabetes mellitus (GDM) (1) are based on the risk of an adverse fetal outcome according to the landmark Hyperglycemia

and Adverse Pregnancy Outcome (HAPO) study. The HAPO study used research-grade methodology to minimize preanalytical glucose loss after an oral glucose tolerance test (OGTT); thus, samples were cooled and plasma separated shortly after phlebotomy (1). This sample processing is the gold standard for GDM-related studies of plasma glucose.

A diagnosis of GDM using IADSPG criteria has been shown to be sensitive to bias in plasma glucose measurement; for example, Daly et al. (2) showed that a modest preanalytical glycolysis-induced difference between research-grade and usual processing of samples resulted in a 2.7-fold difference in the rate of diagnosis of GDM. A significant proportion of women in mid-pregnancy to late pregnancy have plasma glucose values above GDM diagnostic thresholds; also their distribution of glucose, especially fasting glucose, is narrow (1-3). In this setting, a small bias in measured glucose can have large diagnostic consequences.

Minimizing preanalytical glycolysis using research-grade processing is often impractical. Some laboratories therefore recommend using collection tubes containing citrate stabi-

¹**Nonstandard abbreviations:** IADSPG, International Association of Diabetes and Pregnancy Study Group; GDM, gestational diabetes mellitus; HAPO, Hyperglycemia and Adverse Pregnancy Outcome; OGTT, oral glucose tolerance test.

lizer, including for the diagnosis of GDM (3). In nonpregnant populations, citrated tubes may show a small positive bias (4, 5). Also, there is additional debate about liquid (rather than lyophilized) citrate tubes, as the appropriate dilutional correction factor is unclear, and these tubes require a complete fill during venesection (3, 4).

We are unaware of any studies using lyophilized citrate tubes for diagnosing GDM. We therefore undertook an exploratory study comparing three different processing procedures, one of which included lyophilized citrate tubes undergoing routine laboratory processing, in pregnant women undergoing 75-g diagnostic OGTTs.

Plasma glucose results were compared using the following processing methods: (a) immediate plasma separation using lithium-heparin-plasma separator tubes (PSTs) tubes, i.e., optimal "research grade" processing; (b) usual OGTT laboratory processing, using fluoride tubes stored at room temperature, with plasma separation occurring at the end of the OGTT ("batched processing"); and (c) tubes containing lyophilized citrate (Terumo™, Venosafe™, reference number 367375), which underwent usual laboratory processes as for the fluoride tubes; thus, plasma separation occurred at the end of the OGTT. Our labo-

ratory batches the 0-h (fasting) and 1- and 2-h OGTT samples for plasma extraction at the end of the OGTT, followed by analysis in a single run using the hexokinase method (Abbott c8000 Series Analyzer). Nine plasma glucose samples were therefore collected per participant, per OGTT. The study had ethics committee approval and participants gave written consent.

Mean gestation (SD) of the 15 participants was 28 (\pm 2.7) weeks. Table 1 shows plasma glucose results and median phlebotomy-to-analysis times. When comparing results from citrated tubes with the other tubes, differences between mean glucose concentrations were statistically significant across each time point (paired *t*-test; all *P* values < 0.01). Table 1 shows that citrate tubes "read" around 4 mg/dL higher than tubes undergoing optimal processing, a finding that is comparable with nonpregnant populations (4, 5). Fluoride does not fully inhibit glycolysis within the first 2 h of sample collection, and batching of samples delayed processing of the 0- and 1-h OGTT samples. The fluoride tubes showed the expected time-dependent negative bias (Table 1).

While acknowledging that participant numbers were small, it was of interest that, when applying IADSPG diagnostic criteria (fasting glucose \geq 92 mg/dL

and/or 1-h glucose \geq 180 mg/dL and/or 2-h glucose \geq 153 mg/dL), the frequency of GDM was as follows:

- Routine laboratory processing: 3/15 (20%)
- Optimal "research-grade" processing: 5/15 (33%)
- Citrate tubes: 7/15 (47%)

Like Daly et al. (2), we also found that these differences in diagnostic rates were attributable to the fasting result alone.

In conclusion, these findings suggest that discrepancies in GDM diagnostic rates could arise if lyophilized citrated tubes were adopted without proper validation. While the focus of this exploratory study is on GDM diagnosis using IADSPG diagnostic criteria, IADSPG is but one of several diagnostic protocols for GDM, and the consequences of pre-analytical bias may also affect other glucose-based diagnostic and screening tests. Laboratories should at minimum have an understanding of the likely impact of local decisions about sample processing, such as batching of samples from fluoride tubes or use of citrate tubes, on their screening and diagnostic rates for diabetes. The current study does not, of course, cover all collection tube types used to screen and diagnose diabetes;

Table 1. The 75-g OGTT results: comparison of mean glucose concentrations by type of blood collection tube.^a

	Lithium-heparin-PST undergoing immediate plasma separation	Fluoride undergoing routine processing	Citrate undergoing routine processing	Bias: fluoride- PST	Bias: citrate-PST
Fasting glucose					
Venesection to analysis time	–		214 min	–	–
mg/dL	85.4 (6.9) [81.9–88.9]	79.4 (7.5) [75.6–83.2]	88.1 (7.0) [84.6–91.7]	–6.0	+2.7
mmol/L	4.7 (0.4) [4.5–4.9]	4.4 (0.4) [4.2–4.6]	4.9 (0.4) [4.7–5.1]	–0.3	+0.2
1-h glucose					
Venesection to analysis time	–		145 min	–	–
mg/dL	139.0 (39.0) [119.2–158.7]	133.5 (40.2) [113.2–153.8]	144.5 (40.5) [124.0–165.0]	–5.5	+5.5
mmol/L	7.7 (2.2) [6.6–8.8]	7.4 (2.2) [6.3–8.5]	8.0 (2.2) [6.9–9.2]	–0.3	+0.3
2-h glucose					
Venesection to analysis time	–		95 min	–	–
mg/dL	120.7 (34.0) [103.4–137.9]	117.2 (36.3) [98.8–135.6]	125.0 (35.1) [107.3–142.7]	–3.5	+4.3
mmol/L	6.7 (1.9) [5.7–7.7]	6.5 (2.0) [5.5–7.5]	6.9 (1.9) [6.0–7.9]	–0.2	+0.2

^a Data are mean (SD) and [95% CIs for the point estimation of the mean]. The plasma glucose differences from the research grade lithium-heparin-PST results, by collection tube type, are shown in the 2 right-hand columns.

further studies are needed. All of these considerations highlight the critical importance of preanalytical stringency as well as analytical excellence for optimal clinical decision-making.

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