OBJECTIVE
Preanalytical processing of blood samples can affect plasma glucose measurement because ongoing glycolysis by cells prior to centrifugation can lower its concentration. In June 2017, ACT Pathology changed the processing of oral glucose tolerance test (OGTT) blood samples for pregnant women from a delayed to an early centrifugation protocol. The effect of this change on the rate of gestational diabetes mellitus (GDM) diagnosis was determined.

RESEARCH DESIGN AND METHODS
All pregnant women in the Australian Capital Territory (ACT) are recommended for GDM testing with a 75-g OGTT using the World Health Organization diagnostic criteria. From January 2015 to May 2017, OGTT samples were collected into sodium fluoride (NaF) tubes and kept at room temperature until completion of the test (delayed centrifugation). From June 2017 to October 2018, OGTT samples in NaF tubes were centrifuged within 10 min (early centrifugation).

RESULTS
A total of 7,509 women were tested with the delayed centrifugation protocol and 4,808 with the early centrifugation protocol. The mean glucose concentrations for the fasting, 1-h, and 2-h OGTT samples were, respectively, 0.24 mmol/L (5.4%), 0.34 mmol/L (4.9%), and 0.16 mmol/L (2.3%) higher using the early centrifugation protocol (P < 0.0001 for all), increasing the GDM diagnosis rate from 11.6% (n = 869/7,509) to 20.6% (n = 1,007/4,887).

CONCLUSIONS
The findings of this study highlight the critical importance of the preanalytical processing protocol of OGTT blood samples used for diagnosing GDM. Delay in centrifuging of blood collected into NaF tubes will result in substantially lower rates of diagnosis than if blood is centrifuged early.

Gestational diabetes mellitus (GDM) is a hyperglycemic disorder of pregnancy with high prevalence creating a major burden to the provision of maternity health services internationally (1). In 2014, the Australasian Diabetes in Pregnancy Society (ADIPS) set new diagnostic criteria guidelines for GDM for use in Australia that were in line with the World Health Organization (WHO)-endorsed International Association of the
Diabetes and Pregnancy Study Groups (IADPSG) recommendations (2,3). These recommend using a one-step, three point, 75-g oral glucose tolerance test (OGTT) with criteria for the diagnosis of GDM that include at least one elevated plasma glucose reading of $\geq 5.1$ mmol/L fasting, $\geq 10.0$ mmol/L at 1 h, and $\geq 8.5$ mmol/L at 2 h after the glucose load but with no readings diagnostic of diabetes outside of pregnancy (2,3). The OGTT is most often undertaken at 24–26 weeks of gestation, but it may be performed earlier for women with increased risk factors for GDM (3).

Preanalytical processing of blood samples can markedly affect the results of plasma glucose readings because ongoing glycolysis by erythrocytes and leukocytes prior to centrifugation utilizes glucose, lowering its concentration (4–9). Currently, the American Diabetes Association (ADA) recommendation for preanalytical processing for plasma glucose measurements is for collection into sodium fluoride (NaF) tubes with placement in ice-water slurry prior to centrifugation within 30 min (10). An additional recommendation is that citrate tubes be used if a delay in centrifugation is expected because citrate more rapidly inhibits glycolysis (10).

Thus, variability in preanalytic processing of blood for glucose measurement of pregnancy OGTTs could affect GDM diagnostic rates, as predicted using computer modeling and as found in a small prospective study of 155 pregnant women in Ireland (11,12). In the study from Ireland, the rate of GDM using IADPSG criteria in women who were selected for screening because of risk factors and tested between 24 and 32 weeks’ gestation was 2.7-fold higher (38.1% compared with 14.2%, $P < 0.0001$) if the ADA preanalytic protocol was followed compared with the previous standard practice of collecting blood into NaF tubes, leaving them at room temperature, and centrifuging them together after collection of all three samples had occurred (12). Similarly, the impact of long delays in centrifugation for OGTT samples collected into NaF tubes on GDM diagnosis in regional, rural, and remote sites in Western Australia was estimated to be an underdiagnosis rate of 62% (13).

In the Australian Capital Territory (ACT), the new ADIPS/IADPSG/WHO diagnostic criteria for GDM were adopted in January 2015, and testing was recommended for all pregnant women unless they were known to have preexisting diabetes (3). The usual procedure, as in many laboratories in Australia, was to collect all three samples (fasting, 1 h, and 2 h) from the 75-g OGTT before sending them together to the laboratory for processing. From June 2017, ACT Pathology (local, acute hospital-based public pathology service in the ACT) instituted stricter preanalytical processing, namely, all samples were centrifuged at point of collection within 10 min of collection. In this article, we report the consequences of this change in procedure.

**RESULTS**

In 2017, the country/region of birth of women having a baby in the ACT, which reflects the region’s multiethnic population, was as follows: Australia, 63.6%; Asia, 21.9%; Europe, 4.3%; North Africa and the Middle East, 3.4%; Americas, 2.6%; sub-Saharan Africa, 2.1%; and other 2% (14).

Between January 2015 and May 2017, 7,509 women were tested with the delayed centrifugation protocol, of which 7,415 (98.7%) completed all time points. Between June 2017 and October 2018, 4,887 women were tested with the early centrifugation protocol, of which 4,808 (98.4%) completed all time points (Table 1). The mean glucose concentrations for the fasting, 1-h, and 2-h OGTT samples were, respectively, 0.24 mmol/L (5.4%), 0.34 mmol/L (4.9%), and 0.16 mmol/L (2.3%) higher with the early centrifugation protocol ($P < 0.0001$ for all) (Table 1).

The rate of GDM diagnosis almost doubled from 11.6% (869/7,509) to 20.6% (1,007/4,887) with the introduction of the early centrifugation processing protocol ($\chi^2 P < 0.00001$) (Table 2). GDM diagnosis rates on the fasting and the 1-h OGTT samples increased by 127% ($P < 0.00001$) and 66% ($P < 0.01$), respectively, with no significant effect on the rates of diagnosis on the 2-h sample (15% increase, $P = 0.08$) (Table 2). The impact of an increase of 0.2 mmol/L in the plasma glucose test results due to early centrifugation, according to the time point of the OGTT, is also shown in Table 2. It can be seen that the greatest impact of the new protocol on the rates of
GDM diagnosis is a consequence of the increased percentage of women with fasting plasma glucose concentrations in the range of 5.1–5.2 mmol/L (Table 2).

**CONCLUSIONS**

Within a large multiethnic cohort of women undergoing universal diagnostic testing for GDM, we confirm the predictions from modeling and previous small studies that the OGTT preanalytical blood sample handling step is critical for accuracy in GDM diagnosis. We observed an increase in the rate of GDM from 11.6% to 20.6% on changing to a protocol of centrifuging blood collected into NaF tubes within 10 min of venipuncture. An increase in the rate of GDM from 11.6% to 20.6% on changing to a protocol of centrifuging blood collected into NaF tubes within 10 min of venipuncture. An increase in the rate of GDM from 11.6% to 20.6% on changing to a protocol of centrifuging blood collected into NaF tubes within 10 min of venipuncture. An increase in the rate of GDM from 11.6% to 20.6% on changing to a protocol of centrifuging blood collected into NaF tubes within 10 min of venipuncture.

The ADIPS/IADPSG/WHO criteria for diagnosis of GDM by OGTT were derived from analysis of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study results, such that the preanalytical sampling protocol used in that study should be followed if results are to be comparable (2,15). In HAPO, blood samples for all glucose measurements were collected into NaF tubes, placed in an ice slurry immediately after phlebotomy, and kept that way until they could be centrifuged (refrigerated centrifuge) and separated (Boyd E. Metzger, HAPO Lead Investigator, personal communication). The same procedure was followed for cord blood samples collected for glucose measurement in HAPO (16). Of note, the mean OGTT glucose concentrations of the ACT study population using the early centrifugation protocol are similar to those of the multiethnic HAPO study (ACT vs. HAPO; 4.65 vs. 4.49 mmol/L for fasting glucose, 7.33 vs. 7.45 mmol/L for 1-h glucose, and 6.21 vs. 6.16 for 2-h glucose) (15). However, the 0.16 mmol/L higher fasting level using the early centrifugation protocol needs to be considered, as it will be associated with higher GDM diagnosis rates. While it may well be a consequence of the particular study demographic and a general increase in overweight/obesity since HAPO, a delay to centrifugation of fasting samples in HAPO, even though the samples were kept on ice, is another potential contributing factor (16).

The major strength of the current study is the large number of subjects tested prior to and after a well-demarcated change in OGTT protocol. Also, the multiethnic community of the ACT makes the findings relevant internationally. A weakness is the time difference between the two study groups (January 2015 to May 2017, delayed centrifugation; June 2017 to October 2018, early centrifugation) as the true GDM rate is not likely to be static over time. For example, the percentage of women with Australia as their country of birth decreased from 65.8% in 2015 to 63.6% in 2017 (14). However, the dramatic increase in the GDM diagnosis rate was evident within the first 3 months of the change to the early centrifugation preanalytical protocol having been fully implemented (diagnosis rates of 11.9% for March, April, and May 2017 and 19.8% for July, August, and September 2017).

It is clear that the preanalytical blood sampling protocol for OGTTs during pregnancy needs attention and standardization (9). The challenges for small collection centers, including those distant to analytical laboratories, need to be taken into consideration as rapid centrifugation may

### Table 1—The influence of changing sample handling after venesection on OGTT plasma glucose concentrations

| Plasma glucose concentration (mmol/L) | Period A: delayed centrifugation | Period B: early centrifugation |
|--------------------------------------|---------------------------------|------------------------------|
| Fasting                              | Fasting                         | Fasting                      |
| Number                               | 7,509                           | 4,887                        |
| Mean                                 | 4.41*                           | 6.99*                        |
| SD                                   | 0.41                            | 1.63                         |

#### Period A: January 2015 to May 2017, centrifugation of blood samples on completion of OGTT. Period B: June 2017 to October 2018, early centrifugation of samples within 10 min of venipuncture. *t = 32.35, P < 0.0001; t = 11.11, P < 0.0001; t = 6.29, P < 0.0001.

### Table 2—Effect of OGTT preanalytical sample handling protocol on the rates of GDM diagnosis overall and according to the fasting, 1-h, and 2-h plasma glucose results

|                                | Period A: delayed centrifugation | Period B: early centrifugation |
|--------------------------------|---------------------------------|------------------------------|
| Total number                   | 7,509                           | 4,887                        |
| Fasting glucose concentration (mmol/L) | ≥5.1                          | 5.1–5.2¶                     |
| Number (% of total)            | 444*                           | 656* (13.4%)                 |
| Number (% of total)            | 10.0                           | 10.0–10.1¶                   |

#### Period A: January 2015 to May 2017, centrifugation of blood samples on completion of OGTT. Period B: June 2017 to October 2018, early centrifugation of samples within 10 min of venipuncture. ¶Low-band GDM diagnosed if the plasma glucose is in the lowest 0.2 mmol/L of the diagnostic range (likely extra GDM cases using an early compared to late centrifugation protocol). *χ² = 206.6, P < 0.000001; χ² = 44.3, P < 0.01; χ² = 3.1, P = 0.08; χ² = 183.5, P < 0.00001.
not always be possible. Use of citrate tubes could be considered, as suggested by ADA if placement in ice slurry and centrifugation within 30 min is not possible for logistical reasons (9). However, use of citrate tubes (granulated form) may give a positive bias of 0.2 mmol/L, falsely increasing the rate of GDM diagnosis, such that a correction factor to match the OGTG plasma glucose levels of the HAPO study would likely be required (5,17,18). Of note, tubes with citrate in liquid form require accurate blood volume and a correction factor due to blood dilution, such that tubes with citrate in the granulated form would be preferable. It also needs to be considered that early centrifugation of samples collected into NaF tubes may also result in some positive diagnosis bias compared with the preanalytical processing protocol used in HAPO (collection into NaF tubes, placed in ice slurry, some delay in centrifugation). NaF and immediate cooling in an ice slurry does not immediately stop glycolysis (18).

In conclusion, the findings of this study highlight the critical importance of pre-analytical processing of OGTG blood samples used for diagnosing GDM. Delay in centrifuging of blood collected into NaF tubes kept at room temperature will result in substantially lower rates of diagnosis than if blood is centrifuged early. The method used for preanalytical OGTG sample processing should be reported when GDM studies are published, such that study results can be compared. More routine use of collection tubes containing granulated citrate could be considered moving forward, as citrate rapidly inhibits glycolysis; however, this would likely require a correction factor, or adjustment in the cut-points for diagnosis, due to a positive bias in plasma glucose concentrations measured from these tubes. As there is marked heterogeneity in preanalytical OGTG blood sample handling among pathology practices in Australia, this is an issue of major importance for Australian maternity services (13). We expect this is likely not to be unique to Australia.

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