

# Systematic Reduction in Estradiol and Testosterone Measurements due to Serum Separator Gel in Blood Collection Tubes: Implications for At-Home Fertility Testing

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**Background:** Data on the efficacy of at-home capillary testing is limited. We compared 9 fertility-associated analytes [anti-Müllerian hormone, follicle-stimulating hormone, free thyroxine, luteinizing hormone, estradiol (E2), prolactin, sex hormone-binding globulin, thyroid-stimulating hormone, and total testosterone (T)] to assess concordance between venous and capillary tubes and the impact of serum separator gel.

**Methods:** Two trials were conducted. The first (trial 1) used 34 paired blood samples taken from 13 participants collected into 2 capillary storage tubes: serum clot activator tubes (cS-CAT) without serum separator gel and serum separator clot activator tubes (cSS-CAT) with serum separator gel. Trial 2 collected 27 sets of 4 blood samples from 26 participants into cS-CAT, cSS-CAT, venipuncture serum clot activator tubes (vS-CAT) without serum separator gel, and venipuncture serum separator clot activator tubes (vSS-CAT) with serum separator gel. Measurements of the analytes were log-transformed, and each tube type was compared via paired *t*-tests.

**Results:** Significantly lower measurements for both E2 and T (13% and 8%, respectively) were observed for cSS-CAT compared to cS-CAT. Differences were greater between cSS-CAT and vSS-CAT (17% and 11%) and greatest between cSS-CAT and vS-CAT (19% and 14%). No significant differences were observed for the other 7 analytes nor when comparing cS-CAT to vSS-CAT. Additionally, conversion formulas were developed to adjust E2 and T measurements between the most divergent tube types.

**Conclusions:** Capillary blood collection tubes with gel systematically lower E2 and T measurements, showing discrepancies with venous blood collection tubes. These effects did not impact other analytes.

## INTRODUCTION

Whilst the canonical method for blood testing is extraction using venipuncture followed by storage

in a blood collection tube, capillary blood sampling via finger-prick into relatively smaller tubes is used for various diagnostic and monitoring tests, often in remote settings and/or where blood volumes

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## IMPACT STATEMENT

This study has critical implications for providers and users of at-home female and male hormone and fertility tests, particularly for those involved in clinical decision-making. By demonstrating systematic reductions in E2 and T measurements due to serum separator gel, we highlight the need for standardized collection methods to ensure accurate hormone assessments. This research advances knowledge by identifying the preanalytical factors that affect reliability of at-home testing, enabling more informed choices for providers of at-home testing kits and improving their alignment with the gold standard of venous blood collection.

are small. There has been a recent surge in the adoption of capillary blood sampling for at-home testing, largely driven by the COVID-19 pandemic (1, 2). At-home testing provides numerous benefits, including cost-efficiency, the need for only small sample volumes, enabling self-sampling, and minimal discomfort. These factors have established it as the preferred option for conducting tests in remote settings (3–5).

At-home female hormone testing via capillary blood sampling is also currently commercially available for the purpose of fertility and reproductive health assessment. Typical analytes measured in these assessments include anti-Müllerian hormone (AMH), sex hormones such as estradiol (E2) and testosterone (T), thyroid hormones, and gonadotrophins such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Capillary blood collection tubes typically contain a blood-clotting activator, the main component of which is silicon dioxide. Additionally, some collection tubes also contain a separator gel, which includes polymers designed to aid the separation of serum from whole blood. The utilization of tubes with serum separator gel results in shorter sample processing times, higher analyte stability, and higher yield of serum (6).

There is some evidence that prolonged storage of blood in tubes that contain serum separator gel may reduce the stability of some analytes, particularly therapeutic drugs and some steroid

hormones (6, 7). Previous research by our group has shown that capillary tubes containing serum separator gel lead to systematically lower measurements of E2 and T (8). However, to our knowledge, there have been no previous studies comparing the performance of capillary blood collection tubes to venous blood collection tubes for the purpose of at-home fertility hormone tests. The paucity of research on capillary blood samples means the effect of serum separator gels in capillary collection tubes on the measurement of these analytes remains poorly understood.

We therefore aimed to compare venous and capillary serum measurements of analytes commonly used to assess fertility and reproductive health to assess potential differences caused by the collection method.

## MATERIALS AND METHODS

### Participants and Ethical Approval

Trials were conducted using participants who were assigned female at birth, based in the UK, and recruited via Hertility Health Ltd (Research Ethics Committee approval number 20/LO/0265) as per the World Medical Association Declaration of Helsinki. Individuals were eligible to participate if they were  $\geq 18$  years old and provided informed consent following the reading and understanding of a participant information sheet. Individuals

who were not assigned female at birth, were experiencing perimenopause, or had undergone menopause/premature ovarian insufficiency were not eligible to participate due to the increased probability of one or more analytes being below the limit of quantification in their respective assay. Participants who were using hormonal contraception or had preexisting diagnoses of conditions that could affect concentrations of the analytes of interest, such as polycystic ovary syndrome, were not excluded from the study. This was justified as only paired measurements were compared as per the aims of the study, and a wide range of analyte concentration values were beneficial to this assessment. Blood samples were also collected throughout all menstrual cycle phases for the same reason. All venous samples were collected via venipuncture by a trained phlebotomist; all capillary samples were collected by the participant via finger prick. Samples in trial 1 were collected at home, while samples in trial 2 were collected in person at a privately contracted clinic. All samples were posted to Inuvi Diagnostics Ltd. on the same day of collection via a Royal Mail Tracked 24® service, prior to the last post collection time at the drop-off point.

### Trial 1

In February–March 2023 we collected 34 paired blood samples from 13 participants. Specifically, each pair was concurrently collected into a MiniCollect® 1 mL capillary serum clot activator tube (cS-CAT) and a MiniCollect 0.8 mL capillary serum separator clot activator tube (cSS-CAT). Concentrations of 9 analytes—AMH, thyroid-stimulating hormone (TSH), E2, FSH, LH, free thyroxine (FT4), sex hormone-binding globulin, total T, and prolactin (PROL)—were measured in all samples. Overall, 86.6% of the 612 measurements ( $34 \times 2 \times 9$ ) were successfully assayed; the remaining were insufficient, were hemolyzed, or had values outside the quantification limits. This rate varied across analytes, from 55 of 68 successful

measurements for both FSH and LH to 67 of 68 measurements for FT4. This resulted in between 26 and 33 successful paired measurements per analyte (Table 1).

### Trial 2

In September–October 2023 we collected 27 sets of 4 blood samples from 26 participants. Specifically, 2 venous samples were collected in a Vacuette® 4 mL serum clot activator tube (vS-CAT) and a Vacuette 5 mL venipuncture serum separator clot activator tube (vSS-CAT), and 2 capillary samples were collected into a MiniCollect 1 mL cS-CAT and a MiniCollect 0.8 mL cSS-CAT. All 4 samples were obtained within a 30-minute period, in a random order. Concentrations of the same 9 analytes obtained in trial 1 were measured in all samples. Of the 27 quadruplets, one venipuncture sample failed, and of the remaining 107 samples ( $27 \times 4 - 1$ ), 94.2% of the 963 measurements ( $107 \times 9$ ) were successfully assayed, with rates varying from 90 of 107 successful measurements for FSH to 107 of 107 for T. This resulted in between 21 and 27 successful paired measurements per analyte (Table 1).

### Assays

All samples were shipped on the same day of blood collection to Inuvi Diagnostics Ltd.; samples were processed upon arrival in the laboratory in line with laboratory and manufacturer recommendations and analyzed on the same day. Approximately 70% of all samples arrived within 24 hours, a further 28% arrived between 24 and 48 hours, and the remaining 2% arrived between 48 and 72 hours after shipping. All paired samples were processed and assayed within the same run to minimize interassay variability. The Access 2 Immunoassay System (Beckman Coulter Diagnostics) was used to measure concentrations of AMH where described. The Cobas® e801 module (Roche Diagnostics) was used to measure concentrations of E2, LH, FSH, PROL, T, sex

Table 1. Results of paired t-tests between log-transformed values. <sup>a,b,c</sup>										
Analyte	Trial 1			Trial 2						
	CSS-CAT vs cS-CAT	CSS-CAT vs cS-CAT	CSS-CAT vs VSS-CAT	CSS-CAT vs VSS-CAT	CSS-CAT vs VSS-CAT	CSS-CAT vs VSS-CAT	CSS-CAT vs VSS-CAT	VSS-CAT vs VSS-CAT	VSS-CAT vs CS-CAT	VSS-CAT vs CS-CAT
AMH, pmol/L	Paired n = 26 Difference = -0.008 P value = 2.9e-01	Paired n = 26 Difference = -0.016 P value = 5.64e-02	Paired n = 26 Difference = 0.024 P value = 3.02e-02	Paired n = 26 Difference = 0.003 P value = 8.01e-01	Paired n = 25 Difference = 0.027 P value = 1.04e-01	Paired n = 26 Difference = -0.022 P value = 3.22e-02	Paired n = 26 Difference = 0.027 P value = 1.04e-01	Paired n = 26 Difference = -0.022 P value = 3.22e-02	Paired n = 26 Difference = -0.047 P value = 5.43e-03	Paired n = 27 Difference = -0.047 P value = 5.43e-03
TSH, mIU/L	Paired n = 32 Difference = -0.009 P value = 2.0e-01	Paired n = 26 Difference = -0.005 P value = 4.09e-01	Paired n = 26 Difference = -0.030 P value = 2.09e-02	Paired n = 25 Difference = -0.029 P value = 2.34e-02	Paired n = 26 Difference = -0.021 P value = 5.72e-02	Paired n = 26 Difference = 0.005 P value = 5.07e-02	Paired n = 26 Difference = -0.021 P value = 5.72e-02	Paired n = 26 Difference = 0.005 P value = 5.07e-02	Paired n = 27 Difference = 0.024 P value = 2.55e-02	Paired n = 27 Difference = 0.024 P value = 2.55e-02
E2, pmol/L	Paired n = 26 Difference = -0.143 P value = 1.58e-09*	Paired n = 23 Difference = -0.108 P value = 1.79e-07*	Paired n = 25 Difference = -0.184 P value = 1.48e-07*	Paired n = 24 Difference = -0.208 P value = 5.65e-08*	Paired n = 22 Difference = -0.104 P value = 1.31e-04*	Paired n = 24 Difference = -0.022 P value = 1.18e-02	Paired n = 22 Difference = -0.104 P value = 1.31e-04*	Paired n = 24 Difference = -0.022 P value = 1.18e-02	Paired n = 27 Difference = 0.024 P value = 2.55e-02	Paired n = 27 Difference = 0.024 P value = 2.55e-02
FSH, IU/L	Paired n = 24 Difference = -0.008 P value = 1.68e-01	Paired n = 22 Difference = 0.002 P value = 7.26e-01	Paired n = 23 Difference = -0.013 P value = 9.26e-02	Paired n = 22 Difference = -0.010 P value = 1.65e-01	Paired n = 21 Difference = -0.011 P value = 1.39e-01	Paired n = 22 Difference = 0.003 P value = 4.30e-01	Paired n = 21 Difference = -0.011 P value = 1.39e-01	Paired n = 22 Difference = 0.003 P value = 4.30e-01	Paired n = 22 Difference = 0.014 P value = 6.15e-02	Paired n = 22 Difference = 0.014 P value = 6.15e-02
LH, IU/L	Paired n = 24 Difference = -0.016 P value = 5.46e-01	Paired n = 23 Difference = -0.004 P value = 7.69e-01	Paired n = 23 Difference = 0.020 P value = 5.43e-01	Paired n = 22 Difference = 0.002 P value = 9.42e-01	Paired n = 22 Difference = 0.007 P value = 7.79e-01	Paired n = 22 Difference = -0.008 P value = 9.03e-02	Paired n = 22 Difference = 0.007 P value = 7.79e-01	Paired n = 22 Difference = -0.008 P value = 9.03e-02	Paired n = 23 Difference = -0.024 P value = 3.56e-01	Paired n = 23 Difference = -0.024 P value = 3.56e-01
FT4, pmol/L	Paired n = 33 Difference = 0.004 P value = 3.58e-01	Paired n = 25 Difference = 0.005 P value = 1.47e-01	Paired n = 26 Difference = 0.004 P value = 2.89e-01	Paired n = 25 Difference = -0.011 P value = 2.85e-03	Paired n = 25 Difference = -0.015 P value = 1.15e-02	Paired n = 26 Difference = -0.014 P value = 1.43e-04*	Paired n = 25 Difference = -0.015 P value = 1.15e-02	Paired n = 26 Difference = -0.014 P value = 1.43e-04*	Paired n = 26 Difference = 0.000 P value = 9.92e-01	Paired n = 26 Difference = 0.000 P value = 9.92e-01

(continued)

Table 1. Continued.									
Analyte	Trial 1			Trial 2					
	cSS-CAT vs cS-CAT	cSS-CAT vs cS-CAT	cSS-CAT vs cS-CAT	cSS-CAT vs vS-CAT	cS-CAT vs vS-CAT	vSS-CAT vs vS-CAT	vSS-CAT vs cS-CAT		
Sex hormone binding globulin, nmol/L	Paired n = 26 Difference = -0.002 P value = 8.37e-01	Paired n = 25 Difference = 0.013 P value = 6.72e-02	Paired n = 25 Difference = 0.000 P value = 9.69e-01	Paired n = 24 Difference = -0.008 P value = 4.15e-01	Paired n = 24 Difference = -0.021 P value = 1.82e-02	Paired n = 24 Difference = -0.008 P value = 1.77e-01	Paired n = 24 Difference = 0.012 P value = 1.10e-01		
Total T, nmol/L	Paired n = 26 <b>Difference = -0.094</b> <b>P value = 7.88e-05*</b>	Paired n = 27 <b>Difference = -0.066</b> <b>P value = 3.48e-04*</b>	Paired n = 27 <b>Difference = -0.120</b> <b>P value = 7.33e-05*</b>	Paired n = 26 <b>Difference = -0.151</b> <b>P value = 2.43e-05*</b>	Paired n = 26 Difference = -0.084 P value = 4.14e-03	Paired n = 26 Difference = -0.029 P value = 2.13e-02	Paired n = 27 Difference = 0.054 P value = 2.21e-02		
PROL, mIU/L	Paired n = 26 Difference = -0.044 P value = 1.16e-0	Paired n = 26 Difference = 0.007 P value = 4.44e-01	Paired n = 26 Difference = -0.018 P value = 3.96e-01	Paired n = 25 Difference = -0.032 P value = 1.68e-01	Paired n = 26 Difference = -0.039 P value = 4.57e-02	Paired n = 26 <b>Difference = -0.015</b> <b>P value = 6.09e-04*</b>	Paired n = 27 Difference = 0.025 P value = 1.62e-01		

<sup>a</sup>Trial 1 tested for differences between cSS-CAT and cS-CAT. Trial 2 tested all 6 comparisons of cSS-CAT, cS-CAT, vS-CAT, and vSS-CAT. P values reported in bold with asterisk are below the 0.05 alpha significance level adjusted for a total of 9 x 7 multiple tests using the Sidak method (8.14e-04).

<sup>b</sup>cS-CAT: 1 mL tube; cSS-CAT: 0.8 mL tube; vS-CAT: 4 mL tube; vSS-CAT: 5 mL tube.

<sup>c</sup>Difference is mean pairwise difference between log-transformed measurements.

\*Statistically significant. P value at the 0.05 alpha level after adjusting for multiple testing (8.14e-04).

hormone-binding globulin, FT4, and TSH where described. All assays were performed by Inuvi Diagnostics Ltd. All blood collection tubes were supplied by Greiner Bio-One.

### Statistical Analyses

Calculations were performed using the R language and environment for statistical computing, version 4.2.1. Differences between measurements were statistically analyzed using the most appropriate methods, via paired *t*-tests after raw measurements had been log-transformed using base *e*. Trial 1 tested for differences between cSS-CAT and cS-CAT for 9 analytes. Trial 2 tested all 6 differences between cSS-CAT, cS-CAT, vSS-CAT, and vS-CAT for the same 9 analytes. Therefore, the usual 0.05 alpha significance level was adjusted for multiple testing ( $9 \times 7$  tests) using the Sidak method to 0.000814.

## RESULTS

### Comparison of Measurements from Each Tube Type

Both trial 1 and trial 2 compared paired capillary samples between cSS-CAT (with separator gel) and cS-CAT (without separator gel). The results of both trials are notably similar (Table 1, Figs. 1 and 2), showing that E2 measurements differ significantly (trial 1  $P = 1.58\text{e-}09$ , trial 2  $P = 1.79\text{e-}07$ ) and T measurements differ significantly (trial 1  $P = 7.88\text{e-}05$ , trial 2  $P = 3.48\text{e-}04$ ) between tube type, but there was no significant difference in the other 7 analytes measured (accounting for multiple testing). The magnitude of these differences is also notably similar between trials, showing E2 cSS-CAT values were lower than E2 cS-CAT values (Table 1 shows differences in log values of  $-0.143$  from trial 1 and  $-0.108$  from trial 2, which equates to percentage differences in raw values of  $-13.3\%$  and  $-10.2\%$ , respectively) and likewise showing T cSS-CAT values were lower than T

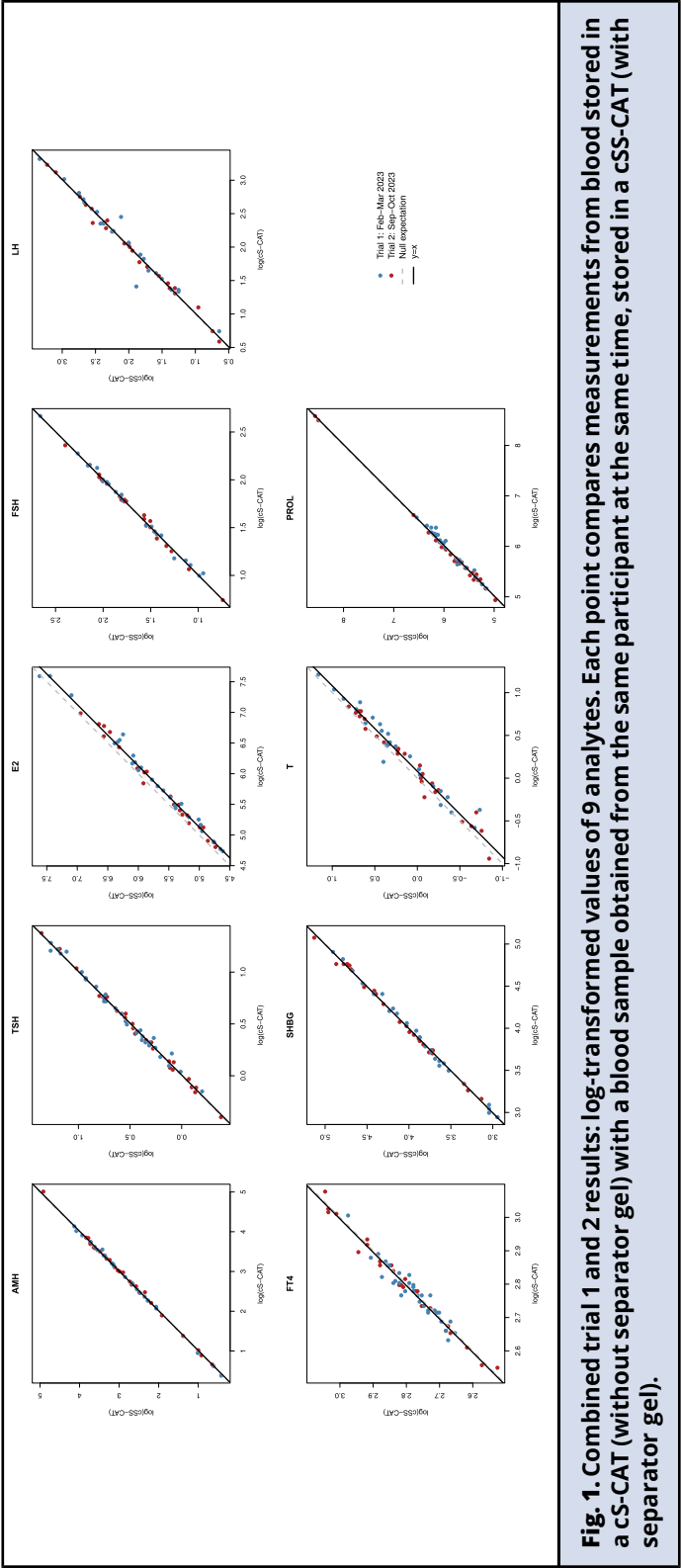
cS-CAT values (Table 1 shows differences in log values of  $-0.094$  from trial 1 and  $-0.066$  from trial 2, which equates to percentage differences in raw values of  $-9.0\%$  and  $-6.4\%$ , respectively).

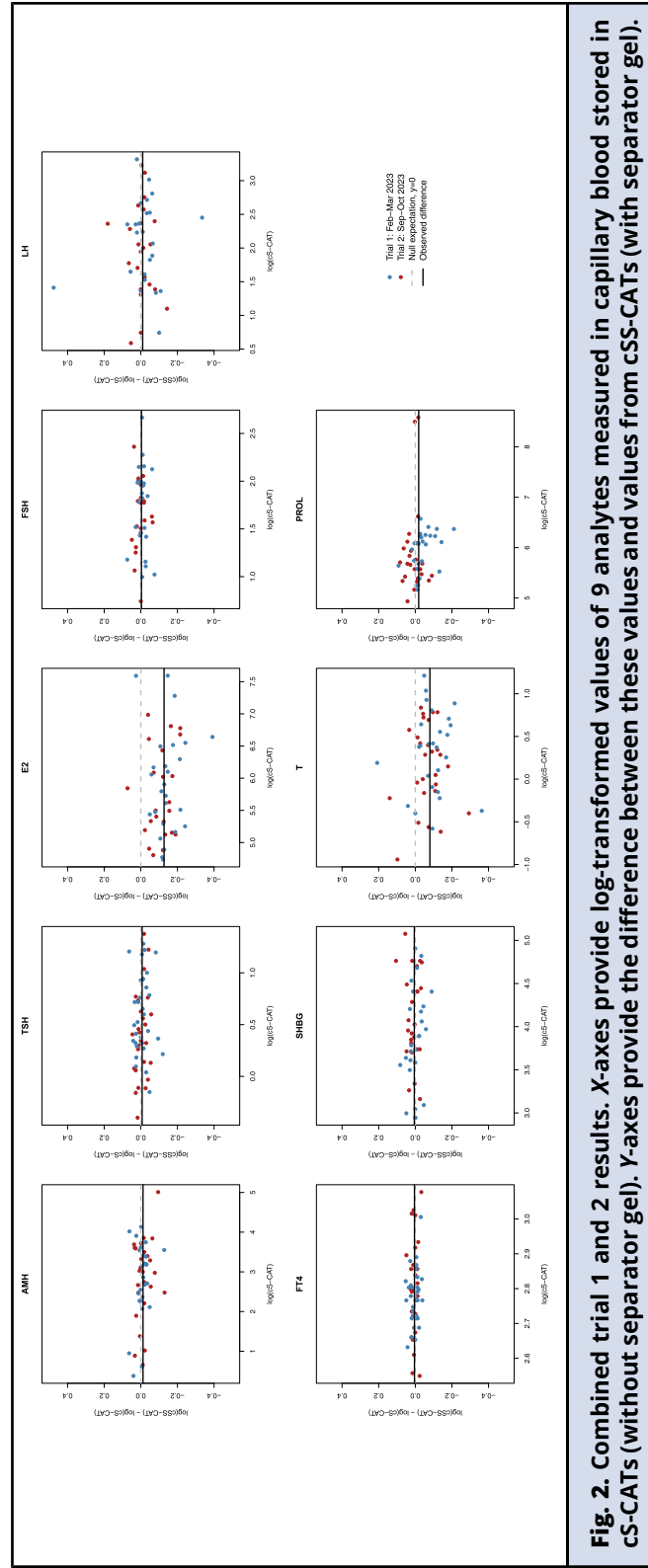
Trial 2 further tested all 6 comparisons between cSS-CAT, cS-CAT, vSS-CAT, and vS-CAT (Table 1, Figs. 1 and 2) revealing that the magnitude of the effect on E2 and T was even greater when comparing cSS-CAT and vSS-CAT measurements (E2 log difference =  $-0.184$ ,  $P = 1.48\text{e-}07$ ; T log difference =  $-0.120$ ,  $P = 7.33\text{e-}05$ ), equating to percentage differences in raw values of  $-16.8\%$  and  $-11.3\%$ , respectively. Furthermore, the magnitude of the effect was greatest between cSS-CAT and vS-CAT (E2 log difference =  $-0.208$ ,  $P = 5.65\text{e-}08$ ; T log difference =  $-0.151$ ,  $P = 2.43\text{e-}05$ ), equating to percentage differences of  $-18.8\%$  and  $-14.0\%$ , respectively. Again, no significant difference was detected for the remaining 7 analytes for either comparison.

Trial 2 also revealed a smaller similar effect between cS-CAT and vS-CAT (neither of which contain separator gel); cS-CAT measurements of E2 were lower with a marginally statistically significant difference (log difference =  $-0.104$ ,  $P = 1.31\text{e-}04$  equating to a  $-9.9\%$  difference in raw values). Similarly, we noted a smaller effect for T, which did not pass our significance threshold adjusted for multiple testing (log difference =  $-0.084$ ,  $P = 4.14\text{e-}03$ ,  $-8.1\%$  in raw values). Again, no significant difference was detected for the remaining 7 analytes.

The relative effect sizes of the statistically significant comparisons are depicted in Fig 3. This figure illustrates that the measurements of both E2 and T increase in the following order: cSS-CAT < cS-CAT < vSS-CAT < vS-CAT.

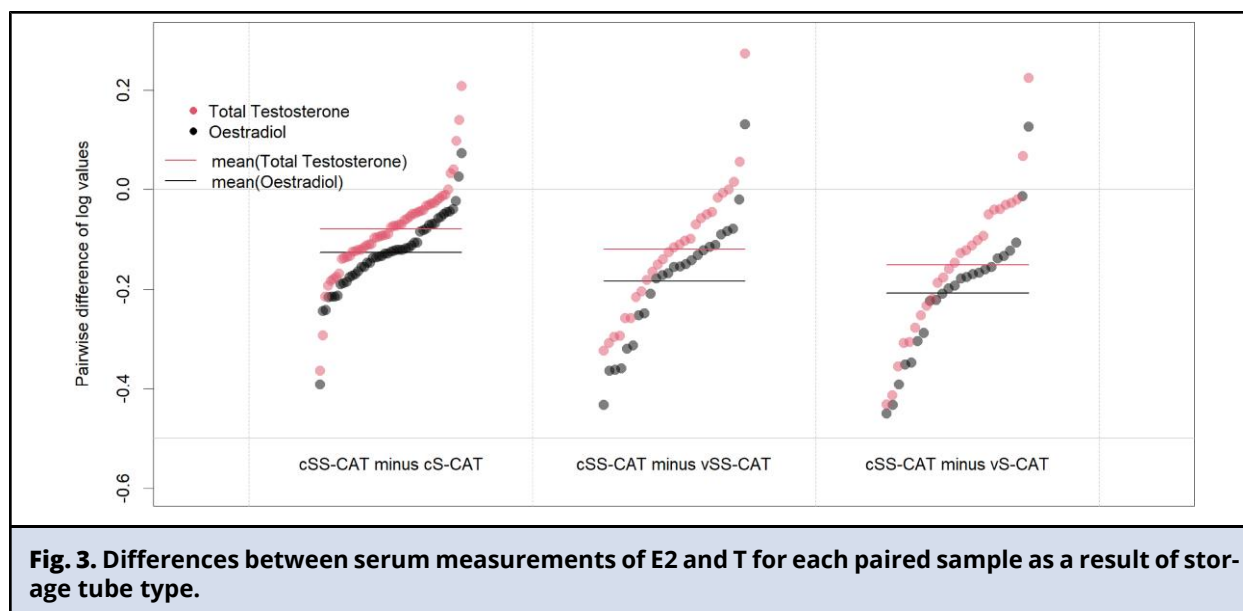
Lastly, comparisons between vSS-CAT and cS-CAT revealed no significant differences for any of the 9 analytes. Comparisons between vSS-CAT and vS-CAT revealed borderline statistical significance for FT4 and PROL ( $1.43\text{e-}04$  and  $6.09\text{e-}04$ , respectively); however, the differences were





**Fig. 2.** Combined trial 1 and 2 results. X-axes provide log-transformed values of 9 analytes measured in capillary blood stored in CS-CATs (without separator gel). Y-axes provide the difference between these values and values from CS-CATs (with separator gel).





almost an order of magnitude smaller than the effect described earlier (log difference = 0.014 and 0.015, respectively, equivalent to 1.4% and 1.5% differences in raw values), and no such effect was detected in these analytes for any of the other 5 tube type comparisons. Overall, therefore, we conclude that the borderline statistical significance is a fluke and not indicative of a real effect.

### Model Comparison and Conversion Formulae

To evaluate the statistical relationship between E2 and T measurements from the tube types where the biggest greatest difference was observed (vS-CAT vs cSS-CAT), we first performed a model comparison of 4 simple linear models (Table 2), fitted to log-transformed data. This balanced the goodness of fit against the model complexity (number of parameters) using the so-called Bayesian Information Criterion. Given the small sample sizes ( $n=37$  and  $31$ , respectively), the best model (lowest Bayesian Information Criterion) was  $y=kx$  for E2 and  $y=x+c$  for T. From these, formulae to convert both raw and log-transformed values were derived (Table 3).

### DISCUSSION

To our knowledge, this is the first study to compare the concordance of common fertility analyte measurements in venous and capillary serum collection tubes both with and without serum separator gel to systematically assess the impact of collection tube type. Overall, our study established 4 key findings.

First, we found that for 7 of the common analytes used in fertility assessments (AMH, TSH, FSH, LH, FT4, sex hormone-binding globulin, and PROL), the collection tube type had no effect, meaning that capillary measurements were comparable to venous measurements regardless of tube type.

Second, we found measurements of 2 common fertility assessment analytes, E2 and T, were significantly lower in blood stored in tubes containing serum separator gel. The effect was greatest between smaller capillary blood collection tubes (cSS-CAT vs cS-CAT, Table 1). The magnitude of this effect was diminished (and not statistically significant given the small paired sample sizes) between larger venous blood collection tubes

**Table 2. Model comparison evaluating 4 plausible linear relationships between analyte values stored in vS-CAT vs cSS-CAT.<sup>a</sup>**

Analyte	Model	Bayesian Information Criterion
E2 paired, n = 37	$y = x$	7.617078
	<b><math>y = kx</math></b>	<b>-24.1495</b>
	$y = x + c$	-23.42914
	$y = kx + c$	-20.63833
Total T paired, n = 31	$y = x$	-10.03093
	$y = kx$	-7.745709
	<b><math>y = x + c</math></b>	<b>-24.51611</b>
	$y = kx + c$	-23.51868

Rows in bold refer to the models with the lowest Bayesian Information Criterion.  
<sup>a</sup>c = y-intercept; k = slope; x = cSS-CAT; y = vS-CAT.

**Table 3. Formulae to convert E2 and total T measurements stored in cSS-CAT to expected values if stored in vS-CAT using parameter estimates from the best fitting models in Table 2.**

Analyte	Formula for natural log-transformed measurements	Formula for raw measurements
E2	$\log(\text{vS-CAT}) = \log(\text{cSS-CAT}) \times 1.037$	$\text{vS-CAT} = \text{cSS-CAT}^{1.037}$
Total T	$\log(\text{vS-CAT}) = \log(\text{cSS-CAT}) + 0.129$	$\text{vS-CAT} = 1.138 \times \text{cSS-CAT}$

(vSS-CAT vs vS-CAT, Table 1). This suggests that the difference in measurements is likely a function of the presence of serum separator gel and the volume of the tube.

Third, we found that capillary measurements of E2 and T were lower than venous measurements. This difference was greatest when serum separator gel was present (cSS-CAT vs vSS-CAT, Table 1), and this effect was greatly reduced when serum separator gel was absent (cS-CAT vs vS-CAT, Table 1). All 4 collection tube types also contain a clot activator; the largest component is silicon dioxide, which has been demonstrated to affect the performance of various laboratory tests (7, 9). We hypothesize this effect is greater in the smaller capillary blood collection tubes due to the higher surface area to volume ratio of gel and whole blood, and is compounded when a

serum separator gel is present in addition to a clot activator. Nevertheless, we cannot exclude the possibility that there may also be some difference in analyte concentrations between venous blood and capillary blood. A further experiment varying the size of the storage tube in both capillary and venous blood may yield a better understanding of the effect size, but we hypothesize this effect decreases as the tube size increases.

Fourth, having previously established that prolonged exposure to serum separator gel in capillary tubes systematically lowers measurements of E2 and T (8), we have provided formulae to adjust for the decrease expected when stored for a period of 24 to 72 hours prior to assaying (Table 3). This was achieved using a statistical model comparison approach that balanced the goodness of fit against model complexity for 4 plausible linear models.

### Implications for At-Home Fertility Assessments

Capillary blood collection is often the preferred method for at-home fertility assessments, with many providers utilizing blood collection tubes that contain serum separator gel (10). Here, we have demonstrated that serum separator gel in capillary collection tubes does not significantly affect most common analytes tested during fertility assessments; however, it substantially lowers measurements of E2 and T. Percentage differences of  $-18.8\%$  and  $-14.0\%$  for E2 and T, respectively, were observed when comparing capillary tubes with serum separator gel to venous tubes without gel. This implies that at-home capillary blood tests using tubes with serum separator gel to measure E2 and/or T may report lower levels than if the blood samples had been collected in standard venous tubes. The total allowable error for E2 and T assays is  $25\%$ ; however, it remains unclear whether this could result in missed diagnoses of conditions such as hypogonadism and polycystic ovary syndrome, or whether a reduction of this magnitude may influence the clinical interpretation of these results (11).

Importantly, the magnitude of this effect was substantially reduced capillary tubes that did not contain serum separator gel, which yielded comparable measurements to venous tubes that did contain serum separator gel (i.e., the most common type used for in-person blood draws via venipuncture at fertility clinics). We therefore reinforce our previous recommendation to utilize capillary tubes without serum separator gel when conducting remote fertility assessments that involve measurement of E2 and/or T (8).

### Comparison with Previous Research

The impact of prolonged contact with serum separator gel is supported by previous research from our group, which found that measurements of E2 and T in serum were lower in capillary tubes

containing serum separator gel compared to those without, and the magnitude of the difference was time-dependent and likely due to adsorption of the analyte by the serum separator gel, which may be due to the lipophilic properties of the analytes (8). The time-dependent absorption by serum separator gel has been evidenced in studies analyzing various therapeutic and recreational drugs in larger serum and plasma draw volumes and with additional experiments extracting gel-bound analytes using high-performance liquid chromatography (12–21).

Serum separator gel did not appear to have an effect on E2 and T measurements from venous tubes in our study, which is consistent with reports of T measurements from venous serum tubes with and without serum separating gel being comparable (22). However, conflicting data showing that E2 and T measurements are lowered in venous tubes with serum separator gel have been reported, suggesting that further investigation may be necessary (23, 24).

Other than E2 and T, there is limited and conflicting published data on the effect of serum separator gel for the other 7 fertility markers investigated in this study. Measurements of FSH, TSH, and FT4 from venous tubes without and without serum separator gel have been described as comparable, suggesting there is minimal interference of serum separator gel on these analytes (24).

There is little previous data regarding the concordance between venous and capillary sample measurements for the fertility markers investigated; however, some research has been conducted for E2 and T using alternative methods of capillary blood collection. Capillary measurements of T collected via volumetric absorptive microsampling have been previously found to be discordant with paired venous measurements; however, it is unknown whether the venous tubes contained serum separator gel (25). There is evidence of a high concordance between E2 measurements

from venous and capillary dried blood spot (DBS) samples; however, the venous collection tube type in this study was again unknown (26). In contrast to the findings in our study, measurements of T in capillary and venous samples collected in tubes containing serum separating gel have recently been found to be highly concordant; however, capillary samples in this study were obtained from the upper arm of a smaller cohort of participants, processed immediately prior to freezing, and assayed after thawing (27).

The previously published data on concordance between venous and capillary measurements for the other fertility markers measured in this study are also inconclusive. One study found AMH measurements from venous and DBS samples to be comparable (28). Another recent study comparing AMH measurements from venous collection tubes, capillary DBS cards, and capillary collection tubes found the latter exhibited the higher concordance with venous measurements of AMH (29). Some historical data has suggested that TSH and FT4 measurements from capillary and venous serum have significant differences (8, 30, 31). However, more recent evidence taken from finger-prick DBS samples shows high concordance for these analytes (32). Early evidence has shown capillary and venous PROL measurements have a high degree of concordance, although details of tube type are unknown (33).

Data on capillary and venous sample concordance for the analytes of interest therefore exists; however, there is little comparative data that has specifically investigated capillary collection tubes and the impact of serum separator gel within these tubes.

### Study Limitations and Future Work

We acknowledge that there are some limitations to this study that may impact the interpretation of results. Samples were collected remotely, resulting in a lag time of 24 to 72 hours between sample collection and assaying. Future studies require

standardization of the time between sample collection and assay given the evidence of a time-dependent interference of serum separator gel and, to a lesser extent, clot activator on E2 and T measurements in capillary tubes. Given previous evidence of similar effects in other sex steroid hormones, further investigation of analytes such as androstenedione and progesterone is warranted, as serum separating gel may similarly impact their measurement (23). It is also important to acknowledge that an increased number of samples and data points may improve precision when estimating the magnitude of the effect. Additional experiments to recover E2 and T from serum separator gel would be beneficial to verify the impact of this blood collection tube component. We recognize the limitations of the conversion formulae presented, as they were derived from samples collected in our study and have not been rigorously tested for known sources of preanalytical variability, such as differences in tube manufacturers and components, nor were the formulae validated against different storage conditions, including time and temperature (7). In order to confidently apply these formulae widely to all E2 and T measurements from capillary blood collected in gel-containing serum separator tubes, the factors outlined here need to be thoroughly investigated.

However, key strengths of this study include it being the first to investigate the performance of capillary blood collection tubes for reproductive health and fertility assessment across a large number of commonly utilized analytes using data collected from a diverse distribution of analyte concentrations provided by human participants.

### CONCLUSION

This study provides compelling evidence that finger-prick capillary blood samples stored in serum capillary collection tubes without serum

separator gel result in comparative measurements to venous sampling for markers utilized in fertility assessments. However, capillary collection tubes containing serum separator gel yield lower

E2 and total T measurements, suggesting that they are not an effective tube type for measurement of these analytes and require the application of the conversion formula provided, if used.

**Nonstandard Abbreviations:** AMH, anti-Müllerian hormone; E2, estradiol; T, testosterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; cS-CAT, capillary serum clot activator tube; cSS-CAT, capillary serum separator clot activator tube; TSH, thyroid-stimulating hormone; FT4, free thyroxine; PROL, prolactin; vS-CAT, venous serum clot activator tube; vSS-CAT, venous serum separator clot activator tube; DBS, dried blood sample.

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