Letter to the Editor

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Capillary blood collection tubes containing serum separator gel result in lower measurements of oestradiol and total testosterone

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To the Editor,

The rise in at-home testing has prompted an increased use of capillary blood sampling for purposes such as fertility assessments, where analytes such anti-Müllerian hormone (AMH), sex hormones, thyroid hormones and gonadotropins are measured in serum. One widely adopted method for storing capillary blood prior to laboratory processing are collection tubes, of which there are several types manufactured for the purpose of separating serum from whole blood prior to assaying.

Despite the increased uptake of capillary blood collection tubes, there has been a paucity of research comparing the concordance of analyte measurements from tubes containing serum separator gel, commonly known as serum separator tubes (SSTs), to equivalent blood collection tubes which do not contain any such gel. This was highlighted by a recently published feature on at-home fertility assessments in the British Medical Journal, which reported that SSTs yield inaccurate measurements of oestradiol (E2), an analyte routinely evaluated during fertility assessments [1].

However, the magnitude and clinical importance of this effect remains unknown. In this correspondence, we present preliminary data that shows SSTs systematically lower measurements of both E2 and total testosterone (T), and estimate the effect size. We did not observe any such effect on other common fertility-related analytes, specifically AMH, follicle-stimulating hormone (FSH), luteinising hormone (LH), free thyroxine (FT4), thyroid-stimulating hormone (TSH), sex hormone-binding globulin (SHBG), and dehydroepiandrosterone sulphate (DHEAS). Additionally, no effect was detected when utilising capillary collection tubes without serum separator gel. By reporting these findings, we hope to inform laboratory practice and clinical interpretation of at-home fertility assessments which may still be using capillary SSTs to measure E2 and T.

We conducted two studies which led to these conclusions; the first was a clinical trial comparing the measurements of fertility-related analytes from capillary blood in collection tubes with serum separator gel (SSTs) to otherwise identical serum tubes without gel (STs). The second was a laboratory experiment to investigate the impact of serum separator gel on E2 measurements.

The first study was a trial conducted in accordance with the Declaration of Helsinki (as revised in 2013). Fourteen premenopausal female participants >18 years old were recruited following informed consent. A total of 34 paired capillary blood samples were collected from the participants via finger prick into MiniCollect® 1 mL STs and 0.8 mL SSTs (Greiner Bio-One, Kremsmünster, Austria) at various menstrual cycle phases. Serum concentrations of AMH, E2, FSH, LH, FT4, TSH, SHBG, T, and DHEAS were measured 24–72 h after collection via the Access 2 Immunoassay System (Beckman Coulter Diagnostics, California, US) (for AMH, E2, LH, FSH and DHEAS) or the Cobas® 8000 modular

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analyser series (Roche Diagnostics, Basel, Switzerland) (for T, SHBG, FT4 and TSH). All assays were performed by Inuvi Diagnostics Limited (UK). In total, the failure rate due to insufficient volume or haemolysis was 10.8 % (66 of 612 measurements), which varied between analytes from 0 % for FT4 (0 of 68 samples failed) to 16.2 % (11 of 68 samples failed) for LH and FSH due to the assay order and differential resistance to haemolysis. Failure rates also varied substantially between individuals; 6 of 14 participants had no failed samples whilst 43 of 66 failed measurements came from three participants.

Following log-transformation using the natural logarithm of base e, we tested for differences between ST and SST analyte measurements using paired t-tests. Table 1 presents the range of raw measurements and paired mean percentage differences with corresponding 95 % confidence intervals (CI) for each analyte, as well as the mean difference of log-transformed measurements (and 95 % CI), t-statistics (t) and p-values calculated from the aforementioned t-tests.

Results from this trial showed that measurements of E2 and T from ST were significantly higher than from SST ($p<0.0001$, n=27 for both) (Table 1), with the magnitude of the effect around 13 and 9 % for raw values of E2 and T respectively. No other statistically significant differences were observed between ST and SST measurements for any other fertility-associated analytes assayed (Table 1).

In response to these findings, experiments were conducted to investigate the impact of serum separating gel exposure on serum measurements of E2. We aliquoted 200 µL of a calibrator containing 19,100 pmol/L of E2 (Beckman Coulter Diagnostics, California, United States) into three MiniCollect® 0.8 mL SSTs. This was repeated using a venous blood sample from a female participant collected into a Vacuette® 4 mL K2E K2EDTA tube (Greiner Bio-One, Kremsmünster, Austria), from which 800–1,000 µL volumes were aliquoted into three MiniCollect® 0.8 mL SSTs. To investigate the effect of serum separator gel on both blood and calibrator samples, one SST from each set of three was stored at 18–22 °C for 4, 72, and 168 h respectively prior to processing and E2 being assayed via the Access 2 Immunoassay System. Table 2 reports the raw E2 measurement and percentage change at each time point.

Our results show a time-dependent decline of E2 in both the calibrator and serum samples (Table 2). Under an assumption that the rate of interaction decays with time, we estimate the decay rate constant λ to be −0.00192 and −0.00239 for the calibrator and whole blood respectively, suggesting a reduction of around 0.2 % per hour of storage within the SST.

Overall, our data suggest that capillary blood collection tubes containing serum separator gel result in a decrease of E2 and T in comparison to tubes without serum separator gel.

Table 1: Statistical analysis of natural log-transformed measurements taken from capillary serum tubes with or without serum-separator gel (SST and ST respectively).

Analyte	Paired n	Raw concentrations			Log-transformed concentrations	
		ST measurement range	SST measurement range	Paired mean percentage difference [95 % CI]	Paired mean log difference [95 % CI]	Test statistic (t); p-value
Anti-Müllerian hormone (AMH)	28	1.46–62.44 pmol/L	1.52–62.34 pmol/L	0.24 % [−7.13, 7.08]	0.0031 [−0.0120, 0.0181]	t=0.4195 p=0.6782
Oestradiol (E2)	27	114–1985 pmol/L	72–2033 pmol/L	13.32 % [2.17, 25.37]	0.1459 [0.1147, 0.1770]	t=9.6215 $p=4.7\times10^{-10}$
Follicle-stimulating hormone (FSH)	25	2.7–14.4 IU/L	2.6–14.3 IU/L	0.77 % [−3.73, 6.43]	0.0080 [−0.0027, 0.0188]	t=1.5478 p=0.1348
Luteinising hormone (LH)	25	2.1–27.6 IU/L	1.9–28.2 IU/L	1.13 % [−28.96, 17.53]	0.0200 [−0.0333, 0.0734]	t=0.7746 p=0.4462
Free thyroxine (FT4)	34	13.9–20.2 pmol/L	14.2–19.6 pmol/L	−0.28 % [−5.09, 3.59]	−0.0025 [−0.0108, 0.0057]	t=−0.6302 p=0.5329
Thyroid-stimulating hormone (TSH)	33	0.86–3.60 mIU/L	0.82–3.56 mIU/L	0.78 % [−4.72, 9.48]	0.0086 [−0.0055, 0.0226]	t=1.2400 p=0.2240
Sex hormone-binding globulin (SHBG)	27	19–135 nmol/L	19–140 nmol/L	0.16 % [−6.42, 6.67]	0.0022 [−0.0125, 0.0169]	t=0.3134 p=0.7565
Total testosterone (T)	27	0.43–3.36 nmol/L	0.48–3.20 nmol/L	8.67 % [−10.77, 23.22]	0.0956 [0.0559, 0.1350]	t=4.9575 $p=3.8\times10^{-5}$
Dehydroepiandrosterone sulphate (DHEAS)	27	3.5–10.5 nmol/L	4.1–10.2 nmol/L	−0.30 % [−3.44, 3.22]	−0.0028 [−0.0110, 0.0054]	t=0.6932 p=0.4943

ST, Minicollect® Serum Clot Activator Tube; SST, Minicollect® Serum Separator Clot Activator Tube; Log, natural log using base e. Differences calculated as ST values minus SST values.

Table 2: Measurements of oestradiol (E2) in E2 calibrator and serum samples stored in capillary serum separator tubes (SST).

	Hours (h) stored in SST prior to assay		
	4 h	**72 h**	**168 h**
Oestradiol (E2) in calibrator sample, pmol/L [percentage change from 4h]	19,683 [0.0]	17,307 [−12.1 %]	14,364 [−27.0 %]
Oestradiol (E2) in serum sample, pmol/L [percentage change from 4h]	170 [0.0]	151 [−11.2 %]	114 [−32.9 %]

This supports previous reports of lower venous measurements of E2 and T in collection tubes containing serum separator gel, which was hypothesised to be due to adsorption by the gel [2, 3]. Numerous commercially available kits designed for at-home assessment of serum E2 and T in capillary blood samples use SSTs. Our findings show that capillary SSTs have a substantial and significant effect on E2 and T measurements which may impact clinical interpretation.

Broadly, serum E2 and T concentrations may be used to assess ovarian, testicular and adrenal function, as well as investigate the Hypothalamic-Pituitary-Gonadal axis. If interpreting measurements of these analytes against a reference range, typically defined by a lower limit at the 2.5th percentile and an upper limit at the 97.5th percentile, there are two scenarios that may occur if laboratory measurements underestimate genuine physiological concentrations. Firstly, physiological levels which moderately exceed the 2.5th percentile may be misclassified as below the reference range. Secondly, if physiological levels moderately exceed the 97.5th percentile, they may be misinterpreted as within the reference range. Depending on the guidance followed, low or high serum concentrations of E2 or T may be used to diagnose conditions such as hypogonadism and polycystic ovary syndrome respectively [4, 5]. Although it is unlikely that measurements of either analyte would be used in isolation for diagnostic purposes, the potential impact of a falsely low or within range result on interpretation and management remains uncertain. According to the manufacturers, the total imprecision specification has been defined as 3–9 % CV at 89–1095 pmol/l of oestradiol and 3.6 % CV at 9 nmol/L of testosterone for the assays utilised in this study. In addition, it is worth noting that the total allowable error for both E2 and T assays is cited as 25 %, which will also impact the clinical significance of the decrease observed in SSTs [6].

There are some limitations to the clinical trial reported in our first study, notably the small sample size and random time lag between blood collection and processing. In the second study, an experiment with additional time-point measurements and several replicates, with ST tubes investigated in parallel, would assist in a better understanding of the time-dependent effects. Additionally the recovery of E2 from the serum separator gel was not attempted. This is particularly important as there are multiple factors other than serum separator gel which are known to interfere with analyte measurement [7]. The same experiment was not conducted on T, however given it has lipophilic properties comparable to E2 (logP values for these analytes are 3.32 and 4.01 respectively), a similar interaction with the serum separator gel is plausible [8–10].

We have shown that SSTs should not be utilised for measuring serum E2 or T in capillary blood collected remotely. However, capillary blood collection tubes without serum separator gel should be encouraged for at-home fertility assessments, as they do not impact serum measurements of fertility-related analytes. In response to these findings, we urge laboratories testing these analytes to undertake verification to ISO 15189 standards, and include investigation of transport stability over the expected temperatures and time periods. As the landscape of at-home testing continues to evolve, investigating the potential impact of capillary blood collection methods on analyte measurements remains a vital area of research to ensure the accuracy and reliability of remote health assessments.

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Research ethics: The local Institutional Review Board, the Health Research Authority (HRA) guidance for Research Ethics Committee (REC), deemed the study exempt from review as the project did not involve the National Health Service and does not come under governance arrangements for REC for NHS Ethics review. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: HON, NG and TW were primarily responsible for the conceptualisation of the study. TV was responsible for drafting the original manuscript; all authors reviewed and provided substantial intellectual input prior to submission. The methodology of the clinical trial was designed by NG, TV and AT. TV was responsible for data collection and recruitment of participants and AT was responsible for statistical analysis. The methodology of gel experiments was designed by TW, and gel experiments were conducted by TW, ER and RB.

Competing interests: HON, NG and TV are employed by and own options at Hertility Health Limited, who provided financial support for this study. TW, ER and RB are employed by Inuvi Diagnostics Limited who conducted all assays described in this manuscript. AT states no conflict of interest.
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Data availability: The raw data can be obtained on request from the corresponding author.

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