RESEARCH

Oral estrogen leads to falsely low concentrations of estradiol in a common immunoassay

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Abstract

Objectives: Recently, an estradiol immunoassay manufacturer (Beckman Coulter, USA) issued an 'important product notice' alerting clinical laboratories that their assay (Access Sensitive Estradiol) was not indicated for patients undergoing exogenous estradiol treatment. The objective of this analysis was to evaluate immunoassay bias relative to liquid chromatography tandem mass spectrometry (LC-MS/MS) in transgender women and to examine the influence of unconjugated estrone on measurements.
Design: Cross-sectional secondary analysis.
Methods: Estradiol concentrations from 89 transgender women were determined by 3 immunoassays (Access Sensitive Estradiol ('New BC') and Access Estradiol assays ('Old BC'), Beckman Coulter; Estradiol III assay ('Roche'), Roche Diagnostics) and LC-MS/MS. Bias was evaluated with and without adjustment for estrone concentrations. The number of participants who shifted between three estradiol concentration ranges for each immunoassay vs LC-MS/MS (>300 pg/mL, 70–300 pg/mL, and <70 pg/mL) was calculated.
Results: The New BC assay had the largest magnitude overall bias (median: −34%) and was −40%, −22%, and −10%, among participants receiving tablet, patch, or injection preparations, respectively. Overall bias was −12% and +17% for the Roche and Old BC assays, respectively. When measured with the New BC assay, 18 participants shifted to a lower estradiol concentration range (vs 9 and 10 participants based on Roche or Old BC assays, respectively). Adjustment for estrone did not minimize bias.
Conclusions: Immunoassay measurement of estradiol in transgender women may lead to falsely decreased concentrations that have the potential to affect management. A multidisciplinary health care approach is needed to ensure if appropriate analytical methods are available.

Key Words
- estradiol
- estrone
- estrogens
- mass spectrometry
- immunoassay
- transgender
- hormone therapy

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Introduction

Estradiol (17β-estradiol) is a natural sex steroid available in several exogenous preparations. The Endocrine Society recommended high-dose exogenous estradiol treatment as one part of feminizing hormone therapy for transgender women (1) – people with a female gender identity who were assigned male at birth. The Endocrine Society and World Professional Association for Transgender Health (WPATH), two international transgender health-focused professional organizations, recommended clinical laboratory monitoring of serum estradiol concentrations during the first year of feminizing hormone therapy (1, 2, 3). Despite these recommendations, neither organization commented on best practices or established challenges associated with determining exogenous estradiol concentrations in clinical settings (4).

Clinicians and laboratory scientists typically determine endogenous estradiol concentrations using commercial immunoassays due to ease of use, rapid result turnaround time, and affordability (5). Despite these advantages, early reports established cross-reactivity between estrogenic metabolites (including unconjugated and conjugated estrone, a weak estrogen) and anti-estradiol antibodies used in immunoassays (4). On April 7, 2021, a manufacturer issued an important product notice alerting customers that a widely used estradiol immunoassay was not indicated for people undergoing exogenous estradiol treatment (Access Sensitive Estradiol, Beckman Coulter, Brea, CA, USA). A related US Food and Drug Administration Medical Device Report documented an occurrence of low measured estradiol concentrations in a patient undergoing exogenous estradiol treatment during an ovarian stimulation protocol (6). Further discussion with the vendor indicated that supraphysiologic circulating estrone concentrations, a metabolite of exogenous estradiol treatment (7, 8), may interfere with estradiol detection (Beckman Coulter Incorporated, personal communication).

We previously reported 10- to 12-fold higher estrone concentrations among transgender women taking exogenous estradiol tablet preparations compared with transgender women taking non-oral estradiol preparations (patch or injection) (9, 10). To examine the effect of unconjugated estrone concentrations on estradiol immunoassay interference, we determined analytical bias relative to liquid chromatography tandem mass spectrometry (LC-MS/MS) in three estradiol commercial immunoassays among transgender women undergoing feminizing hormone therapy with tablet and non-oral estradiol preparations.

Materials and methods

Samples

We prospectively collected single whole blood specimens (5-mL gold-top serum separator tube) from a cohort of transgender women between 2017 and 2018 at two US lesbian, gay, bisexual, transgender, queer (LGBTQ)-focused clinics to determine estradiol and estrone concentrations, as described previously (9, 10). Among 89 participants with available samples for this analysis, all participants underwent at least 12 months of feminizing hormone therapy based on clinical need with estradiol tablets (n = 51, total amount (range): 2–8 mg daily), patches (n = 9, total amount (range): 100–200 μg daily), or injections (n = 29, total amount (range): 3–10 mg weekly) at time of specimen collection (1, 10). We separated and stored serum aliquots at –80° until analysis. This study was approved by the Western Institutional Review Board and University of Iowa Institutional Review Board and all participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Estradiol and estrone assays

In this analysis, we determined serum estradiol concentrations using Access Sensitive Estradiol assay run on the DxI800 ('New BC,' Beckman Coulter) (11). We previously determined estradiol concentrations using two other commercial immunoassays as described (9): (1) Estradiol III assay run on Cobas E601 analyzer ('Roche', Roche Diagnostics GmbH) and (2) Access Estradiol assay run on a DxI800 analyzer, the previous generation estradiol assay ('Old BC', Beckman Coulter). Table 1 summarizes apparent cross-reactivities of four estradiol immunoassays reported in package insert information, including three immunoassays used in the present study (11, 12, 13, 14). We previously determined serum estradiol and estrone concentrations by a LC-MS/MS method (6500 QTrap, Applied Biosystem Sciex) with linearity over calibration ranges of 4–700 pg/mL for estradiol and 10–5000 pg/mL for estrone (10).

Data analysis

We evaluated each estradiol immunoassay method vs LC-MS/MS using Bland-Altman plots (15). Because we previously observed statistically significant differences in estrone concentrations between oral and non-oral estradiol preparations (10), we evaluated bias by estradiol

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Table 1 Apparent estradiol cross-reactivities reported in assay package inserts (11, 12, 13, 14).

Compound[a]	Beckman Access Estradiol ('Old BC')	Beckman Access Sensitive Estradiol ('New BC')	Roche Estradiol II	Roche Estradiol III
Estradiol	100%	100%	100%	100%
Estrone	1.98%	0.40%	0.811%	0.757%
Estrone-3-sulfate	0.01%	0.0010%	0.006%	0.002%
Estrone-3-glucuronide	No cross-reactivity detected	0.0010%	0.002%	0.003%
2-Hydroxyestrone	No data	No data	No data	No data
4-Hydroxyestrone	No data	No data	No data	0.754%
16α-Hydroxyestrone	No data	No data	No data	No data
Estriol	0.50%	0.050%	0.218%	0.233%
Ethinyl estradiol	0.37%	0.030%	0.231%	0.334%

Roche Estradiol II was not utilized in the present study but was included for comparison of cross-reactivity.
[a]Estrone is the main metabolite of estradiol. Other compounds (except ethinyl estradiol) are additional estrone metabolites. Ethinyl estradiol is a synthetic estrogen common in oral contraceptives but not used in feminizing hormone therapy.

preparation (tablet, patch, and injection). We tested data normality using the Shapiro–Wilk test ($P > 0.05$) and performed non-parametric Kruskal–Wallis one-way ANOVA for all four estradiol assay methods with Dunn's test for multiple comparisons. We summarized continuous variables as medians and interquartile ranges. A two-sided P-value < 0.05 was considered statistically significant. Statistical analysis was performed using SAS software (SAS Institute Inc., Cary, NC, USA).

To associate inaccuracies in immunoassay-measured estradiol concentrations with potential changes in clinical management of feminizing hormone therapy (i.e., estradiol dose adjustment), we grouped LC-MS/MS estradiol results according to concentration ranges designated conservatively as 'supraphysiologic' (>300 pg/mL), 'within desired range' (70–300 pg/mL), and 'sub-physiologic' (<70 pg/mL). We selected these estradiol concentration ranges using a combined approach of empirical guideline-based ranges (1), prospectively derived estradiol concentration ranges determined previously within this cohort (9), and consensus among gender care providers in the community.

Results

Overall, the New BC assay demonstrated median bias of −34% relative to LC-MS/MS ($n = 89$; Fig. 1A). We observed the greatest magnitude of bias within the tablet subgroup ($n = 51$, median: −40%) relative to the patch or injection subgroups ($n = 9$, −22% and $n = 29$, −10%, respectively). When considering the influence of estrone concentrations, the magnitude of bias in measured estradiol concentrations appeared to increase as estrone concentrations increased (Fig. 1D) but was constant when estrone concentration was plotted relative to estradiol concentration (estrone/estradiol ratio) (Fig. 2A).

For the Roche assay, we observed median overall bias of −12%. Within the tablet, patch, or injection subgroups, median bias was −14%, −13%, and −3% (Fig. 1B). Bias was relatively constant across a range of estradiol concentrations (Fig. 1E) and estrone/estradiol ratios (Fig. 2B). For the Old BC assay, we observed median overall bias of +17% ($n = 88$) relative to LC-MS/MS. Similar to the New BC assay, we observed the greatest magnitude of bias within the tablet subgroup (median: +23%) relative to the patch or injection subgroups (−17%, $n = 8$ and +8%, respectively) (Fig. 1C). Bias increased with increasing estrone concentration (Fig. 1F) but was relatively constant across a range of estrone/estradiol ratios (Fig. 2C).

When comparing measured estradiol concentrations among all estradiol assay methods, participants taking estradiol tablets had statistically significantly lower median estradiol concentrations using the New BC assay compared with LC-MS/MS assay (90 pg/mL vs 152 pg/mL, respectively, $P < 0.0001$) (Supplementary Table 1, see section on supplementary materials given at the end of this article) (11, 12, 13, 14). New BC median estradiol concentrations were statistically significantly lower compared with the Old BC assay (201 pg/mL, $P < 0.001$) and Roche assay (132 pg/mL, $P = 0.0001$). Among participants taking estradiol patch or injection, estradiol concentrations were similar between LC-MS/MS and all three estradiol immunoassays (overall P values: patch, $P = 0.6852$; injection, $P = 0.4200$).

Using the New BC assay, 71 (of 89, 79.8%) of measured estradiol concentrations did not shift between concentration groups relative to LC-MS/MS (Fig. 3A). In the >300 pg/mL concentration group, seven participants shifted to the lower adjacent concentration group (70–300 pg/mL). In the 70–300 pg/mL group, 11 participants

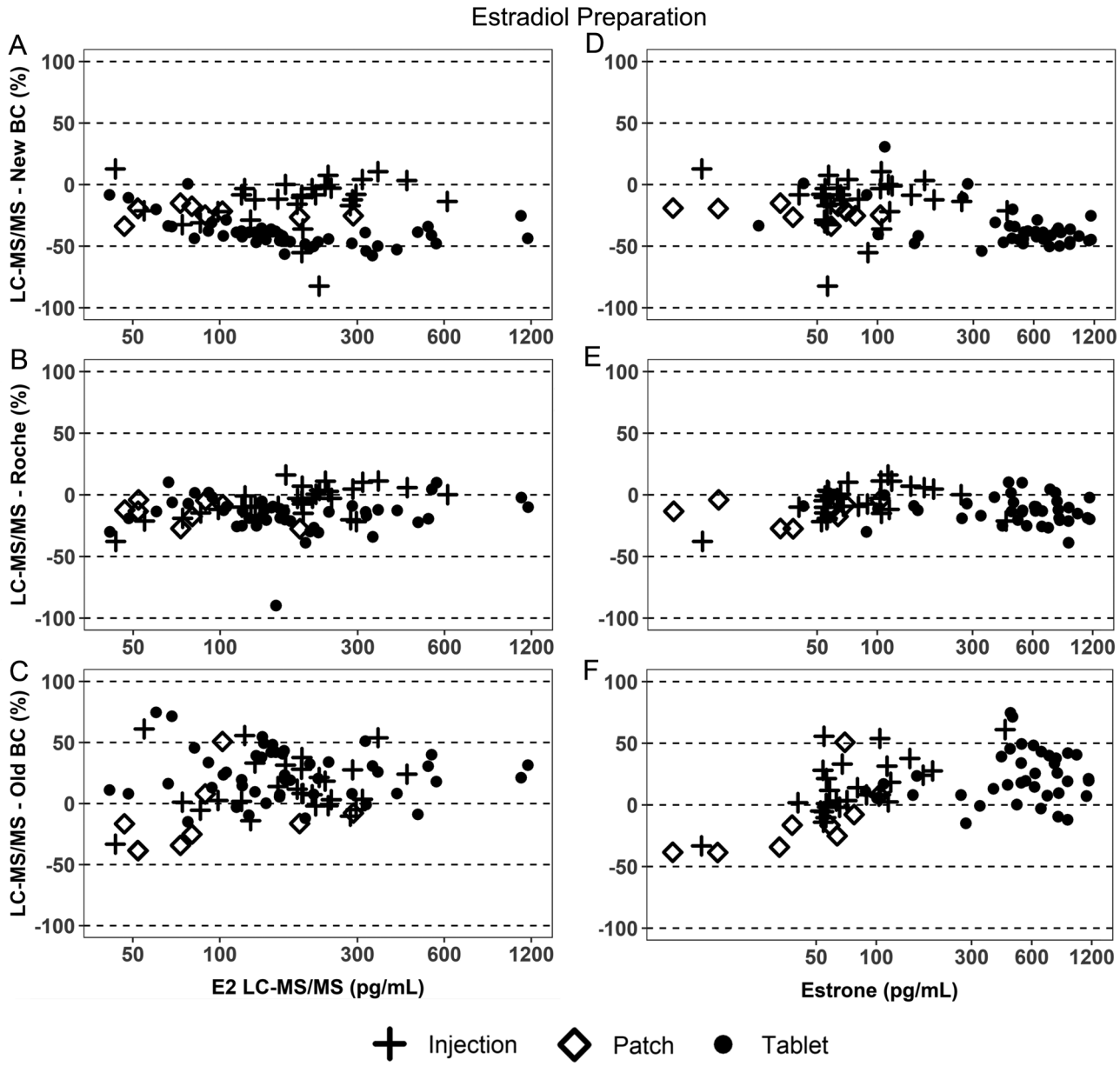

Figure 1
Bland–Altman plot of percent bias between immunoassays and LC-MS/MS estradiol according to LC-MS/MS estradiol (A, B, and C) and LC-MS/MS estrone (D and E) concentrations (New BC, panels A and D; Roche, panels B and E; Old BC, panels C and F).

shifted to the lower adjacent concentration group based on the New BC assay (<70 pg/mL).

Using the Roche assay, 79 (of 88, 89.8%) of measured estradiol concentrations did not shift concentration groups relative to LC-MS/MS (Fig. 3B). In the >300 pg/mL concentration group, three participants shifted to the lower adjacent concentration group (70–300 pg/mL). In the 70–-300 pg/mL group, four participants shifted to the lower adjacent concentration group (<70 pg/mL), whereas one participant shifted to the higher concentration group.

In the <70 pg/mL concentration group, one participant shifted to the higher adjacent concentration group. Using the Old BC assay, 78 (of 88, 88.6%) of measured estradiol concentrations did not shift concentration groups relative to LC-MS/MS (Fig. 3C). In the >300 pg/mL concentration group, no participants shifted to lower concentration groups. In the 70–300 pg/mL group, three participants shifted to the lower adjacent concentration group (<70 pg/mL), whereas three participants shifted to the higher concentration group. In the <70 pg/mL concentration

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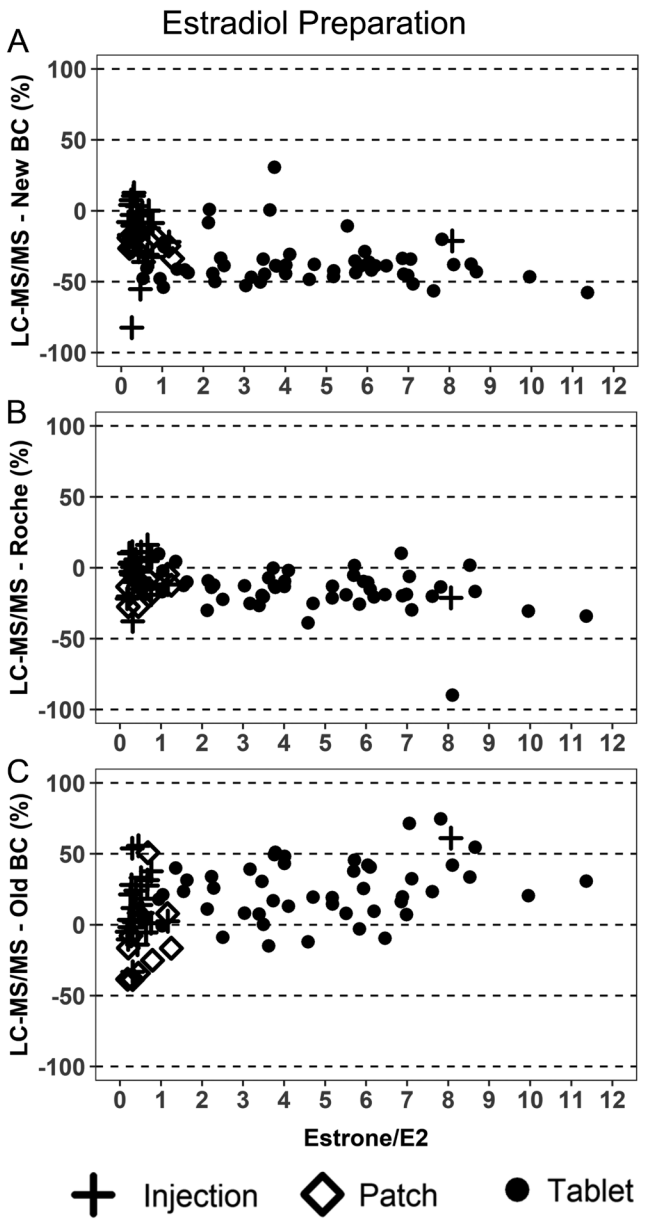

Figure 2
Bland–Altman plot of percent bias between immunoassays and LC-MS/MS according to LC-MS/MS estrone-to-estradiol ratio.

group, four participants shifted to the higher adjacent concentration group.

Discussion

We are the first investigators to evaluate bias across three estradiol immunoassays relative to LC-MS/MS in a clinical cohort of transgender women undergoing feminizing hormone therapy. Motivated by a recent manufacturer notice regarding analytical interference for estradiol immunoassays related to circulating estradiol metabolites, we observed considerable negative bias using the New BC assay (Access Sensitive Estradiol, Beckman Coulter) relative to LC-MS/MS (−34%). The observed bias was more pronounced using the New BC assay relative to the Roche and Old BC estradiol immunoassays. When comparing estradiol concentrations between each assay method across estradiol preparations, New BC assay estradiol concentrations were statistically significantly lower compared with those measured using LC-MS/MS. These findings conflict with package insert data from 87 samples, in which New BC assay showed strong positive correlation relative to LC-MS/MS assay over estradiol concentrations 17–2119 pg/mL (r = 0.97) (11). We recommend that clinicians and laboratory scientists determine which estradiol preparation patients are taking to facilitate measurement with a different immunoassay or via LC-MS/MS among transgender adults taking oral estradiol tablets.

Using the New BC assay, we observed nearly half of participants in the >300 pg/mL concentration group (determined by LC-MS/MS) shifted to the 70–300 pg/mL estradiol concentration range (7 of 15 participants). Because the Endocrine Society and WPATH recommended monitoring estradiol concentrations to detect 'supraphysiologic' concentrations during feminizing hormone therapy (1, 2, 3), clinicians may dose-decrease estradiol treatment in response to high estradiol measures. Our findings suggest that care providers using the New BC assay may not detect clinically actionable estradiol concentrations, specifically measures that warrant dose-decreased estradiol treatment. This exposure may place transgender patients at risk for estradiol exposure-related adverse events (1), although exact estradiol concentrations associated with increased risk of cardiovascular toxicities remain to be determined for transgender patients (16).

Although data are lacking for transgender women undergoing estrogen treatment, several investigators examined immunoassay performance among adults taking oral exogenous estradiol treatment for either ovarian stimulation or menopausal hormone therapy (17, 18). Dancoine *et al.* observed agreement between an automated chemiluminescent estradiol assay method (Immulite, Diagnostic Products Corp., Los Angeles, CA, USA) and RIA among 41 cisgender women undergoing exogenous estradiol treatment as part of ovarian stimulation protocols, although most participants took non-oral estradiol preparations (17). Cook *et al.* observed discrepant estradiol concentrations among three commercial immunoassays and a RIA method among cisgender women undergoing

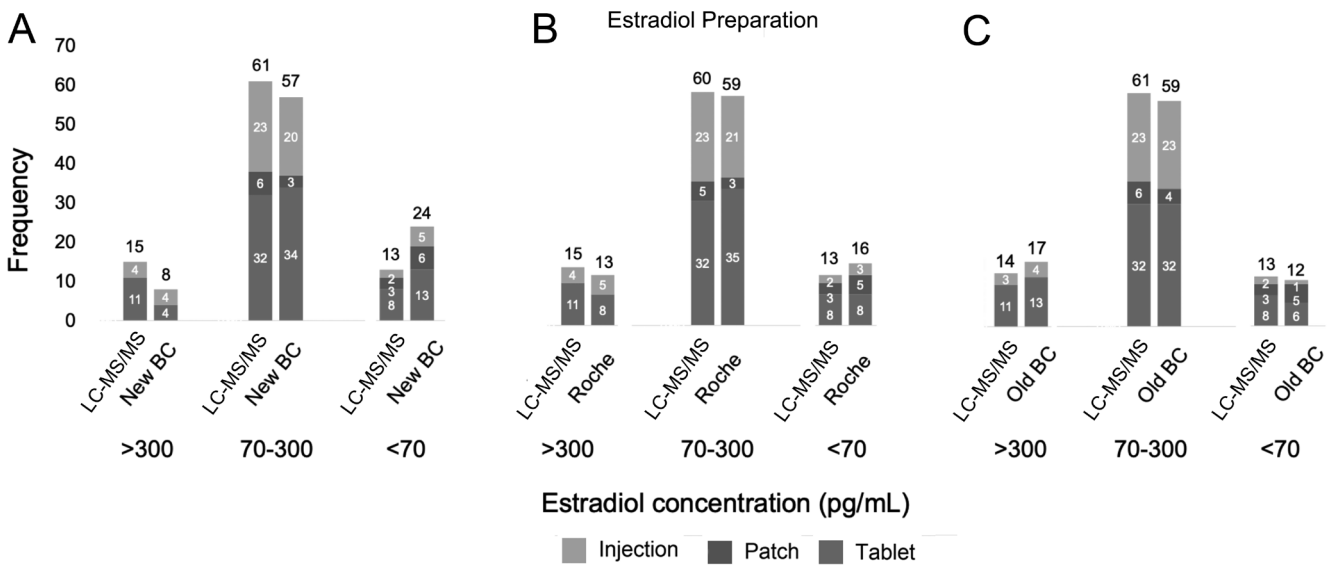

Figure 3
Frequency of participants who shifted between estradiol concentration groups ('supraphysiologic' estradiol concentration range: >300 pg/mL; 'within desired range' estradiol concentration range: 70–300 pg/mL; and 'sub-physiologic' estradiol concentration range: <70 pg/mL) based on immunoassay versus vs liquid chromatography and tandem mass spectrometry (LC-MS/MS). (A) Estradiol concentration group shifts between LC-MS/MS vs New BC. (B) Estradiol concentration group shifts between LC-MS/MS vs Roche. (C) Estradiol concentration group shifts between LC-MS/MS vs Old BC.

oral menopausal hormone therapy, although all participants were taking oral conjugated estrogens, which has established cross-reactivity with commercial estradiol immunoassay methods (18, 19). Cao *et al.* observed unconjugated estriol, an active estradiol metabolite that is elevated during pregnancy, led to negative interference in an estradiol microparticle enzyme immunoassay (AxSYM, Abbott Laboratories), although this finding was based on assay proficiency testing samples (20, 21).

We previously reported statistically significantly higher median estrone concentrations among participants taking estradiol tablets within this cohort (693.0 pg/mL) compared with those taking non-oral preparations (patch: 58.6 pg/mL, injection: 67.5 pg/mL, both $P < 0.001$ vs tablet) (10). Other investigators similarly observed numerically higher estrone concentrations among transgender women taking oral estradiol tablets compared with estradiol patches (410 pg/mL (95% CI, 347–473 pg/mL) vs 51 pg/mL (95% CI, 41–60 pg/mL), respectively) (22). These findings are consistent with extensive first-pass metabolism of oral estradiol tablets to the metabolite estrone, which circulates predominantly as estrone-3-sulfate and is further metabolized to estrone glucuronide, 2-hydroxyestrone, 4-hydroxyestrone, 16α-hydroxyestrone, and estriol (21).

Typically, investigators suggest estradiol metabolites to contribute to potential cross-reactivity with assay antisera leading to positive bias in estradiol concentration measurements (18, 23). Immunoassay package insert cross-reactivity data predicted minimal interference by estrone and its conjugates on measured estradiol concentrations (11, 12, 13, 24). Of note, the New BC assay package insert reported lower cross-reactivity with estrone and estrone-3-sulfate compared with the Old BC assay (Table 1) (11, 12, 13, 14). This may indicate that interference was not detected by routine cross-reactivity testing protocols commonly reported in assay package inserts. Alternatively, other interferences, including well-established elevations in sex hormone-binding globulin during oral estradiol treatment (22), may lead to underestimated estradiol concentrations (18, 23). One limitation of this analysis is that we only measured unconjugated estrone metabolite using LC-MS/MS. Future studies should analyze a broader panel of estrone metabolites, including the conjugated and hydroxylated metabolites, and sex hormone-binding globulins to better characterize their role in estradiol assay interference among transgender adults.

Negative assay bias, as we observed in this analysis, may place patients at risk for inappropriately high estradiol dosing. The safety implications of prolonged, supratherapeutic estradiol concentrations for transgender adults are largely unknown (16), although accurate estradiol measures are an important tool for determining estradiol concentrations associated with increased venous thromboembolic risks. Despite its importance in transgender medical care, estradiol concentration determination in clinical settings is complicated

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(25, 26, 27). Because transgender people face disparities in all aspects of health care including in clinical laboratory settings (28), accurate estradiol measurement is a crucial component of safe, equitable medical care access for transgender adults. Clinical laboratories are often siloed from other areas of the healthcare team, and laboratory scientists typically do not have immediate access to the clinical indication for serum estradiol laboratory orders. Although there is increased interest in adapting electronic medical records to include sexual orientation and gender identity data for clinical decision support (29), additional work is needed to systematically alert laboratory personnel about whether a specific immunoassay may be required for a transgender person undergoing feminizing hormone therapy.

Despite a lack of evidence to support traditional therapeutic drug monitoring of estradiol during feminizing hormone therapy, this approach appears to persist in clinical practice (30). Current transgender health-focused guides do not endorse therapeutic drug monitoring for estradiol treatment; instead, they recommend monitoring estradiol treatment for 'supraphysiologic' concentrations (1, 2, 3), although this definition may vary depending on expert opinion and laboratory reference intervals. Importantly, no data are available to establish specific serologic data with estradiol efficacy or safety during feminizing hormone therapy (e.g. breast development and cardiovascular risks) (16, 31), which is essential to justify therapeutic monitoring of any drug (32). Given the potential for interference by exogenous estradiol and negative bias reported in our analysis, these factors need to be examined collectively for laboratory scientists to make informed recommendations on how to determine estradiol concentrations in clinical settings and when to dose-adjust estradiol treatment among transgender patients. As new, highly sensitive immunoassays come to the market, manufacturers and regulatory bodies need to consider the transgender population and its medical needs when evaluating new devices to ensure potential health care disparities are mitigated.

Conclusion

The Beckman Coulter Access Sensitive Estradiol assay demonstrated considerable negative bias when used among transgender women undergoing feminizing hormone therapy. Clinicians and laboratory scientists currently lack strategies to systematically select patients who may benefit from specific immunoassay or LC-MS/MS methods. Professional societies involved with transgender medicine should speak to this issue to increase awareness among clinicians and laboratory scientists.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-21-0550.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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