# Reproductive Endocrinology Reference Intervals for Transgender Men on Stable Hormone Therapy

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**Background:** Gender-affirming therapy with testosterone is commonly prescribed to aid in the masculinization of transgender men. Sex-hormone concentrations are routinely measured, but interpretation of results can be difficult due to the lack of published reference intervals.

**Methods:** Healthy transgender individuals who had been prescribed testosterone ($n = 82$) for at least a year were recruited from internal medicine and primary care clinics that specialize in transgender medical care. Total testosterone and estradiol were measured using immunoassay and mass spectrometry; LH, FSH, SHBG, prolactin, progesterone, anti-Müllerian hormone (AMH), and dehydroepiandrosterone sulfate (DHEAS) were measured using immunoassay; free testosterone was calculated. Reference intervals (central 95%) were calculated according to Clinical Laboratory Standards Institute guidelines.

**Results:** When evaluating general endocrine laboratory tests in people using masculinizing hormones, reference intervals for cisgender men can be applied for total and free testosterone and SHBG and reference intervals for cisgender women can be applied for prolactin. Reference intervals for estradiol, LH, FSH, AMH, and DHEAS differ from those used for cisgender men and cisgender women, and therefore should be interpreted using intervals specific to the transmasculine population. For testosterone and estradiol, results from immunoassays were clinically equivalent to mass spectrometry.

**Conclusion:** Masculinizing hormones will alter the concentrations of commonly evaluated endocrine hormones. Providers and laboratories should use appropriate reference intervals to interpret the results of these tests.

## INTRODUCTION

Gender-affirming hormone therapy is standard of care for transgender people who seek to medically transition (1). Testosterone is prescribed to people assigned female at birth that identify as male (transgender male) or identify as being masculine of center (nonbinary; transmasculine). Testosterone administration promotes masculinizing secondary sex characteristics such as

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## IMPACT STATEMENT

Gender-affirming hormone therapy is standard of care for transgender and nonbinary people who seek to medically transition. When masculinization is the goal, testosterone administration allows for several phenotypically male characteristics to develop. Testosterone use will also alter the systemic concentration of sex-specific laboratory tests, such as hemoglobin and hematocrit. In this study, reference intervals for commonly measured endocrine reference intervals were established.

increased muscle mass, increase in facial and body terminal hair development, decreased vocal pitch, and cessation of menses (2). Additionally, testosterone administration will alter the concentrations of routinely monitored laboratory values, such as hemoglobin and hematocrit (3). Recent studies have shown that people administering gender-affirming testosterone for at least a year will have hematological parameters that parallel those of cisgender men (3).

Serum testosterone concentrations are commonly monitored in people who are prescribed gender-affirming hormone therapy (1). The Endocrine Society Guidelines state that testosterone should be measured every 3 months for the first year and at least annually thereafter. Suggested treatment goals are concentrations of 400–700 ng/mL, or to fall within the age-matched cisgender male reference interval (1). This recommendation is not graded within the guideline, indicating there is limited evidence in the medical literature to support the interval and that the range is empirically derived through clinical judgment. The guidelines have no mention of the expected or appropriate concentration interval for other endocrine markers specific to people on masculinizing hormones, although estradiol will commonly be measured, and in some cases, particularly if fertility is a goal, progesterone, LH, and/or FSH concentrations may also be evaluated (4, 5).

Interpretation of endocrine laboratory values in people on masculinizing therapy is limited by the lack of published literature establishing reference intervals specific to this population (6). The objective of this study was to establish reference intervals for common endocrine laboratory measurements in transgender people receiving masculinizing hormone therapy, specifically testosterone, estradiol, SHBG, FSH, LH, progesterone, prolactin, AMH, and DHEA-S. We selected these analytes because they are the most commonly measured endocrine-related analytes in the clinical laboratory and they have sex-specific reference intervals. Further, testosterone and estradiol are almost always evaluated before and after initiation of gender-affirming therapy, even if threshold concentrations are empirically derived. An additional objective was to evaluate the adequacy of immunoassay measurements relative to mass spectrometry for testosterone and estradiol monitoring in this population.

## METHODS

### Patient Recruitment, Questionnaires, and Sample Collection

Study participants were prospectively recruited from 2 lesbian, gay, bisexual, transgender, and queer (LGBTQ)-oriented primary care and internal medicine clinics in Seattle, Washington, and Iowa City, Iowa, between November 1, 2017, and July 1,

2018. Consent was obtained for venipuncture collection of whole blood (2 × 5 mL into gold top serum separator tube). Basic demographic information and hormonal therapy (dose, mode of administration, duration of therapy) were collected using a standardized questionnaire. Study numbers were used in place of participant names; no patient identifiers were retained.

Participants were at least 18 years old, self-identified as transgender or gender nonbinary, had been prescribed testosterone gender-affirming hormone therapy for at least 1 year, and consented to collection of relevant samples and information. Specific demographics of the study participants have been previously published (3). Exclusion criteria included past history of diabetes, severe cardiovascular event (e.g., myocardial infarction or stroke), clotting or blood cell disorders (e.g., sickle cell anemia, deep venous thrombosis), HIV infection, obstructive sleep apnea, active cigarette use, current pregnancy or current body mass index (BMI) >30. Eligibility to participate was not influenced by any other criteria. The Western Institutional Review Board (IRB) approved the protocol for samples collected in Seattle (study number 1179338). The University of Iowa IRB approved the protocol for samples collected in Iowa (study number 201710702).

### Sample Analysis

Total testosterone, estradiol, FSH, LH, SHBG, prolactin, and progesterone were measured using the Beckman Coulter DxI and the Roche Cobas immunoassay instruments; LH, anti-Müllerian hormone (AMH), and dehydroepiandrosterone sulfate (DHEAS) were measured on the Cobas only. For testosterone results from each platform used, free testosterone was calculated from total testosterone and SHBG using the equation derived by Ly and Handelsman (7). Additionally, total testosterone and estradiol were quantified using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) at The Permanente Medical Group Regional Laboratory in Northern California and Seattle Children's Hospital, respectively. Measurements were performed either within 8 hours of serum collection (Seattle cohort on DxI) or frozen immediately, stored at −80 °C and measured within 3 months of collection (Iowa cohort on DxI; Seattle and Iowa cohorts on the Cobas; all samples for mass spectrometry). The participating laboratories followed standard quality practices and are accredited through the College of American Pathologists.

### Statistical Analysis

Data were analyzed following CLSI C28-A3 (8). In brief, distributions were transformed to achieve normality using a Box–Cox transformation. Normality was assessed using the Shapiro–Wilk test. Outliers were evaluated using the Tukey test on the transformed distributions. In some cases, it was not possible to transform the data to a normal distribution using Box–Cox transformation. In those cases, we identified outliers by visual inspection of histograms. Confidence intervals for reference limits were calculated using bootstrapping. Statistical calculations were performed using STATA 16 (STATA LLP).

## RESULTS

### Study Participants

Serum samples were collected from 82 adults using masculinizing hormones for at least one year (median 3 years; range 1–17 years; IQR 1.8–5.0 years). The demographics of these cohorts have been previously published (3). Briefly, the median age was 27 years (range 19–55 years; IQR 23–33 years). The majority of participants ($n = 76$, 93%) administered testosterone intramuscularly or subcutaneously (median dose 80 mg/week; range and IQR 50–100 mg/week); 5 participants (6%) administered testosterone topically (median

**Table 1. Reference intervals and confidence limits for common endocrine laboratory measurements in a cohort of healthy transgender men on stable gender-affirming hormone therapy across instruments.**

| | | Reference limits | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Analyte** | **Platform** | **2.5** | **Low CI** | **High CI** | **97.5** | **Low CI** | **High CI** | ***N*** |
| AMH (ng/mL) | Roche | 0.02 | NC | NC | 14.0 | 10.5 | 17.6 | 78 |
| DHEAS (μg/dL) | Roche | 51 | 14 | 88 | 642 | 471 | 813 | 78 |
| Estradiol (pg/mL) | DxI | 29 | NC | NC | 87 | 64 | 110 | 77 |
| Estradiol (pg/mL) | Roche | 4.0 | 2.7 | 5.3 | 77 | 53 | 101 | 75 |
| Estradiol (pg/mL) | LC–MS/MS | 10.6 | 8.2 | 13.0 | 115 | 63 | 168 | 79 |
| FSH (mIU/mL) | DxI | 1.0 | 0.8 | 1.1 | 42 | 28 | 56 | 77 |
| FSH (mIU/mL) | Roche | 0.3 | 0.1 | 0.5 | 28 | 12 | 46 | 75 |
| LH (mIU/mL) | Roche | <0.1 | 0.0 | 0.2 | 42 | 17 | 66 | 77 |
| Prolactin (ng/mL) | DxI | 3.0 | 1.8 | 4.3 | 21.0 | 18.0 | 24.0 | 80 |
| Prolactin (ng/mL) | Roche | 3.9 | 1.8 | 6.0 | 29.4 | 25.0 | 33.8 | 78 |
| Progesterone (ng/mL) | DxI | 0.1 | 0.08 | 0.11 | 1.2 | 1.0 | 1.4 | 78 |
| Progesterone (ng/mL) | Roche | 0.1 | NC | NC | 0.5 | 0.4 | 0.6 | 76 |
| SHBG (nmol/L) | DxI | 7.0 | 3.5 | 10.4 | 65.6 | 47.6 | 83.6 | 80 |
| SHBG (nmol/L) | Roche | 10.1 | 6.9 | 13.1 | 86.1 | 65.1 | 107.0 | 78 |
| Testosterone, free (pg/mL) | DxI | 16.5 | 2.8 | 30.1 | 161 | 137 | 184 | 82 |
| Testosterone, free (pg/mL) | LC–MS/MS | 15.7 | 0.0 | 35.1 | 221.8 | 160.2 | 283.4 | 82 |
| Testosterone, free (pg/mL) | Roche | 5.8 | 0.0 | 17.0 | 186.1 | 151.4 | 220.8 | 77 |
| Testosterone, total (ng/dL) | DxI | 180 | 127 | 233 | 930 | 800 | 1069 | 80 |
| Testosterone, total (ng/dL) | LC–MS/MS | 199 | 150 | 248 | 1149 | 1074 | 1224 | 80 |
| Testosterone, total (ng/dL) | Roche | 158 | 135 | 181 | 1115 | 865 | 1366 | 76 |

dose 50 mg/week; range and IQR 50–100 mg/week); 1 participant administered testosterone both topically and intramuscularly.

## Reference Intervals

Reference intervals and confidence limits for all hormones evaluated were calculated and are listed in Table 1. Description statistics are listed in Table 2.

## Total and Free Testosterone by Immunoassay and Mass Spectrometry

A qualitative assessment of total versus free testosterone indicated that clinically, free testosterone and total testosterone will allow for the same interpretation (Fig. 1). Linear regression analysis showed this was true for all 3 methods tested: DxI ($y = 0.19x - 11.5$; $R^2 = 0.97$), Roche ($y = 0.18x + -9.4$; $R^2 = 0.94$), and the LC–MS/MS ($y = 0.19x + b$; $R^2 = 0.97$) assays.

Total testosterone measured by immunoassay resulted in clinically equivalent concentrations relative to LC–MS/MS for both the DxI ($y = 0.69x + -79.9$; $R^2 = 0.84$) and the Roche ($y = 0.95x + -13.5$; $R^2 = 0.97$) assays (Fig. 2).

## Estradiol by Immunoassay and Mass Spectrometry

Estradiol measured by immunoassay generally resulted in clinically equivalent concentrations relative to LC–MS/MS for both the DxI

**Table 2. Distribution statistics for common endocrine laboratory measurements in a cohort of healthy transgender men on stable gender-affirming hormone therapy.**

| Analyte | Platform | Distribution statistics | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | min | 25th | 50th | 75th | max | mean | SD | *N* |
| AMH (ng/mL) | Roche | 0.02 | 1.07 | 2.33 | 4.57 | 15.76 | 3.30 | 3.47 | 78 |
| DHEAS (μg/dL) | Roche | 49 | 153 | 222 | 302 | 730 | 245 | 126 | 78 |
| Estradiol (pg/mL) | DxI | 29 | 29 | 36 | 51 | 106 | 42 | 16 | 77 |
| Estradiol (pg/mL) | Roche | 4 | 13 | 25 | 33 | 83 | 26 | 16 | 75 |
| Estradiol (pg/mL) | LC–MS/MS | 10 | 18 | 26 | 39 | 149 | 33 | 23 | 79 |
| FSH (mIU/mL) | DxI | <1 | 3 | 7 | 9 | 146 | 12 | 22 | 77 |
| FSH (mIU/mL) | Roche | 0.3 | 2 | 5 | 7 | 15 | 6 | 6 | 75 |
| LH (mIU/mL) | Roche | <0.1 | 1.5 | 5.2 | 9.1 | 53.5 | 7.0 | 8.6 | 77 |
| Prolactin (ng/mL) | DxI | 3 | 7 | 10 | 12 | 22 | 10 | 4 | 78 |
| Prolactin (ng/mL) | Roche | 3.8 | 9.7 | 12.9 | 18 | 29.5 | 14.0 | 5.7 | 78 |
| Progesterone (ng/mL) | DxI | <0.1 | 0.3 | 0.4 | 0.7 | 1.3 | 0.5 | 0.3 | 78 |
| Progesterone (ng/mL) | Roche | 0.1 | 0.1 | 0.2 | 0.3 | 0.6 | 0.2 | 0.1 | 76 |
| SHBG (nmol/L) | DxI | 5 | 18 | 26 | 36 | 68 | 28 | 13 | 80 |
| SHBG (nmol/L) | Roche | 10 | 24 | 34 | 46 | 89 | 36 | 16 | 78 |
| Testosterone, free (pg/mL) | DxI | 3.7 | 47.3 | 74.8 | 112.9 | 184.9 | 79.4 | 41.9 | 80 |
| Testosterone, free (pg/mL) | LC–MS/MS | 2 | 58 | 94 | 132 | 274 | 98 | 53 | 82 |
| Testosterone, free (pg/mL) | Roche | 1 | 50 | 83 | 112 | 208 | 83 | 45 | 77 |
| Testosterone, total (ng/dL) | DxI | 130 | 320 | 460 | 630 | 1050 | 481 | 211 | 79 |
| Testosterone, total (ng/dL) | LC–MS/MS | 150 | 360 | 551 | 710 | 1179 | 568 | 257 | 80 |
| Testosterone, total (ng/dL) | Roche | 147 | 318 | 528 | 656 | 1331 | 529 | 246 | 76 |

($y = 0.94x + 15$; $R^2 = 0.74$) and the Roche ($y = 0.80x + b$; $R^2 = 0.77$) assays (Fig. 3).

## DISCUSSION

The reference intervals for transgender men on masculinizing hormone therapy are similar to cisgender men for total testosterone, free testosterone, and SHBG (Fig. 4). While this is somewhat circular thinking, since the primary aim of masculinizing therapy has been to titrate hormone concentrations into the respective ranges, these results align with the guideline recommendations to use the age-matched cisgender male reference interval for people using masculinizing therapy (1). However, aiming for the guideline-recommended 400–700 ng/mL may be too tight of a range, as most reference intervals for cisgender men are much wider on both the lower and upper ends. Trying to titrate the serum testosterone concentration to between 400–700 ng/mL is unforgiving for the timing of the serum test relative to the IM dose, likely leading to frustrations for both the patient and provider. These results bolster the preferred indication of "male" in the medical record for people on masculinizing hormones (9). Recognition of the male gender by the laboratory information system (LIS) will append reference intervals that allow for proper interpretation of SHBG and testosterone in people on masculinizing hormones.

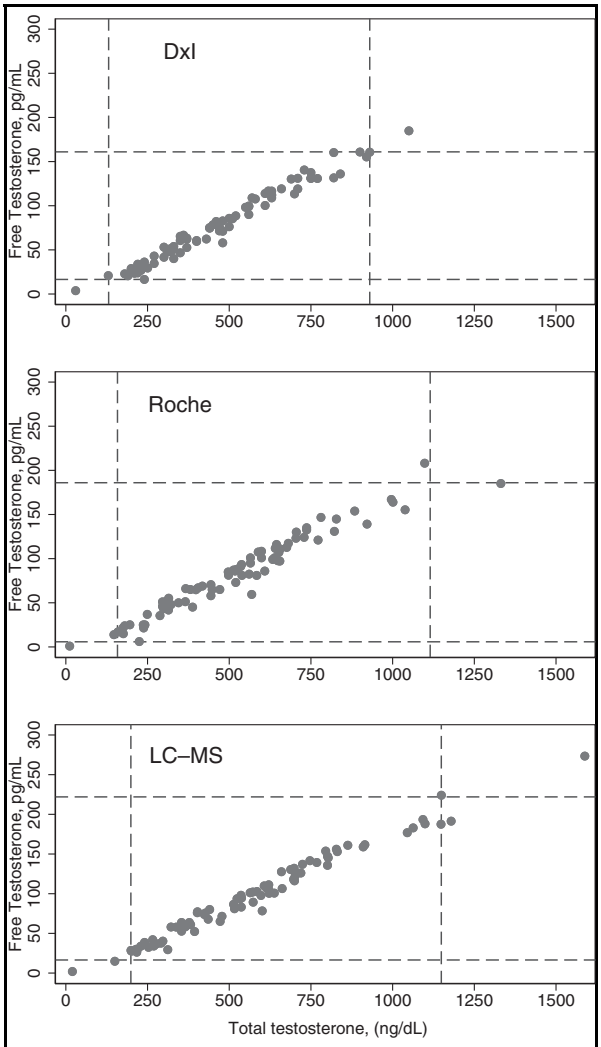


**Fig. 1. Comparison of total and free testosterone on 2 immunoassay platforms, the DxI (top panel) and the Roche (middle panel), and LC–MS/MS (bottom panel) indicates that free and total testosterone will give clinically equivalent information for the majority of patients. Dashed lines indicate 2.5 and 97.5% reference intervals established in the current study.**

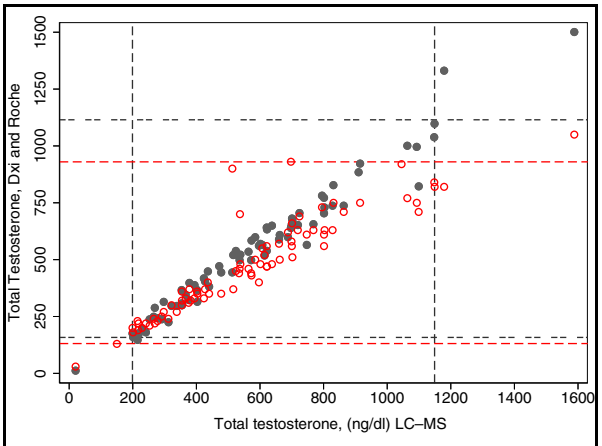


**Fig. 2. Comparison of total testosterone by LC–MS/MS to 2 immunoassay platforms indicates that the methods will provide clinically equivalent information for the majority of specimens. Relative to LC–MS/MS concentrations obtained through measurements on the DxI are indicated in red ($y = 0.69x + -79.9$; $R^2 = 0.84$), Roche in black ($y = 0.95x + -13.5$; $R^2 = 0.97$). Dashed lines indicate 2.5 and 97.5% reference intervals for the DxI (red; hollow circles) and the Roche (black; shaded circles).**

In contrast, cisgender men have low estradiol concentrations relative to transgender men (Fig. 4). The results of our study indicate that the cisgender male reference interval for estradiol may be too stringent for application to people on masculinizing therapy, and likely set unrealistic expectations for estradiol suppression. Using the cisgender male reference interval of <45 pg/mL, ~18% of our cohort would have been flagged high. A solution to over-flagging within the binary gender electronic systems would be to build an estradiol test specific to people on masculinizing hormones. This would allow for appending a reference interval applicable to transgender and nonbinary people suppressing estradiol with exogenous testosterone administration. A limitation is that the burden would be on the provider to order the correct test. These results, and their subsequent laboratory workarounds, highlight the importance of improving how gender (typically limited to a binary male/female) is designated in the LIS and electronic medical record (EMR) for transgender patients (10).

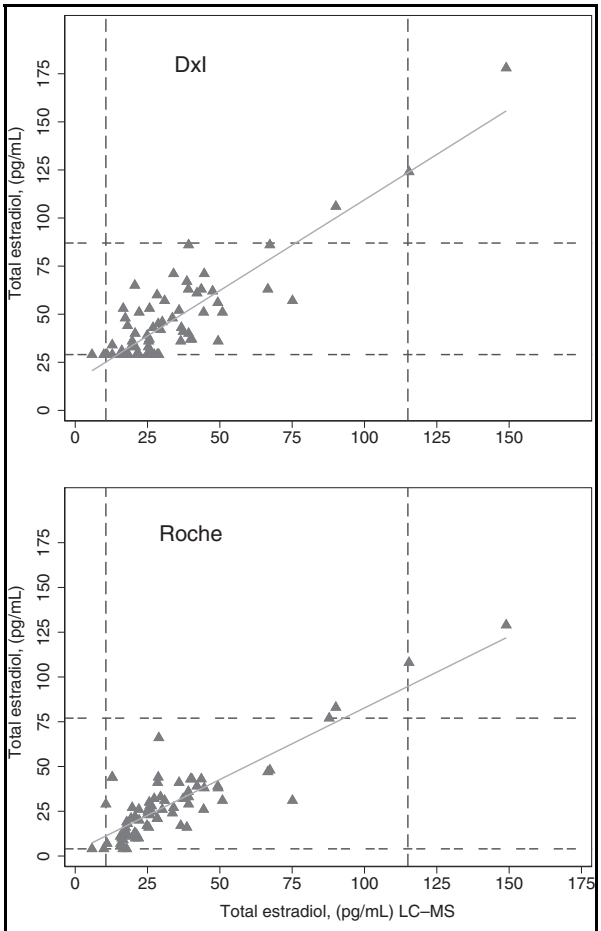


**Fig. 3. Comparison of estradiol by LC–MS/MS to the DxI (top panel; $y = 0.94x + 15$; $R^2 = 0.74$) and the Roche (bottom panel; $y = 0.80x + b$; $R^2 = 0.77$) immunoassays indicates that the methods will provide clinically equivalent information for the majority of patients in this population. Dashed lines indicate 2.5 and 97.5% reference intervals established in the current study.**

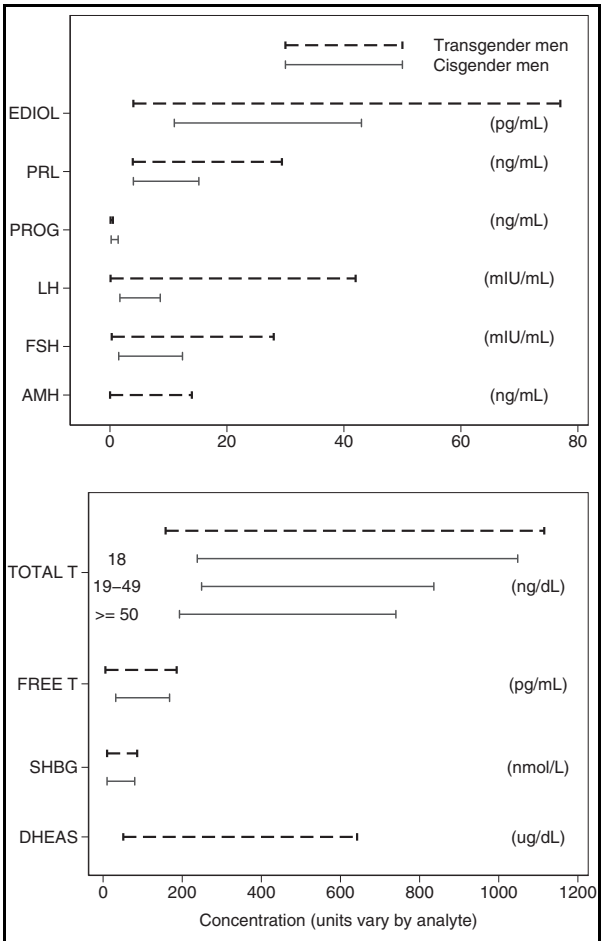


**Fig. 4. Comparison of manufacturer-recommended and/or laboratory-established reference limits for cisgender men to the reference intervals derived in this study for transgender men. Comparisons utilize the ranges established with LC–MS/MS assay for testosterone and the Roche immunoassays for all other analytes. Numeric values indicate age range in years. Dashed black lines indicate the transgender cohort; solid black lines are manufacturer's ranges for cisgender men. The specific numeric values illustrated can be found in the online Supplemental Table 1.**

Similar to estradiol, the concentration distributions of progesterone, LH, and FSH in our masculinizing cohort differ relative to cisgender men and women. The intervals most closely resemble the follicular phase for cisgender women, having a higher upper reference limit compared to cisgender men, but a lower upper limit compared to the ovulatory phase of cisgender women. These analytes are not routinely ordered in transgender people, but if measured for fertility or specific endocrine conditions, the provider should

understand that progesterone, FSH and LH concentrations for people administering masculinizing therapy might not be adequately interpreted if cisgender reference intervals are used. Since these analytes should only be evaluated by endocrinologists or reproductive specialists, we do not believe that the laboratory will need to implement measures to address these ranges, as those providers are generally much more equipped to interpret these values on a case-by-case basis, in relation to the individual clinical needs.

AMH is a marker of ovarian reserve and is therefore mainly measured in people with fertility concerns, most commonly cisgender women (11). Less commonly, AMH may be ordered to assess menopausal status, as a diagnostic tool for polycystic ovarian syndrome, or in the workup of differences of sexual differentiation (11, 12). However, AMH measurements could be indicated in any transgender or nonbinary person with a desire to understand their reproductive potential. Higher AMH concentrations are associated with increased ovarian reserve. Our results indicated that people on masculinizing hormones have a slightly higher upper reference limit relative to cisgender women, which was an unexpected result given that one of the treatment goals for administering masculinizing hormone is amenorrhea. However, previous studies have shown that the lower and upper reference limits for cisgender adult men are significantly higher than that of age-matched cisgender women (13). Two previous studies have assessed impact of testosterone on AMH in transgender men. A 12-week observational study showed no significant impact on AMH compared to baseline for testosterone therapy (14). In contrast, a 16-week study of transgender men receiving testosterone, an aromatase inhibitor, and a GnRH agonist (to suppress endogenous hormone secretion) showed significant decrease in AMH (15). This latter study differs from the present study in the use of aromatase inhibitor and GnRH agonist in addition to testosterone.

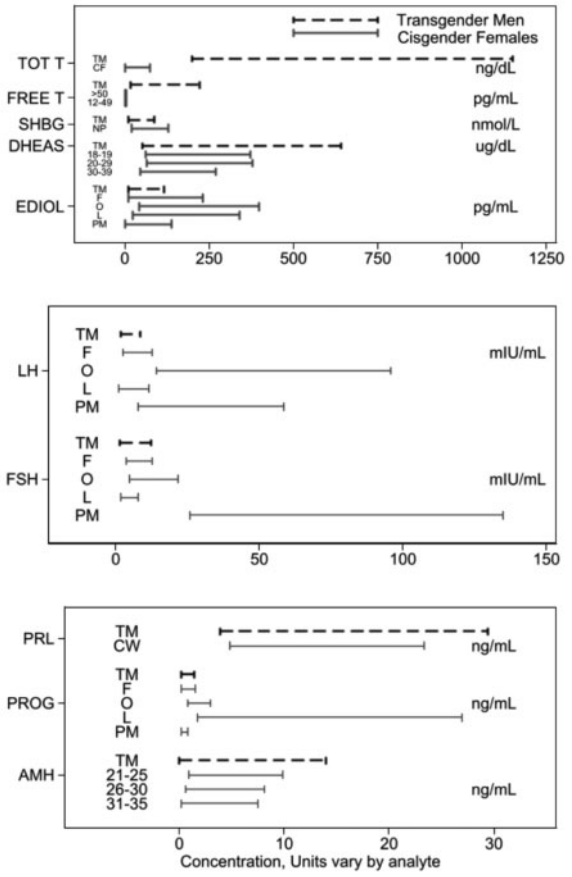


**Fig. 5. Comparison of manufacturer-recommended and/or laboratory-established reference limits for cisgender women (CW) to the reference intervals derived in this study for transgender men (TM). Comparisons utilize the ranges established with LC–MS/MS assay for testosterone and the Roche immunoassays for all other analytes. F l, O l, L, and PM indicate the follicular, ovulatory, luteal, and postmenopausal reproductive stages, respectively. NP indicates nonpregnant. Dashed black lines indicate the transgender cohort; solid black lines are manufacturer's ranges for cisgender women. The specific numeric values illustrated can be found in Supplemental Table 2.**

Prolactin concentrations in people on masculinizing hormones were similar to concentrations in cisgender women when evaluated on the DxI

(upper reference limit of 21 versus 24 ng/mL for cisgender women), but had a higher upper reference limit when evaluated on the Roche (32 versus 23 ng/mL for cisgender women). Regression analysis indicated that the bias between the assays was systematic ($y = 1.4x - 0.1$ where $y$ is Roche values and $x$ is DxI), indicating that there may have just been a positive bias on the Roche assay during the completion of this study. Overall, the data indicate that for some instruments the cisgender women reference interval would be an appropriate comparator (Fig. 5).

The upper reference limit for DHEAS was higher relative to cisgender men and cisgender women, but most closely resembled that of cisgender men between 20 and 29 years old. The biology and regulation of DHEAS is complicated and not entirely understood. DHEAS serum concentrations are consistently higher in males compared to females throughout the lifespan starting at puberty, a finding also seen in nonhuman primates (16, 17). A prior study of transgender men treated with intramuscular testosterone undeconoate for 12 months showed no significant difference in DHEAs compared to baseline (18). In contrast, the 16-week study of transgender men receiving testosterone, an aromatase inhibitor, and a GnRH agonist (mentioned above with respect to AMH) showed significant increase in DHEAS (15).

A common question for laboratories is "when is mass spectrometry required to evaluate hormone concentrations?" Our data suggest that the majority of the time, immunoassay is sufficient to clinically evaluate total and free testosterone and estradiol in the trans masculine population. In any circumstance where these values are inconsistent with presentation, mass spectrometry should be relied on for confirmatory testing.

The reference intervals derived in this manuscript are suitable for clinical use. However, there are several limitations to this study. First, the sample size did not reach 120 individuals. Second, the timing of last testosterone dose was not reported, which means that some participants may have been at peak testosterone concentrations and others at trough or mid-cycle. Also, sample size was not sufficient to group participants by age, mode of testosterone administration, gender-affirming surgery, or any other subgroup analysis. In addition, the therapy regimens used at the 2 study sites did not include modalities such as GnRH agonists (an important approach for the adolescent transgender population receiving testosterone) or aromatase inhibitors. Last, while most transmasculine people would not be prescribed oral contraception, we did not evaluate how any type of pharmaceutical contraceptive might influence the intervals. These could be focus of future studies.

In conclusion, we have derived reference intervals for common endocrine hormones in transgender people using testosterone therapy. These reference intervals can aid laboratories and providers in interpreting hormone concentrations in this population.

## SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

**Author Contributions:** *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

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## REFERENCES

1. Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2017;102:3869–903.
2. Irwig MS. Testosterone therapy for transgender men. Lancet Diabetes Endocrinol 2017;5:301–11.
3. Greene DN, McPherson GW, Rongitsch J, Imborek KL, Schmidt RL, Humble RM, et al. Hematology reference intervals for transgender adults on stable hormone therapy. Clin Chim Acta 2019;492:84–90.
4. Leung A, Sakkas D, Pang S, Thornton K, Resetkova N. Assisted reproductive technology outcomes in female-to-male transgender patients compared with cisgender patients: a new frontier in reproductive medicine. Fertil Steril 2019;112:858–65.
5. Wang B, Hengel R, Ren R, Tong S, Bach PV. Fertility considerations in transgender patients. Curr Opin Urol 2020;30:349–54.
6. Goldstein Z, Corneil TA, Greene DN. When gender identity doesn't equal sex recorded at birth: the role of the laboratory in providing effective healthcare to the transgender community. Clin Chem 2017;63:1342–52.
7. Ly LP, Handelsman DJ. Empirical estimation of free testosterone from testosterone and sex hormone-binding globulin immunoassays. Eur J Endocrinol 2005; 152:471–8.
8. CLSI. CLSI document C28-A3. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline. 3rd Ed. Wayne (PA): CLSI; 2008.
9. Deutsch MB, Buchholz D. Electronic health records and transgender patients–practical recommendations for the collection of gender identity data. J Gen Intern Med 2015; 30:843–7.
10. Imborek KL, Nisly NL, Hesseltine MJ, Grienke J, Zikmund TA, Dreyer NR, et al. Preferred names, preferred pronouns, and gender identity in the electronic medical record and laboratory information system: is pathology ready? J Pathol Inform 2017;8:42.
11. Oh SR, Choe SY, Cho YJ. Clinical application of serum anti-Mullerian hormone in women. Clin Exp Reprod Med 2019;46:50–9.
12. Freire AV, Grinspon RP, Rey RA. Importance of serum testicular protein hormone measurement in the assessment of disorders of sex development. Sex Dev 2018;12:30–40.
13. Lu J, Holt C, Choi M, Kalp K, Straseski J. Quantitation of anti-Müllerian hormone by the AnshLabs picoAMH ELISA assay. Poster session presented at American Society for Clinical Chemistry; 2014 Jul 27–31; Chicago, IL.
14. Taub RL, Adriane Ellis S, Neal-Perry G, Magaret AS, Prager SW, Micks EA. The effect of testosterone on ovulatory function in transmasculine individuals. Am J Obstet Gynecol 2020;223:229.e1–8.
15. Caanen MR, Soleman RS, Kuijper EA, Kreukels BP, De Roo C, Tilleman K, et al. AntiMullerian hormone levels decrease in female-to-male transsexuals using testosterone as cross-sex therapy. Fertil Steril 2015;103: 1340–5.
16. Labrie F, Belanger A, Luu-The V, Labrie C, Simard J, Cusan L, et al. DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: its role during aging. Steroids 1998;63:322–8.
17. Sorwell KG, Urbanski HF. Causes and consequences of age-related steroid hormone changes: insights gained from nonhuman primates. J Neuroendocrinol 2013;25: 1062–9.
18. Wierckx K, Van Caenegem E, Schreiner T, Haraldsen I, Fisher AD, Toye K, et al. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: results from the European network for the investigation of gender incongruence. J Sex Med 2014;11:1999–2011.