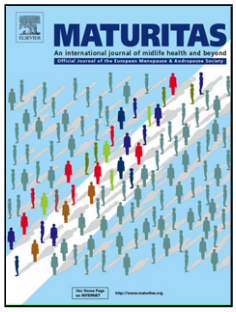
Accepted Manuscript



Title: Measuring testosterone in women and men

Authors: George A. Kanakis, Christos P. Tsametis, Dimitrios G. Goulis

PII: S0378-5122(19)30019-2
DOI: https://doi.org/10.1016/j.maturitas.2019.04.203
Reference: MAT 7173

To appear in: *Maturitas*

Received date: 11 January 2019
Revised date: 31 March 2019
Accepted date: 2 April 2019

Please cite this article as: Kanakis GA, Tsametis CP, Goulis DG, Measuring testosterone in women and men, *Maturitas* (2019), https://doi.org/10.1016/j.maturitas.2019.04.203

Measuring testosterone in women and men

George A. Kanakis[a], Christos P. Tsametis[b], Dimitrios G. Goulis[b]

[a]Department of Endocrinology, Athens Naval and Veteran Affairs Hospital, Greece

[b]Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynaecology, Medical School, Aristotle University of Thessaloniki, Greece

Corresponding author

George A. Kanakis

Athens Naval & VA Hospital

70 Deinokratous Str., 11525, Athens, Greece

Tel. +30 210 770 92 48

Email: geokan@endo.gr

Highlights

- Despite the advances achieved in steroid hormone assessment, substantial variability exists in testosterone measurement within and across laboratories.

- Universally accepted age- and sex-matched reference intervals for testosterone are still lacking.
- Standardization of testosterone testing among current commercial assays should aim to improve performance at lower concentrations of testosterone.

Highlights

- Despite the advances achieved in steroid hormone assessment, substantial variability exists in T measurement within and across laboratories
- Universally accepted age- and sex-matched reference intervals for T are still lacking.
- Standardization of T testing among current commercial assays should aim to improve performance at lower T concentrations.

Abstract

Measurement of serum testosterone (T) level is of utmost importance for the evaluation of hypogonadism in men and androgen excess in women. Despite the advances in steroid hormone assessment, substantial variability exists regarding measurement of T concentrations. Several factors affect T measurement in men, including circadian rhythms, intra-individual daily variability and transient stressors, while T concentrations in women vary mainly according to the phase of the menstrual cycle. Most of the available immunoassays lack the required accuracy when dealing with T concentrations at the lower end of the normal range for men and across the entire range for females. Consequently, there is no universally accepted lower T threshold for healthy adult men and most immunoassays fail to detect states of mild androgen excess in women. Mass spectrometry is considered the gold-standard method for T measurement; however, due to its complexity and cost, it has not been widely adopted. To increase accuracy, T in men should be measured with a fasting morning sample and repeated if the level is found to be low; in women, measurement must be performed at the follicular phase of the cycle. In both cases, borderline results may be clarified by the assessment of free testosterone (fT). Since most fT assays are unreliable, calculated surrogates should be used instead. Collaborative efforts have

been undertaken, with rigorous internal and external quality controls and the establishment of reference methods, to harmonise the commercial assays.

Keywords

Testosterone; male hypogonadism; androgen excess; mass spectrometry

Introduction

Testosterone (T) plays a crucial role in male sexual differentiation, sexual maturation and reproductive health, as well as in the general health and quality of life of men. In women, abnormal circulating levels of T are associated with several diseases and chronic conditions. Despite the advances achieved in steroid hormone assessment, substantial variability exists in T measurement within and across laboratories; universally adopted sex- and age-related normal ranges are still lacking [1].

In this mini-review, the clinical indications for measurement of T will be discussed, together with the pitfalls in T evaluation. In addition, the measures that have been applied to improve the quality of T measurements and the prospects for T testing will be considered.

Testosterone physiology and indications of measurement

The testes secrete the vast majority (>95%) of T in men, under the control of the hypothalamus-pituitary-testicular (HPT) axis. In women, 50% of T is produced by the conversion of androgens in the periphery, while the ovaries and the adrenal glands contribute equally to the rest of the T that circulates in the blood (25% each) [2]. Most circulating T is conjugated with proteins; the majority (66%) is bound to sex hormone-binding globulin (SHBG) and a lower fraction (30%) to

albumin. Only 2-4% of T remains uncoupled (free T; fT), which is considered to be its active form. The bond between T and albumin is relatively weak, allowing its dissociation at the capillary level and biological action. The sum of albumin-bound T plus the fT fraction equates to the bioavailable T [3].

Serum T measurement is of utmost importance in the evaluation of conditions suggesting hypogonadism in adult males (Table 1). Regarding adult women, measurement of T is useful for the assessment of signs of androgen excess (hirsutism, acne), particularly when the presence of an androgen-secreting tumour is suspected (virilization). Evaluation of T deficiency for women with sexual dysfunction is still the subject of debate [4].

Challenges in the interpretation of testosterone concentrations

Although T assays were originally designed to assess T values within the normal adult male range, reliability problems emerge when assessing concentrations at the lower end of that range; thus, there is no universally accepted lower T threshold for healthy adult men. According to a consensus statement endorsed by five andrology societies, T concentrations >12 nmol/L (350 ng/dL) should be considered normal and do not require further evaluation or treatment. Concentrations <8 nmol/L (230 ng/dL) are subnormal and definitely require T replacement therapy (TRT). For values that lie between, the evaluation of fT is recommended to establish a diagnosis of hypogonadism [5]. The US Endocrine Society, in its latest guidelines on TRT in men, adopted a threshold of 9.2 nmol/L (264 ng/dL), corresponding to the normal range of a population of healthy non-obese European and American men aged 19 to 39 years [6,7].

Evaluation of T in men may also be influenced by circadian variation: T concentrations obtained at 16:00 are 20–25% lower than those received at 08:00, whereas food intake mildly suppresses T concentrations [8,9]. In addition, there is considerable intra-individual daily variability; 30% of men with an initial low T concentration may have a normal one on repeat measurement, rendering the confirmation of low T imperative [10]. Various stressors can cause T concentrations to fluctuate; reductions of up to 30% can occur during the acute phase of illness [11]. Furthermore, several medications (e.g. glucocorticoids) may also temporarily affect T secretion.

T concentrations in women vary with the phase of the menstrual cycle and the body mass index, making the establishment of normal ranges more cumbersome [12]. A recent review of studies using T assays based on liquid chromatography tandem mass spectrometry (LC-MS/MS) demonstrated a clear bimodal distribution of T concentration between healthy men and women, with the upper end of the female range being 4-5 times lower than the lower end of the adult male range (0.4–2.0 nmol/L; 12-58 ng/dL). Women with mild androgen excess, for instance in association with polycystic ovary syndrome (PCOS) or congenital adrenal hyperplasia (CAH), lie above the normal female range [0.34 – 5.5 nmol/L (10-159 ng/dL) and 1.32–5.62 nmol/L (39-162 ng/dL), respectively], but still below the normal male range. Women with T concentrations 3 times higher than the upper limit of normal usually have overt signs of virilisation, which should prompt evaluation for an androgen-secreting tumour [13].

Testosterone assays and pitfalls in testing

Most of the current commercial methods for measuring T are immunoassays (IAs). There are several types of IAs, depending on the tracer used, which may be a radioisotope (radioimmunoassay, RIA), an enzyme (enzyme immunoassay, ELISA), or a fluorescent (fluoroimmunoassay, FIA) or a chemiluminescent compound (CLIA). These IAs initially required extraction and/or chromatography to avoid protein interference and cross-reactivity with other steroids (steroid purification step). Modern IAs may be performed directly on serum or plasma [14]; however, at lower concentrations of T they lack the accuracy and precision required. When blinded samples corresponding to the normal female range were processed by different laboratories using the same methodology, the results for the same sample varied 2- to 6-fold and the coefficients of variation ranged from 13 to 32%. These problems may be attributed to analytical bias, such as low antibody specificity, lack of assay linearity and non-uniform calibration. Other potential sources of bias, such as data entry errors, reagent lot-to-lot variability, and specimen handling and preparation inconsistency, cannot be excluded [1].

Currently, mass spectrometry (MS) is considered the gold-standard method for T measurement. This technique includes isolation of the steroid hormones from proteins (chromatographic separation), followed by compound identification before quantification. MS has higher specificity and lower intra- and interassay variability compared with IAs. However, due to its

complexity and high cost, MS had not been widely adopted for commercial use. The replacement of gas chromatography by liquid chromatography and the introduction of LC-MS/MS have improved the method's accuracy while increasing its throughput [15]. Nonetheless, even for MS, considerable inter-laboratory variability exists, though much less pronounced compared with the IAs [16].

Older studies comparing the accuracy of commercially available IAs and MS have shown that 7 out of 10 IAs diverged significantly from MS below a concentration of 8.0 nmol/L (230 ng/dL) [17]. Similarly, a study conducted with a European population demonstrated high correlation ($r=0.93$, $p<0.001$) between a common platform IA and MS over a broad range of T concentrations; however, this correlation was less robust in the hypogonadal range (T<11 nmol/L; $r=0.72$, $p<0.001$) [18]. In a recent study comparing five automated IAs with a reference LC-MS/MS, most of the IAs performed satisfactorily for T concentrations in the adult male range, but at concentrations <1.9 nmol/L (55 ng/dL) their accuracy was compromised, with the measurement bias ranging from -14.2 to 63.8% [19].

Solutions for improving testosterone testing

In order to increase specificity, most societies recommend reserving T measurement for men with conditions associated with male hypogonadism (Table 1) and symptoms suggestive of T deficiency [6]. Moreover, T should be measured with a fasting morning sample and repeated, if found to be low (<12 nmol/L). If T values lie near the lower limit of the adult male range (8-12 nmol/L), particularly if conditions that alter SHBG are present (Table 2), confirmation of the result with the assessment of fT is recommended (Figure 1) [6]. To assess androgen excess in women, T measurement is advised to be performed during the follicular phase [1].

The gold-standard method to measure fT is equilibrium dialysis, which retains the protein-bound fraction of hormones via a semipermeable membrane. Measurement is performed on T that crosses this membrane, which is considered to represent fT [20]; however, this procedure is time-consuming and demands expertise. Measurement of fT with direct IAs is even more problematic than that of tT due to its very low plasma concentrations, leading most experts to strongly discourage their use [6,21]. In contrast, calculated surrogates of fT correlate substantially with equilibrium dialysis ($r=0.96$) and pose as the most useful estimates of fT in clinical practice [22].

Calculated-fT (CfT) is based on the equilibrium dissociation constants for the binding of T to SHBG and albumin. Relevant calculators are available on-line (http://www.issam.ch/freetesto.html) and CfT values above 225 pmol/L (6.45 ng/dL) are considered normal [23]. On the other hand, the Free Androgen Index (FAI), defined as the ratio of T/SHBG (in nmol/L) × 100, correlates better with fT in women than in men, due to the abundance of SHBG in women [24]. Most clinicians consider a FAI value above 6.0-6.5 in a woman of reproductive age to be abnormal; however, a universally accepted reference range has not yet been established.

Collaborative efforts have been undertaken to harmonize the commercial assays, including rigorous internal and external quality control, to avoid pre-analytical errors, as well as the establishment of reference methods. In 2007, the Endocrine Society published a position statement that stressed the limitations and pitfalls in measuring T [1]. This was followed by a consensus statement that set out the necessary steps to achieve excellence in T testing [25]. The US Centers for Disease Control and Prevention (CDC) responded by funding the Hormone Standardization (HoSt) Program to improve T (and oestradiol) measurements. This program has run in the US since 2007 and sets the standards for certification of laboratories that measure T, by using the same stable calibrators. This policy seems to be effective, at least regarding MS methods, as the between-laboratory variability declined by approximately 50% from 2007 to 2011. Recently, a large study using a CDC reference IA was conducted on a sample of more than 9,000 community-dwelling men included in four hallmark cohort studies in the US and Europe: the Framingham Heart Study; the European Male Aging Study; the Osteoporotic Fractures in Men Study; and the Male Sibling Study of Osteoporosis. This established a harmonized normal range of a healthy non-obese population of European and American men aged 19 to 39 years (264 to 916 ng/dL) [7].

Conclusions

The reliability of T measurement remains questionable, despite its clinical importance in the diagnosis and treatment of hypogonadism in men and androgen excess in women. MS, despite of offering better performance compared with IAs, is still an expensive and cumbersome option. Efforts should be made to increase the precision of current IAs and to reinforce standardization

programs, a strategy that has been very efficient in the measurement of other analytes, such as cholesterol.

Practice points

- Testosterone (T) in men should be measured with a fasting morning sample and repeated if found to be low; in women, T measurement must be performed at the follicular phase of the cycle.
- Currently, mass spectrometry (MS) is considered the gold-standard method for T measurement; immunoassays (IA) can be used instead, at least for measuring T in men, provided that a reliable method is used, optimally one certified by a standardization or quality control program.
- Borderline results may be clarified by the assessment of free T (fT). Since most fT assays are not reliable, calculated surrogates should be used.

Research agenda

- Universally accepted age- and sex-matched reference intervals for T should be established.
- Most of the current commercial IAs would benefit from assay improvement at lower T concentrations.
- Standardization of T testing among different IAs and across the entire community of laboratories should be reinforced, including rigorous internal and external control, and uniform calibrators.

Contributors

All authors contributed to the literature review and the writing and revision of the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

No funding was received specifically for the preparation of this review.

Provenance and peer review

This article has undergone peer review.

Declarations of interest: none

References

[1] W. Rosner, R.J. Auchus, R. Azziz, P.M. Sluss, H. Raff, Position statement: Utility, limitations, and pitfalls in measuring testosterone: An endocrine society position statement, J. Clin. Endocrinol. Metab. 92 (2007) 405–413. doi:10.1210/jc.2006-1864.

[2] F. Labrie, V. Luu-The, C. Labrie, A. Bélanger, J. Simard, S.-X. Lin, G. Pelletier, Endocrine and Intracrine Sources of Androgens in Women: Inhibition of Breast Cancer and Other Roles of Androgens and Their Precursor Dehydroepiandrosterone, Endocr. Rev. 24 (2003) 152–182. doi:10.1210/er.2001-0031.

[3] A. Matsumoto, W. Bremner, Male Hypogonadism, in: S. Melmed, K. Polonsky, P. Larsen, H. Kroneneberg (Eds.), Williams Textb. Endocrinol., 12th ed., Saunders, Philadelphia, 2011: pp. 709–755.

[4] A.K Korkidakis, R.L. Reid, Testosterone in Women: Measurement and Therapeutic Use, J. Obstet. Gynaecol. Canada. 39 (2017) 124–130. doi:10.1016/j.jogc.2017.01.006.

[5] C. Wang, E. Nieschlag, R. Swerdloff, H.M. Behre, W.J. Hellstrom, L.J. Gooren, J.M. Kaufman, J.J. Legros, B. Lunenfeld, A. Morales, J.E. Morley, C. Schulman, I.M. Thompson, W. Weidner, F.C.W. Wu, Investigation, treatment and monitoring of late-onset hypogonadism in males, Int. J. Androl. 32 (2009) 1–10. doi:10.1111/j.1365-2605.2008.00924.x.

[6] S. Bhasin, G.R. Cunningham, F.J. Hayes, A.M. Matsumoto, P.J. Snyder, R.S. Swerdloff, V.M. Montori, E.S. Task Force, Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline, J Clin Endocrinol Metab. 103 (2018) 1–30. doi:10.1210/jc.2009-2354\r10.1210/jc.2009-2354.

[7] T.G. Travison, H.W. Vesper, E. Orwoll, F. Wu, J.M. Kaufman, Y. Wang, B. Lapauw, T. Fiers, A.M. Matsumoto, S. Bhasin, Harmonized Reference Ranges for Circulating Testosterone Levels in Men of Four Cohort Studies in the United States and Europe., J. Clin. Endocrinol. Metab. 102 (2017) 1161–1173. doi:10.1210/jc.2016-2935.

[8] S.R. Plymate, J.S. Tenover, W.J. Bremner, Circadian variation in testosterone, sex hormone-binding globulin, and calculated non-sex hormone-binding globulin bound

testosterone in healthy young and elderly men., J. Androl. 10 (n.d.) 366–71. http://www.ncbi.nlm.nih.gov/pubmed/2592266.

[9] M. Lehtihet, S. Arver, I. Bartuseviciene, A. Pousette, S-testosterone decrease after a mixed meal in healthy men independent of SHBG and gonadotrophin levels., Andrologia. 44 (2012) 405–10. doi:10.1111/j.1439-0272.2012.01296.x.

[10] D.J. Brambilla, A.B. O'Donnell, A.M. Matsumoto, J.B. McKinlay, Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men., Clin. Endocrinol. (Oxf). 67 (2007) 853–62. doi:10.1111/j.1365-2265.2007.02976.x.

[11] M.P. Muehlenbein, J.L. Hirschtick, J.Z. Bonner, A.M. Swartz, Toward quantifying the usage costs of human immunity: Altered metabolic rates and hormone levels during acute immune activation in men., Am. J. Hum. Biol. 22 (n.d.) 546–56. doi:10.1002/ajhb.21045.

[12] W.Y. Chang, E.S. Knochenhauer, A.A. Bartolucci, R. Azziz, Phenotypic spectrum of polycystic ovary syndrome: clinical and biochemical characterization of the three major clinical subgroups., Fertil. Steril. 83 (2005) 1717–23. doi:10.1016/j.fertnstert.2005.01.096.

[13] R. V. Clark, J.A. Wald, R.S. Swerdloff, C. Wang, F.C.W. Wu, L.D. Bowers, A.M. Matsumoto, Large divergence in testosterone concentrations between men and women: Frame of reference for elite athletes in sex-specific competition in sports, a narrative review, Clin. Endocrinol. (Oxf). (2018) 0–3. doi:10.1111/cen.13840.

[14] H. Vesper, Y. Wang, J. Botelho, Challenges and improvements in testosterone and estradiol testing, Asian J. Androl. (2014). doi:10.4103/1008-682X.122338.

[15] F.Z. Stanczyk, N.J. Clarke, Advantages and challenges of mass spectrometry assays for steroid hormones., J. Steroid Biochem. Mol. Biol. 121 (2010) 491–5. doi:10.1016/j.jsbmb.2010.05.001.

[16] H.W. Vesper, S. Bhasin, C. Wang, S.S. Tai, L.A. Dodge, R.J. Singh, J. Nelson, S. Ohorodnik, N.J. Clarke, W.A. Salameh, C.R. Parker, R. Razdan, E.A. Monsell, G.L. Myers, Interlaboratory comparison study of serum total testosterone [corrected] measurements performed by mass spectrometry methods., Steroids. 74 (2009) 498–503. doi:10.1016/j.steroids.2009.01.004.

[17] J. Taieb, B. Mathian, F. Millot, M.-C. Patricot, E. Mathieu, N. Queyrel, I. Lacroix, C.

Somma-Delpero, P. Boudou, Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children., Clin. Chem. 49 (2003) 1381–95. http://www.ncbi.nlm.nih.gov/pubmed/12881456.

[18] I.T. Huhtaniemi, A. Tajar, D.M. Lee, T.W. O'Neill, J.D. Finn, G. Bartfai, S. Boonen, F.F. Casanueva, A. Giwercman, T.S. Han, K. Kula, F. Labrie, M.E.J. Lean, N. Pendleton, M. Punab, A.J. Silman, D. Vanderschueren, G. Forti, F.C.W. Wu, EMAS Group, Comparison of serum testosterone and estradiol measurements in 3174 European men using platform immunoassay and mass spectrometry; relevance for the diagnostics in aging men, Eur. J. Endocrinol. 166 (2012) 983–991. doi:10.1530/EJE-11-1051.

[19] S.L. La'ulu, K.J. Kalp, J.A. Straseski, How low can you go? Analytical performance of five automated testosterone immunoassays, Clin. Biochem. 58 (2018) 64–71. doi:10.1016/j.clinbiochem.2018.05.008.

[20] K. Van Uytfanghe, D. Stöckl, J.M. Kaufman, T. Fiers, H.A. Ross, A.P. De Leenheer, L.M. Thienpont, Evaluation of a candidate reference measurement procedure for serum free testosterone based on ultrafiltration and isotope dilution-gas chromatography-mass spectrometry., Clin. Chem. 50 (2004) 2101–10. doi:10.1373/clinchem.2004.037358.

[21] H.J. Teede, M.L. Misso, M.F. Costello, A. Dokras, J. Laven, L. Moran, T. Piltonen, R.J. Norman, M. Andersen, R. Azziz, A. Balen, E. Baye, J. Boyle, L. Brennan, F. Broekmans, P. Dabadghao, L. Devoto, D. Dewailly, L. Downes, B. Fauser, S. Franks, R.M. Garad, M. Gibson-Helm, C. Harrison, R. Hart, R. Hawkes, A. Hirschberg, K. Hoeger, F. Hohmann, S. Hutchison, A. Joham, L. Johnson, C. Jordan, J. Kulkarni, R.S. Legro, R. Li, M. Lujan, J. Malhotra, D. Mansfield, K. Marsh, V. McAllister, E. Mocanu, B.W. Mol, E. Ng, S. Oberfield, S. Ottey, A. Peña, J. Qiao, L. Redman, R. Rodgers, L. Rombauts, D. Romualdi, D. Shah, J. Speight, P.M. Spritzer, E. Stener-Victorin, N. Stepto, J.S. Tapanainen, E.C. Tassone, S. Thangaratinam, M. Thondan, C.-R. Tzeng, Z. van der Spuy, E. Vanky, M. Vogiatzi, A. Wan, C. Wijeyaratne, S. Witchel, J. Woolcock, B.O. Yildiz, Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome, Fertil. Steril. 110 (2018) 364–379. doi:10.1016/j.fertnstert.2018.05.004.

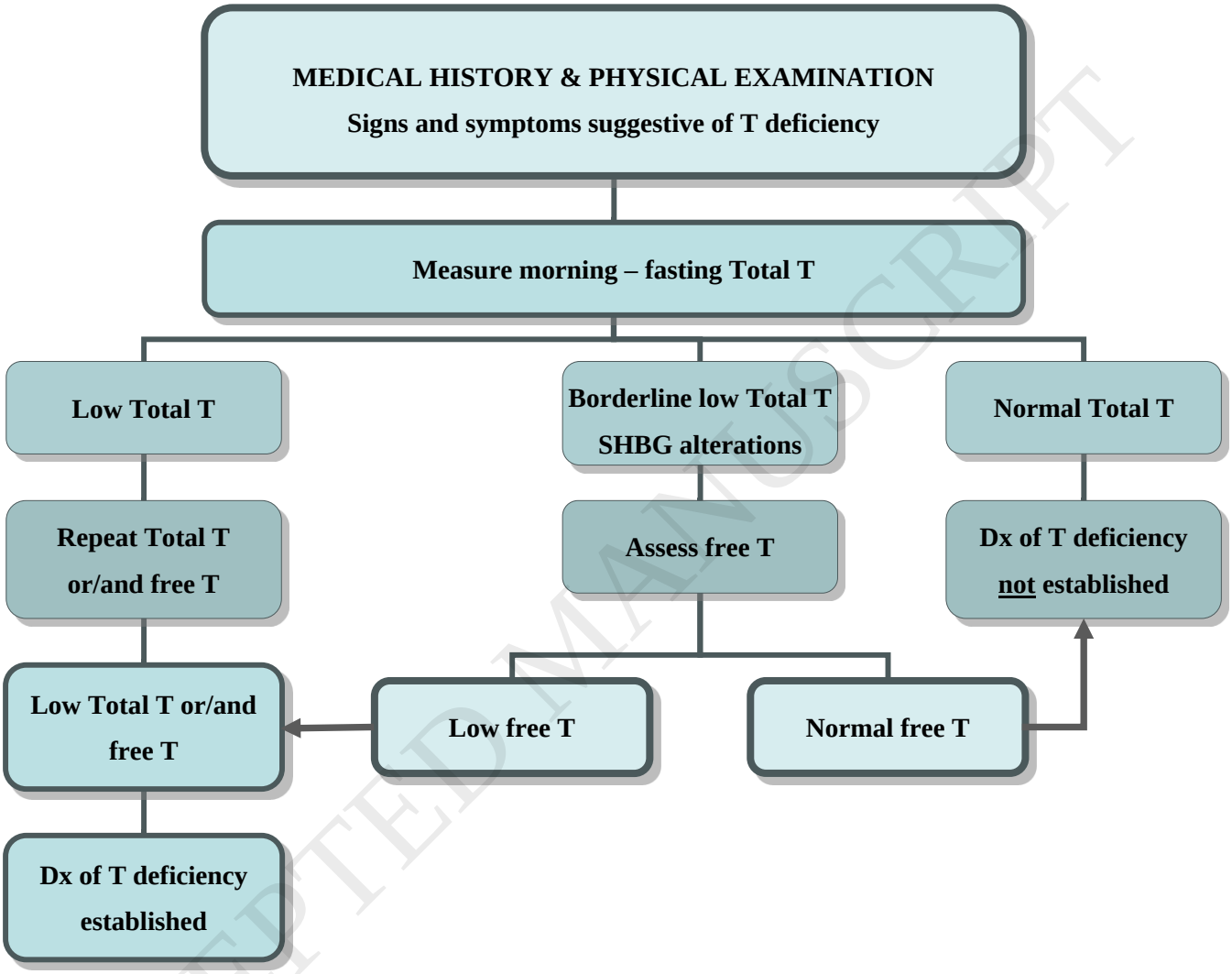
[22] W. de Ronde, Y.T. van der Schouw, H.A.P. Pols, L.J.G. Gooren, M. Muller, D.E. Grobbee, F.H. de Jong, Calculation of bioavailable and free testosterone in men: a comparison of 5 published algorithms., Clin. Chem. 52 (2006) 1777–84. doi:10.1373/clinchem.2005.063354.

[23] A. Vermeulen, Commentary: Androgen replacement therapy in the aging male - A critical evaluation, J. Clin. Endocrinol. Metab. 86 (2001) 2380–2390. doi:10.1210/jc.86.6.2380.

[24] P. Kapoor, B.M. Luttrell, D. Williams, The free androgen index is not valid for adult males., J. Steroid Biochem. Mol. Biol. 45 (1993) 325–6.

[25] W. Rosner, H. Vesper, Toward excellence in testosterone testing: A consensus statement, J. Clin. Endocrinol. Metab. 95 (2010) 4542–4548. doi:10.1210/jc.2010-1314.

Figure 1. Algorithm for the diagnosis of T deficiency in adult men.



Dx: diagnosis; SHBG: sex hormone-binding globulin; T: testosterone.

Modified from reference 6.

Table 1. Conditions associated with a high prevalence of hypogonadism.

• Tumours / infiltrating lesions of the sellar region
• Radiation to the pituitary region
• Use of medications that disrupt T production or metabolism
• Withdrawal of AAS following long-term use
• Weight loss associated with HIV infection
• Infertility
• Osteoporosis and / or low-impact fracture
• Low libido and / or erectile dysfunction

AAS: androgen anabolic steroids; HIV: human immunodeficiency virus; T: testosterone.

Modified from reference 6.

Table 2. Conditions associated with altered SHBG concentrations.

Increased SHBG concentrations	**Decreased SHBG concentrations**
Ageing	Obesity
HIV disease	Diabetes mellitus
Hyperthyroidism	Hypothyroidism
Cirrhosis and hepatitis	Nephrotic syndrome
Use of some anticonvulsants	Acromegaly
Use of oestrogens	Use of glucocorticoids, some progestins and androgenic steroids
Polymorphisms in the SHBG gene	Polymorphisms in the SHBG gene

HIV: human immunodeficiency virus; SHBG: sex hormone-binding globulin.

Modified from reference 5.