

Serum Total Testosterone Measurement Using Point of Care Test (POCT) I-CHROMA™ Testosterone Method in Healthy Male Volunteers

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ABSTRACT

The estimation of testosterone levels in the blood is an important part of urological practice. Recently, the point of care i-CHROMA™ testosterone method was found to correlate well with traditional laboratory methods. In this study, the i-CHROMA™ testosterone method was used to estimate testosterone levels in 91 healthy male volunteers with no current disease. The testosterone levels in the 91 healthy male volunteers ranged between 1.155 ng/mL-9.485 ng/mL, with a mean of 4.25 ng/mL. The serum testosterone levels did not vary significantly between the age ranges. The average testosterone levels for the age ranges 41-50 years, 51-60 years, 61-70 years and 71-80 years, were 4.05 ng/mL, 3.92 ng/mL, 4.25 ng/mL and 4.21 ng/mL, respectively. The total testosterone range data generated on a healthy male population using the i-CHROMA™ testosterone method was consistent with the reference range on the product leaflet of 2-8 ng/mL and the recognised testosterone levels expected in the age group of the volunteers.

INTRODUCTION

The estimation of testosterone levels in the blood is an important part of urological practice. The estimation of serum testosterone is vital in the understanding of conditions presenting as fatigue, loss of libido, impaired cognition, decreased muscle mass, anaemia, osteoporosis and erectile dysfunction [1-10]. We recently studied the performance of the point of care i-CHROMA™ testosterone method with other laboratory testosterone methods such as the Abbott Architect 2nd generation, Abbott Architect, BioMerieux Vidas, Roche COBAS® 6000/8000, Siemens Centaur XP/XPT/Classic, Siemens/DPC Immulite 2000/2500, Siemens/DPC Immulite 1000, Beckman DXI 600/800, Roche Elecsys, Diasorin, Liaison, Roche Cobas 4000/e411, Roche Modular E170, Beckman, Access/LXi725, SNIBE Maglumi analysers, Tosoh, and Ortho Vitros 3600/5600/ECi enrolled in the RIQAS EQA programme^[11]. This point of care method compared well with the other laboratory testosterone method. The normal total testosterone of a healthy non obese population of European and American men aged 10-39 years of age was described as 264 (2.64)-916 (9.16) ng/dL (ng/mL) ^[12]. In a study conducted in the UK on men with an age range of 40-94 years with a mean age of 60.3 years, the mean testosterone was 415.2 ng/dL (4.15 ng/mL) ^[13]. In this study, we studied the distribution of total testosterone in a population of healthy males attending a prostate cancer screening program using the recently described point of care i-CHROMA™ testosterone method to see how it compared with other reference ranges.

MATERIALS

Whole blood venous samples were taken from healthy male volunteers undergoing a prostate cancer screening session ^[13]. Ninety-eight samples were assayed in duplicate using the i-CHROMA™ testosterone method as described. Internal quality control material provided by the manufacturer was run with each batch in duplicate using the i-CHROMA™ testosterone method as described. These samples were taken in the afternoon.

The ages of the volunteers for these testosterone estimations ranged from 40-77 years, with a mean of 65.7 years, see distribution of ages of volunteers in Figure 1.

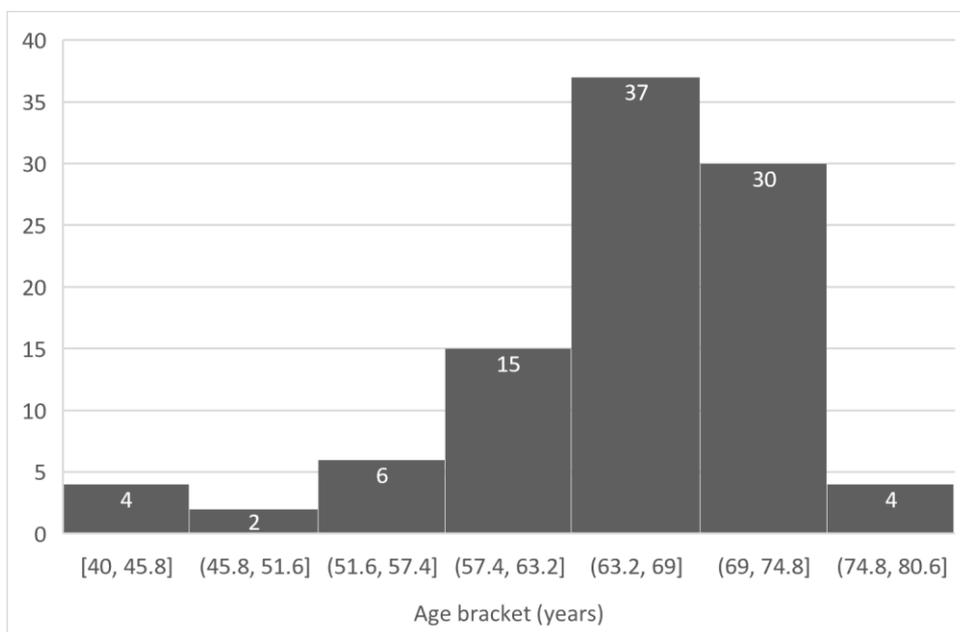


Figure 1. showing age range distribution.

METHOD

i-CHROMA™ testosterone method uses a sandwich immuno-detection principle, such that the fluorescence-labelled detector antibody binds to the target protein in the sample. The sample is then applied onto a test strip and the fluorescence labelled antigen-antibody complex is captured by a second antibody embedded in the solid phase. The signal intensity of fluorescence of the captured complex is directly proportional to the amount of testosterone present and thus allows for the calculation of sample testosterone concentration and the result is displayed on the reader as nanograms per millilitre (ng/mL).

TESTOSTERONE CONCENTRATION ESTIMATION

The assay was performed following the manufacturer’s instructions:

1. Transfer 30 µL of displacing reagent to the sample mixing tube.
2. Transfer 75 µL of sample serum using a pipette to a sample mixing tube containing the displacing reagent.
3. Close the lid of the sample mixing tube.
4. Shake the tube up and down 10 times or more.
5. Incubate at room temperature for 3 minutes.
6. Pipette out 75 µL of a sample mixture and dispense it into the tube containing detection buffer.
7. Shake the tube up and down 10 times or more.
8. Transfer the mixture onto the sample well of the test device.
9. Incubate at room temperature for 12 minutes.
10. Insert test cartridge into Test Cartridge holder in the i-CHROMA™ Reader.
11. Press “Select”.
12. Read the result on the display screen.

RESULTS

The internal control for the i-CHROMA™ was within the expected values. The i-CHROMA™ testosterone estimations reported an undetectable level (<1.00 ng/mL in 7 (7.1%) of the healthy volunteers. In the remaining 91, the testosterone estimations ranged between 1.15-9.48 ng/mL with a mean of 4.25 ng/mL: Fourteen (14.3%) of the volunteers’ levels were between 1.15 and 2.55 ng/mL, 37 (38%) of the volunteers’ levels were between 2.55 and 3.95 ng/mL, 14 (14.3%) of the volunteers’ levels were between 3.95 and 5.35 ng/mL, 16 (16.3%) of the healthy volunteers’ levels were between 5.35 and 6.75 ng/mL, 9 (19.2%) of the healthy volunteers’ levels were between 6.75 and 8.15 ng/mL and 1 (1%) of the volunteers’ levels was between 8.15 and 9.55 ng/mL (Figure 2).

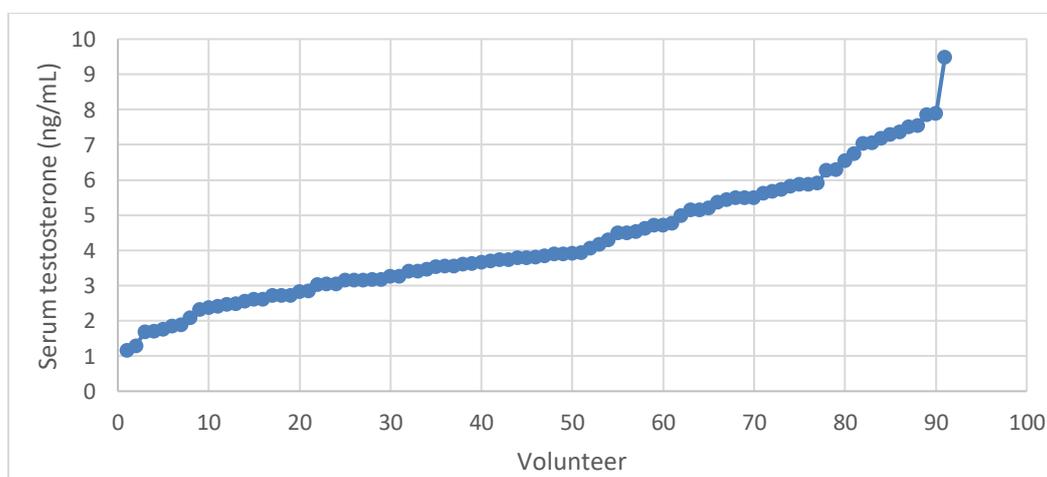


Figure 2. Distribution of testosterone in 91 of the healthy volunteers (7 had total testosterone level <0.1 ng/mL not included in this figure).

Looking at the age range distribution of the volunteers, there were 5 (5.1%) healthy volunteers aged between 41-50 years, 15 (15.3%) volunteers were aged between 51-60 years, 51 (52%) volunteers were aged between 61-70 years and 27 (28%) volunteers were aged between 71-80 years. The average testosterone levels for the age ranges 41-50 years, 51-60 years, 61-70 years and 71-80 years, were 4.05 ng/mL, 3.92 ng/mL, 4.01 ng/mL and 4.09 ng/mL, respectively (Table 1). The ages of the 7 volunteers with total testosterone estimations <1.00 ng/mL ranged between 62-72 years (Table 2).

Age range in years	Total Testosterone concentration (ng/mL)
41-50 years	4.05 ng/mL
51-60 years	3.92 ng/mL
61-70 years	4.01 ng/mL
71-80 years	4.09 ng/mL

Table 1. Showing ages of healthy male volunteers and estimated testosterone levels with i-CHROMA™ Testosterone method (The 7 volunteers with levels detected as <1.0 ng/mL, were allocated 1.00 ng/mL for this table).

	Testosterone Concentration (ng/mL)	Age (years)
1	<1.0	62
2	<1.0	64
3	<1.0	64
4	<1.0	68
5	<1.0	69
6	<1.0	70
7	<1.0	72

Table 2. Showing ages of healthy male volunteers with testosterone levels <1.0 ng/mL).

DISCUSSION

The diurnal rhythm of serum total testosterone level is highest in the early hours of the morning and it is recommended that the best time to take the sample is between 7 am and 11 am [15], however, there is information to indicate that testosterone levels were unchanged in a study of 3006 men with a mean of 60.3 years who were attending for prostate cancer screening very similar to the population in our study. Serum testosterone levels were consistent between 6 am to 2.00 pm and only decreasing by 13% between 2.00 pm and 6.00 pm [13]. This study concluded that it was no longer necessary to discount testosterone estimations made in the afternoon in elderly males [13]. In this study, the testosterone samples were taken in the afternoon in the 91 healthy male volunteers. The testosterone levels ranged between 1.155 ng/mL-9.485 ng/mL, with a mean of 4.25 ng/mL. This mean testosterone value was consistent with the normal mean testosterone level of 4.15 ng/mL observed in another study looking at testosterone levels in a very similar population of men [13]. Another observation sometimes observed in testosterone levels was that there is decline in the levels over the decades. In this study, the average testosterone levels for the age ranges 41-50 years, 51-60 years, 61-70 years and 71-80 years, were 4.05 ng/mL, 3.92 ng/mL, 4.25 ng/mL and 4.21 ng/mL, respectively and did not show a decline with age. This is quite consistent with observations seen in a study in healthy Japanese male volunteers, where the mean total testosterone values

for each age decade for the 20 s to the 70 s ranged between 4.0 and 4.7 ng/mL and failed to demonstrate a decline with age [16]. Individuals with total testosterone levels <0.180 nmol/L are considered to have hypogonadism and suggested to require testosterone therapy [17,18]. In this study, using the reference range derived using the i-CHROMA™ testosterone method, we found that twelve of the volunteers had testosterone levels <0.180 nmol/L (12.2%) fulfilled such criteria. In a study carried out in a similar population, the authors found that the percentage of men with testosterone levels <300 ng/dL and <231 ng/dL, were 31% and 14.1%, respectively [13]. In this study, we found very comparable data, the percentage of men with testosterone levels <300 ng/dL and <231 ng/dL, were 29% and 15.3%, respectively.

In conclusion, the point of care i-CHROMA™ testosterone method was able to determine a reference range and have features consistent and comparable with other reference range studies. This is the first time that a quantitative immunoassay total testosterone point of care method has been shown to be demonstrate comparable information to those obtained with traditional testosterone laboratory methods.

CONFLICT OF INTEREST

JB is a consultant advisor to Boditech Med Inc. and Tae Kyum Kim is an employee of Boditech Med Inc. Boditech Med Inc is the manufacturer of the iCHROMA™.

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