

Testosterone Quantitative Test

A Rapid "Sandwich" Immunochromatographic Test for Quantitative Detection of Total Testosterone in human finger-prick Blood or Serum

REF 4003-Q25

For in vitro Diagnostic use only

Read Instructions before use

INTENDED USE

SPARK Testosterone Quantitative Test is an immunochromatography-based one-step in vitro test. It is designed for the quantitative determination of total Testosterone in human finger-prick blood or serum. This assay provides a preliminary diagnostic test result and can be used for screening Testosterone abnormalities. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays or other quantitative immunoassays are recommended to further confirm the diagnostic test results.

SUMMARY AND EXPLANATION

Testosterone is a very important and powerful steroid hormone in both men and women. In males, Testosterone is secreted primarily by the Leydig cells of the testes. In females, 50 % of circulating Testosterone is derived from the peripheral conversion of androstenedione, 25% from the ovary, and 25% from the adrenal glands. Circulating testosterone is 98% protein-bound in males, with slightly less bound in females. The proteins responsible for binding testosterone are serum albumin and Sex Hormone Binding Globulin (SHBG), also referred to as Testosterone Binding Globulin (TeBG).

Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal state. In men, high levels of testosterone are associated with the hypothalamic-diseases, testicular tumors, congenital adrenal hyperplasia, and prostate cancer. Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, Testicular feminization, Orchidectomy and Cryptorchidism, enzymatic defects, and some autoimmune diseases. LowTestosterone levels can cause changes in sexual function, including low libido, impotence, erectile dysfunction (ED), and infertility. Other signs of low Testosterone levels include changes in sleep patterns, difficulty in concentrating, lack of motivation, reduced muscle mass and strength, decreased bone density, large breasts in men, depression, and fatigue.

In women, high levels of Testosterone are generally found in hirsutism and virilization, polycystic ovaries, ovarian tumors, adrenal tumors, and adrenal hyperplasia. Excess Testosterone in a woman's bloodstream can cause loss of scalp hair, acne, irregular or absent menses, growth of facial hair, and infertility. Low testosterone in women can also cause fertility problems, in addition to weak bones and loss of libido.

Therefore, now detecting and monitoring testosterone levels is considered extremely important to maintain and improving overall health and well-being.

EXPECTED NORMAL VALUES

There are different types of Testosterone tests, but the most accurate one measures total testosterone in the blood. Test results are measured in ng/ml or ng/dL. It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for testosterone normal ranges may be used as guidelines:

Male:

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Stages of Life Normal Level (ng/ml)		Stages of Life	Normal Level (ng/ml)	
Adulthood	3.0 – 10.0	Adulthood	0.15 - 0.7	

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TEST PRINCIPLE

SPARK Testosterone Quantitative Test utilizes the principle of Immunochromatography, a unique two-site "Sandwich" immunoassay on a membrane. The test employs a very "Exclusive" pair of anti-Testosterone Monoclonal Antibodies; one conjugated with colloidal gold and another one immobilized in the solid phase. This will selectively detect Testosterone with a high degree of sensitivity and specificity.

As the test sample flows through the membrane assembly within the test device, the colored anti-Testosterone-colloidal gold conjugate complexes with Testosterone from the sample. This complex moves further on the membrane by the capillary action to the test region (T) where it is immobilized by another anti-Testosterone coated on the membrane, leading to the formation of a pink/purple colored band, which confirms a positive test result. The intensity of the colored band in the test line region is Testosterone concentration-dependent, the higher the concentration of Testosterone in the tested sample darker the colored band. A control line is present in the test window to work as procedural control. This colored band should always appear on the control line region (C) if the test device is stored in good condition and the test is performed appropriately.

MATERIALS PROVIDED

(1) SPARK Testosterone Test Cassette (1x)

- (2) Buffer tube (1x)
- (3) Capillary Collection tube (1x)
- (4) Camera Test Card (1x)
- (5) Package Insert (1x)
- (6) Lancet (1x)
- (7) Alcohol swab (1x)

MATERIALS REQUIRED BUT NOT PROVIDED

- 1.Smart Phone
- 2. Spark Dx Mobile App
- 3. Time or clock

STORAGE AND STABILITY

The test device should be stored at 4°C to 30°C and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being opened. Any improperly sealed product should be discarded.

Test Device		
Buffer		
Sampler		
Camera Card		
Lancet		
Alcohol Pad		

Note: Alcohol pad and Lancet are medical devices meeting the provisions of Directive 93/42/EEC. Lancet is sterile and bears CE0197.

PRECAUTIONS

- 1. For in vitro diagnostic use only.
- 2. Do not use the product beyond the expiration date.
- 3. Handle all specimens as potentially infectious.
- 4. Humidity-sensitive products, do not open the foil pouch until it is ready to be tested.
- 5. CAUTIONI Do not use if (a) Test device seal is broken, (b) Box contents are missing, or (c) inside contents are damaged or broken.

QUALITY CONTROL

Good Laboratory Practice recommends the frequent use of control materials to validate the reliability of the test device. If control values do not fall within the established range, assay results are invalid. The SPARK Testosterone Quantitative Test device provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless of the presence of Testosterone. If the control line does not appear, the test device should be discarded, and the obtained result is invalid. The presence of this control band in the control region verifies 1) that sufficient sample volume is added, and 2) proper sample flow is obtained.

CAUTION!

SPARK Testosterone Quantitative Test device has been designed for "Decision-Point" Finger-prick Blood (or Serum) samples ONLY. NO Anticoagulated Blood or Plasma samples should be used for testing the SPARK Testosterone Quantitative Test device as Anticoagulants may impact the test results.

DIRECTIONS FOR USE

Specimen Collection and Preparation

Before performing the test, please make sure that all components are brought to room temperature (15-30°C). Cold buffer solution or moisture condensation on the membrane can lead to invalid test results. Take a tube with buffer solution out of the kit. Specimen Collection and Preparation Before performing the test, please make sure that all components are brought to room temperature (15-30°C). Cold buffer solution or moisture condensation on the

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Blood Sample Collection

- Wash hands with soap and rinse with clean warm water
- ⊳ Get the lancet and carefully pull off and dispose of the cap of the lancet
- Use an alcohol pad to clean the fingertip of the middle or ring finger ⊳
- ≽ Press the lancet against the fingertip to obtain a blood drop for a sample
- ≽ Contact the capillary collection tube (Sampler) to the blood drop without squeezing the capillary bulk dropper. Fill the capillary collection tube with blood up to the line indicated on the capillary dropper. Massage the fingertip to obtain more blood to fill the capillary to the line. Avoid air bubbles

Testing with Testosterone Test Cassette

- ⊳ Bring the SPARK Testosterone test cassette to room temperature before opening it. Once the test cassette is removed from the sealed pouch, use it as soon as possible.
- ⊳ Place the cassette on a clean and level surface.
- ⊳ Drop the collected blood from the Sampler into the sample well of the cassette marked "S" by squeezing on the dropper bulk of the blood collection tube.
- ⊳ Wait for the blood to be totally dispensed in the well.
- ⋗ Unscrew the cap of the buffer bottle and add 2 drops of buffer into the sample well of the cassette.
- ≽ Wait for the colored line(s) to appear. Read the results in 15 minutes with the Spark Reader app. Do not read the results after 20 minutes.

See illustration below.



1. The SAMPLER Device contains a Collection Tube (left) and a Blood Collector with Cap (right).



5. Correct compression of Buffer and Sampler



2. Use a Lancet to draw finger-prick blood.

Incorrect



3. Gently touch the tip of Blood Collector to blood droplet. Capillary action will completely fill 10 µl of blood and stop.



6. Shake the SAMPLER with "Jerk" 3-4 times to completely take out blood from Blood Collector into the Sample Buffer, followed by complete mixing.



7. Remove the Cap of the SAMPLER



4. Fully Insert the Blood from the Blood Collector into the Collection Tube and push firmly to close tightly.



8. Invert the SAMPLER Device and gently squeeze 3 drop of pre-mix blood into the Sample Well (S) of the Test Cassette.

CAUTION!

1.Complete (100%) PRE-MIXING of finger-prick blood with sample buffer is an "EXTREMELY" important and critical step to get the correct result.

- 2. This can be determined by checking the UNIFORM red color of pre-mix blood in Collection Tube and Blood Collector.
- 3. Incomplete mixing of Blood with Buffer means Sample Preparation has been compromised, and the test result is likely to show lower values.
- 4. Pressing of the Sampler should be "GENTLE" to get three full drops of pre-mix blood into the sample well (S).

PROCEDURE:

- 1. Follow the Instruction on how to use the SPARK-Testosterone app to get the results.
- 2.Bring all materials and specimens to room temperature (between 21°C 24°C).
- 3. Remove the test card from the sealed foil pouch and place it on a hard flat surface.
- 4. Follow the Instructions to use Sampling Device.

Disposal

After use, dispose of all components of the SPARK-Testosterone Quantitative Test as biohazard waste.

Serum Protocol:

The same Protocol is also used for testing Testosterone controls. SPARK-Testosterone Quantitative Test has been designed for human finger-prick blood. However, Serum samples can be used for testing. Instead of taking fingerprick blood with a blood collector, apply 5µl of serum into the Collection Tube using Micropipette (not provided with the Kit) and follow the "Instructions to Use Sampler Device".

Important Note: Result after 15 minutes may not be accurate.

Instructions on how to use the SPARK DX Reader App (REF 8002-RF):

INTENDED USE OF APP:

sample application).

your storage, when needed (see privacy policy)

The SPARKDx Reader mobile app is designed to read the SPARK Testosterone test (4003-Q25). The mobile app scans the completed SPARK Testosterone test using the camera function of a mobile phone (or smartphone) to determine qualitative readings of Testosterone threshold levels of the test specimen and store the results in the phone.

Open the app and follow the below instruction to perform the test after preparing the test cassette.

1. About App: SparkDx App is a universal app that enables its user to run different Spark tests on a single platform.

INSTRUCTIONS TO USE THE APP:

1. Open SPARKDx Reader App (One-Time): By using the App, the user, can either create their own Spark account or Sign-in with their Google account (1a). Once logged in, the home screens appear as shown in the figure below (1b). Upon using 'Test', the user can navigate the wide range of tests offered by Spark (1c).

2.Start Test Instructions and Timer SPARKDx ReaderApp: After selecting a particular test, the user can see the test instructions by clicking on "Start test" (2a). The procedure for preparation of the test cassette is given step by step with an image guide on the screen.

Follow all the steps until step 7 (2b). At the end of the instructions, the user will receive a

prompt to set a 15 minutes timer (2c) for the test completion (before the next steps). Alternatively, the user can skip the timer to directly scan the test cassettes after the

completion of 15 min (The user should make sure that 15 min. has been completed after the

3. Camera Permissions SPARKDx ReaderApp (One-Time): In order to perform the test,



4.Scan Test Cassette in SPARKDx Reader App: After 15 minutes timer completion (in Step 3), the camera will start to take images (4a). Important: Use the "CAMERA CARD" provided with the package as background as shown in the screen below (4b). Keep the test cassette as shown in the picture below on the Camera Card before taking the image. Click "Next" to take a total of 3 images.



5. Display Result SPARK ReaderApp: After the 3 Scans are completed the result will be displayed on the screen as shown (5). Click "Save report" to save the results in the history screen or Export them as pdf.

PRECAUTIONS FOR APP:

1. Use Camera Scan functions only after completion of a 5 minutes timer.

2. Camera reading should be taken immediately after 15 minutes since start of test.

3. Always use a camera card to take pictures.

PERFORMANCE CHARACTERISTICS:

Sensitivity:

The sensitivity of the **SPARK Testosterone** Quantitative Test device is 0.07 ng/ml (LOD).

Detection Range:

The Detection Range of the **SPARK Testosterone** Quantitative Test is from 0.07 ng/ml to 25 ng/ml. Accuracy:

The accuracy of the **SPARK Testosterone** Quantitative Test was also evaluated using 20 serum samples in comparison with LC-MS/MS Assay ("Gold Standard" for Testosterone measurement). The comparison result showed a linear regression with a slope of 1.02 and a Correlation Coefficient of 98%. In conclusion, SPARK Testosterone Quantitative Test results agree closely with the true values generated from LC-MS/MS assay.

The accuracy of the **SPARK Testosterone** Quantitative Test was evaluated using human finger-prick blood samples in comparison with a reference Testosterone ELISA assay using corresponding serum samples. The comparison result showed a linear regression with a slope of 0.98 and a Correlation Coefficient of 92%. In conclusion, **SPARK Testosterone** Quantitative Test results of human blood samples showed good agreement with the ELISA results of corresponding serum samples.

Precision: Intra Lot

Sample	No. of Lot	No. of Replicates	Mean ng/ml	Coefficient Variation (CV)			
Serum -1	3	20	6.31	7.9%			
Serum -2	3	20	0.30	12.0%			
Serum -1	3	10	3.50	9.7%			

Intra Lot

Sample	No. of Lot	No. of Replicates	Mean ng/ml	Coefficient Variation (CV)
Serum -1	3	60	6.25	8.2%
Serum -2	3	60	0.29	12.8%
Serum -1	3	15	3.45	10.7%

Specificity:

30 Testosterone free serum samples were tested, and all showed negative results: suggesting 100% Specificity.

EXPECTED RESULTS

SPARK Testosterone Quantitative Test is a Rapid Quantitative assay. The test is intended to use for screening individuals to identify Testosterone levels. This assay provides only a preliminary analytical test result. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays or quantitative immunoassays is recommended to confirm the analytical result.

REFERENCES

- 1. Total Testosterone: Health Encyclopedia: https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=testosterone_total.
- 2. Testosterone Levels by Age: <u>https://www.healthline.com/health/low-testosterone/testosterone-levels-by-age.</u>
- 3. High Testosterone Levels in Women: <u>https://www.healthline.com/health/high-testosterone-in-women.</u>
- 4. Testosterone, Serum testosterone: https://www.mountsinai.org/health-library/tests/testosterone
- 5. Chen, A., Bookstein, J.J., Meldrum, D.R., Diagnosis of a testosterone-secreting adrenal adenoma by selective venous catheterization. Fertil. Steril., 1991; 55: 1202-1203.
- 6. Granoff, A.B. and Abraham, G.E., Peripheral and adrenal venous levels of steroids in a patient with virilizing adrenal adenoma. Obstet. Gynecol., 1979; 53:111-115.

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