Testosterone Quantitative Test

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

CE REF **1173Q-25**

For in vitro Diagnostic use only ***Read Instructions before use***

INTENDED USE

Global Diagnostics B's **TestNOW® - 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 of Testosterone abnormalities. The 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 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 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 being bound in females. The proteins responsible for biding 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 states. In men, high levels of testosterone are associated to the hypothalamic pituitary unit 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. Low testosterone levels can cause changes in sexual function, including: low libido, impotence, erectile dysfunction (ED), infertility. Other signs of low testosterone levels include: changes in sleep patterns, difficulty concentrating, lack of motivation, reduced muscle bulk 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, 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 level is considered extremely important to maintain and improve 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 guideline:

Male:

Stages of Life	Normal Level (ng/ml)
Prepubertal (late)	0.1 – 0.2
Adulthood	3.0 – 10.0

Female:

Stages of Life	Normal Level (ng/ml)
Prepubertal (late)	0.1 – 0.2
Follicular phase	0.2 – 0.8
Luteal phase	0.2 – 0.8
Post-menopausal	0.08 – 0.35

TEST PRINCIPLE

TestNOW® - 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 on 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 formation of a pink / purple colored band, which confirms a positive test result. The intensity of colored band in the test line region is Testosterone concentration-dependent, higher the concentration of Testosterone in the tested sample, the stronger the colored band is. 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. **TestNOW® - Testosterone** Quantitative Test (Kit Size: 25 Tests/Box)
2. **UniSampler™** Collection Tube (sealed Sampler Buffer Tubes – 26 pieces)
3. **UniSampler™** Blood Collector (26 pieces)
4. RFID Card (provides result in ng/ml) - 1
5. Instructions for use – 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer or clock
2. Lancet
3. Alcohol Swab
4. ***RapiRead™*** CUBE Reader (CE Marked) – To be purchased separately

STORAGE AND STABILITY

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

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 product, do not open foil pouch until it is ready to be tested.
5. **TestNOW® - Testosterone** Quantitative Test device must be quantified with ***RapiRead™*** CUBE Reader only.
6. RFID Card is Lot Specific and cannot be interchanged with another Lot.

QUALITY CONTROL

Good Laboratory Practice recommends the frequent use of control materials to validate the reliability of test device. If control values do not fall within established range, assay results are invalid.

The **TestNOW® - 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 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 serves as 1) verification that sufficient sample volume is added, 2) that proper sample flow is obtained.

CAUTION!

TestNOW® - 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 **TestNOW® - Testosterone** Quantitative Test device as Anticoagulants may impact the test results.

SPECIMEN COLLECTION AND PREPARATION

1. Wash your hand thoroughly and dry completely.
2. Rub and Wipe your ring or middle finger of non-dominant hand.
3. Using safety lancet puncture the side of your finger.
4. Collect 10 µl blood using Blood Collector (See instructions below) and perform testing immediately.

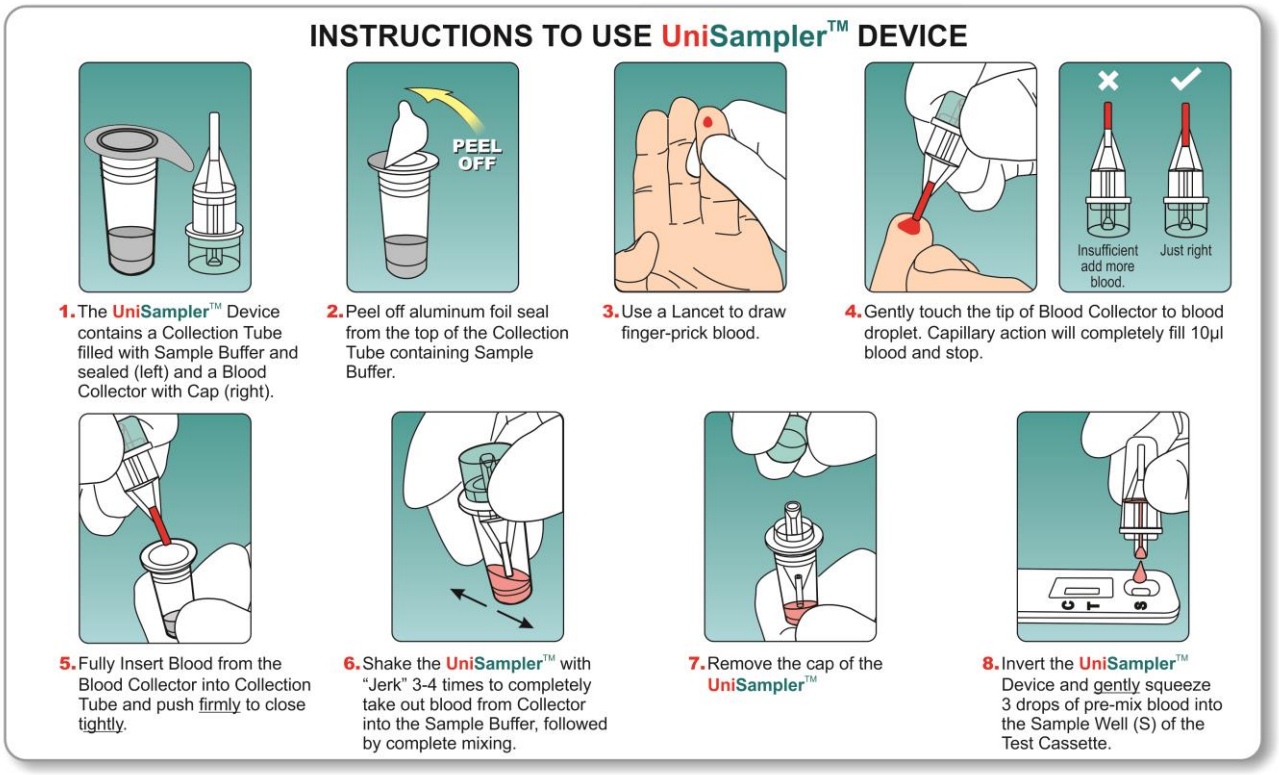
PROCEDURE:

1. Bring all materials and specimens to room temperature (between 21°C – 24°C).
2. Remove the test card from the sealed foil pouch and place it on a hard flat surface.
3. Follow Instructions to use **UniSampler™** Device.
4. After applying 3 drops of pre-mix blood into the sample well (S), read and record the results at 15 Minutes by ***RapiRead™*** CUBE Reader.

TESTOSTERONE SERUM PROTOCOL:

TestNOW® - Testosterone Quantitative Test has been designed for human finger-prick blood. However, Testosterone Serum sample can be used for testing. Instead of taking finger prick blood with blood collector, apply 5µl of Testosterone Serum into the Collection Tube using Micropipette (not provided with the Kit) and follow "Instructions to Use **UniSampler™** Device".

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

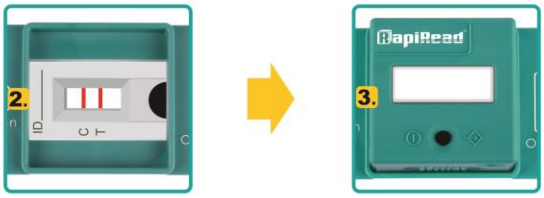


CAUTION!

- Complete (100%) PRE-MIXING of finger-prick blood with sample buffer is "EXTREMELY" important and CRITICAL Step to get correct result. This can be determined by checking the UNIFORM red color of pre-mix blood in Collection Tube and Blood Collector.
- Incomplete mixing of Blood with Buffer means Sample Preparation has been compromised, and the test result is likely to show lower values.
- Pressing of **UniSampler™** should be "GENTLE" to get three full drops of pre-mix blood into the sample well (S).

QUANTITATIVE DETECTION USING *RapiRead*™ CUBE READER

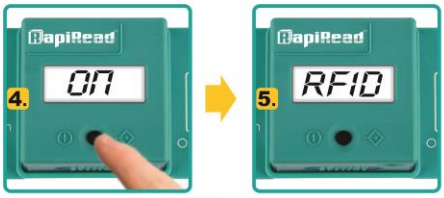
1. Check the "Correct Orientation" shown on the Adaptor for the Test Device and *RapiRead*™ CUBE Reader.



2. Place the Adapter on top of the Test Device correctly.

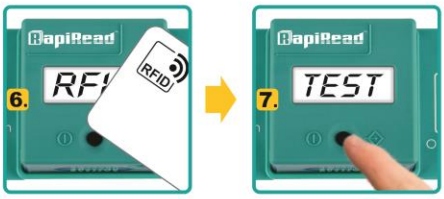
3. Place the *RapiRead*™ CUBE Reader on top of the Adaptor correctly
- For Non-Timer Protocol: After 15 Minutes of testing.
- For Timer Protocol: After adding 3 drops of pre-mix blood into the Sample Well (S) of the Test Cassette.

NON-TIMER PROTOCOL



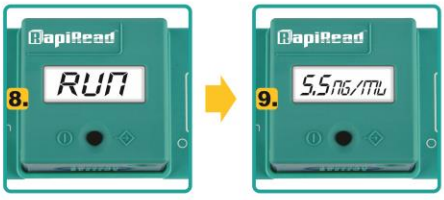
4. Turn-on the *RapiRead*™ by pressing the black button. Reader runs a self-test, during the self-test "WAIT" is displayed. After an audible beep signal, "ON" is displayed. To perform a reading, press the black button again once for 1 second.

5. The display will show "RFID".



6. Place the Lot specific RFID Card provided with the Kit onto the top side of the *RapiRead*™. This will upload Vitamin D test specific Calibration data from RFID Card to *RapiRead*™.

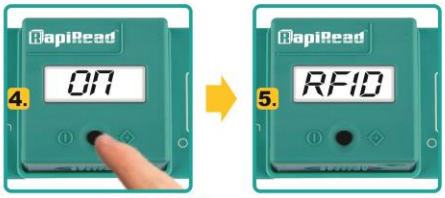
7. Following an audible beep signal, "TEST' is displayed. Press the black button, the Reader displays "RUN".



8. After successful data transmission the measurement will start.

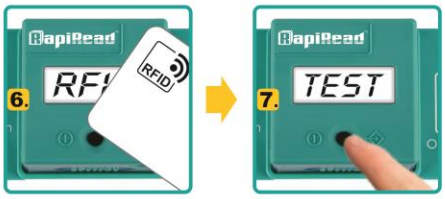
9. Testosterone concentration is displayed as ng/ml followed by Result with an audible beep signal.

TIMER PROTOCOL



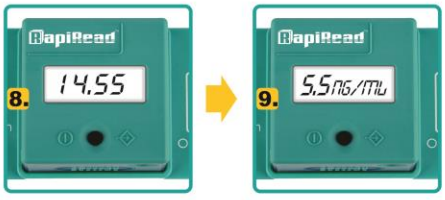
4. Turn-on the *RapiRead*™ by pressing the black button. Reader runs a self-test, during the self-test "WAIT" is displayed. After an audible beep signal, "ON" is displayed. Keep pressing black button till display shows RFID.

5. The display will show "RFID".



6. Place the Lot specific RFID Card provided with the Kit onto the top side of the *RapiRead*™. This will upload Vitamin D test specific Calibration data from RFID Card to *RapiRead*™.

7. Following an audible beep signal, "TEST' is displayed. Press the black button, the countdown timer will start.

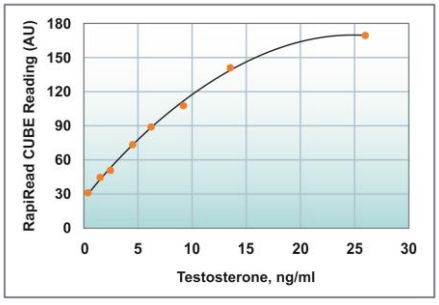


8. Countdown timer display will start.

9. After 15 minutes Testosterone concentration is automatically displayed as ng/ml followed by Result with an audible beep signal.

STANDARD CURVE USING *RapiRead*™ CUBE READER

A typical standard curve is illustrated on right side. The reading AU is automatically converted into ng/ml in *RapiRead*™ Reader.



INTERPRETATION OF RESULTS:

The ***RapiRead***™ CUBE analyzer automatically determines the final result by comparing the AU for each sample against a pre-established calibration curve. Testosterone levels are expressed in ng/ml. Please refer to Table on Page-1.

PERFORMANCE CHARACTERISTICS:

Sensitivity:

The sensitivity of **TestNOW® - Testosterone** Quantitative Test device is 0.07 ng/ml (LOD).

Detection Range:

The Detection Range of **TestNOW® - Testosterone** Quantitative Test with ***RapiRead***™ CUBE Reader is from 0.07 ng/ml to 25 ng/ml.

Accuracy:

The accuracy of **TestNOW® - 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 the slope of 1.02 and Correlation Coefficient of 98%. In conclusion, **TestNOW® - Testosterone** Quantitative Test results agree closely to the true values generated from LC-MS/MS assay.

The accuracy of **TestNOW® - 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 slope of 0.98 and Correlation Coefficient of 92%. In conclusion, **TestNOW® - 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%
Blood - 1	3	10	3.50	9.7%

Inter 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%
Blood - 1	3	15	3.45	10.7%

Specificity:

30 Testosterone free serum samples were tested, and all showed negative results: suggesting 100% Specificity.
No interference and cross reactivity were observed with Bilirubin, Triglycerides, Cholesterol, Vitamin B12 and Vitamin C.

EXPECTED RESULTS

TestNOW® - Testosterone Quantitative Test is a Rapid Quantitative assay. The test is intended to use for screening individuals to identify Testosterone level. This assay provides only a preliminary analytical test result. The liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays or quantitative immunoassays are 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.
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3. High Testosterone Levels in Women: https://www.healthline.com/health/high-testosterone-in-women.
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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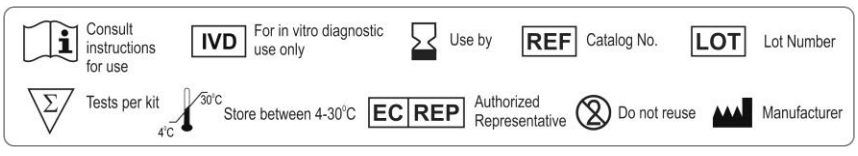
SHELF LIFE: 18 Months

INDEX OF CE SYMBOLS



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