



## Cortisol Quantitative Test

**A Rapid “Sandwich” Immunochromatographic Test for Quantitative Detection of total Cortisol in human finger-prick Blood or Serum**



**For *in vitro* Diagnostic use only**

**Read Instructions before use**

### INTENDED USE

Affimedix's **TestNOW® - Cortisol** Quantitative Test is an immunochromatography-based one step *in vitro* test. It is designed for the quantitative determination of total Cortisol in human finger-prick blood or serum. This assay provides a preliminary diagnostic test result and can be used for screening of Cortisol 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

Cortisol is a glucocorticoid hormone released by the adrenal cortex and plays an important role in the body's physiological processes and functions. Cortisol levels vary during the day, being the highest in the early morning (30 min after awaking, 50–250 ng/ml) and the lowest before bedtime (30–130 ng/ml). Elevated cortisol levels with repeated activation leads to Cushing's syndrome, which can result in severe fatigue, depression, anxiety, cognitive difficulties, obesity and cardiovascular disease. Studies have shown that the cortisol level increases in response to both physical and psychological stress, and has therefore been identified as a key biomarker of stress. Cortisol secretion in response to physical or emotional stress increases blood pressure to provide fat and glucose in muscles and brain for successfully coping with stress. However, prolonged elevation of cortisol can damage health and produce serious problems, such as hypertension, particularly in older and/or unhealthy individuals. Monitoring cortisol levels can reveal physiological states for both prognosis and diagnosis purposes.

The lack of sufficient cortisol in the body results in Addison's disease. The determination of cortisol is used for the recognition and treatment of functional disorders of the adrenal gland. Acute adrenal crisis is a life-threatening state caused by insufficient levels of cortisol — a hormone produced and released by the adrenal gland. Adrenal insufficiency is a rare condition, not always easy to identify but requiring immediate diagnosis and treatment to resolve.

Cortisol at different physiological concentrations can be found in a variety of biofluids, such as blood, urine, saliva, interstitial fluid and sweat. Most cortisol in the blood is bound to corticosteroid-binding globulins (CBG); only a small percentage is “free” and biologically active. Blood cortisol testing evaluates both protein-bound and free cortisol while urine and saliva testing evaluate only free cortisol, which should correlate with the levels of free cortisol in the blood. Blood analysis is the gold standard in monitoring the physiological state of individuals.

Cortisol hormone affects almost every organ and tissue in your body. It plays a critical role in helping to: Respond to stress, Fight infection, Regulate blood sugar, Maintain blood pressure, Regulate metabolism, and the process of how the body uses food and energy. Therefore, now detecting Cortisol level is considered as very important Diagnostic Test to maintain and improve overall health and well-being. Recent literature has suggested the following ranges for the classification of Cortisol status:

| Cortisol Level | Morning (8:00AM) Reference Level (ng/ml) | Evening (4:00PM) Reference Level (ng/ml) |
|----------------|--|--|
| Low            | < 50                                     | < 30                                     |
| Normal         | 50 - 250                                 | 30-130                                   |
| High           | > 250                                    | > 130                                    |

### TEST PRINCIPLE

**TestNOW® - Cortisol** 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-Cortisol Monoclonal Antibodies; one conjugated with colloidal gold and another one immobilized on the solid phase. This will selectively detect Cortisol with a high degree of sensitivity and specificity. As the test sample flows through the membrane assembly within the test device, the colored anti-Cortisol-colloidal gold conjugate complexes with Cortisol 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-Cortisol 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 Cortisol concentration-dependent, higher the concentration of Cortisol 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® - Cortisol** 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 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 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® - Cortisol** 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® - Cortisol** 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 Cortisol. 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® - Cortisol** 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® - Cortisol** 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.

#### CORTISOL SERUM PROTOCOL:

**TestNOW® - Cortisol** Quantitative Test has been designed for human finger-prick blood. However, Cortisol Serum sample can be used for testing. Instead of taking finger prick blood with blood collector, apply 5µl of Cortisol 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.**

### INSTRUCTIONS TO USE **UniSampler™** DEVICE

1. The **UniSampler™** Device contains a Collection Tube filled with Sample Buffer and sealed (left) and a Blood Collector with Cap (right).
2. Tap the Buffer tube on a Flat surface to ensure that all the liquid has come at the bottom.
3. Peel off aluminum foil seal from the top of the Collection Tube containing Sample Buffer.
4. Use a Lancet to draw finger-prick blood.
5. Gently touch the tip of Blood Collector to blood droplet. Capillary action will completely fill 10µl blood and stop.
6. Check the level of Blood in Blood Collector.
 

Insufficient  
add more  
blood.

Just right
7. Fully Insert Blood from the Blood Collector into Collection Tube and push firmly to close tightly.
8. Shake the **UniSampler™** with “Jerk” 3-4 times to completely take out blood from Collector into the Sample Buffer, followed by complete mixing.
9. Remove the cap of the **UniSampler™**
10. Invert the **UniSampler™** Device and gently squeeze 3 drops of pre-mix blood into the Sample Well (S) of the Test Cassette.

#### 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.



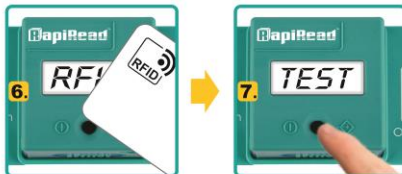
2. Place the Adapter on top of the Test Device correctly.
3. Place the *RapiRead*™ CUBE Reader on top of the Adaptor correctly
  - For Non-Timer Protocol: After 15 Minutes of testing.
  - For Timer Protocol: After adding 3 drops of pre-mix blood into the Sample Well (S) of the Test Cassette.

### 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 Cortisol 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.

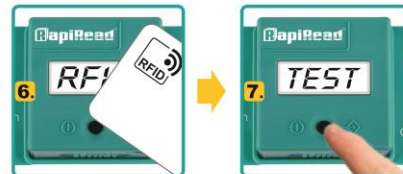
9. Cortisol concentration is displayed as ng/ml with an audible beep signal.

### 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 Cortisol 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.

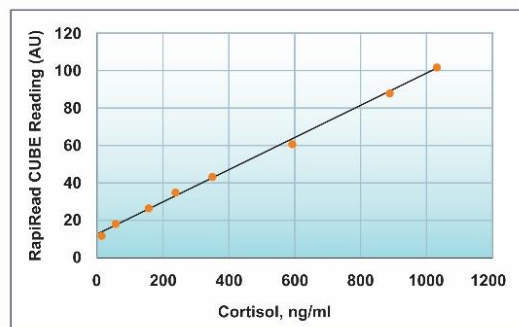


8. Countdown timer display will start.

9. After 15 minutes Cortisol concentration is automatically displayed as ng/ml 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. The cortisol values of serum calibrators are assigned by using a competitive antibody-binding assay and Standard Reference Material 921a (NIST).



## INTERPRETATION OF RESULTS:

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

## PERFORMANCE CHARACTERISTICS:

### Sensitivity:

The sensitivity of **TestNOW® - Cortisol** Quantitative Test device is 30 ng/ml (LOD).

### Detection Range:

The Detection Range of **TestNOW® - Cortisol** Quantitative Test with **RapiRead™** CUBE Reader is from 30 ng/ml to 1000 ng/ml.

### Accuracy:

The accuracy of **TestNOW® - Cortisol** Quantitative Test was evaluated using human finger-prick blood samples in comparison with a reference Cortisol ELISA assay using corresponding serum samples. The comparison result showed a linear regression with slope of 0.91 and Correlation Coefficient of 90%. In conclusion, **TestNOW® - Cortisol** Quantitative Test results of human blood samples showed good agreement with the ELISA results of corresponding serum samples.

The accuracy of **TestNOW® - Cortisol** Quantitative Test was also evaluated using 20 serum samples in comparison with LC-MS/MS Assay ("Gold Standard" for Cortisol measurement). The comparison result showed a linear regression with the slope of 0.95 and Correlation Coefficient of 94%. In conclusion, **TestNOW® - Cortisol** Quantitative Test results agree closely to the true values generated from LC-MS/MS assay.

### Precision:

#### Intra Lot

| Sample    | No. of Lot | No. of Replicates | Mean ng/ml | Coefficient Variation (CV) |
|-----------|------------|-------------------|------------|----------------------------|
| Serum -1  | 3          | 20                | 230.0      | 7.9%                       |
| Serum -2  | 3          | 20                | 55.1       | 12.5%                      |
| Blood - 1 | 3          | 10                | 150.0      | 9.2%                       |

#### Inter Lo

| Sample    | No. of Lot | No. of Replicates | Mean ng/ml | Coefficient Variation (CV) |
|-----------|------------|-------------------|------------|----------------------------|
| Serum -1  | 3          | 60                | 231.9      | 8.1%                       |
| Serum -2  | 3          | 60                | 53.5       | 12.9%                      |
| Blood - 1 | 3          | 15                | 148.6      | 10.1%                      |

### Specificity:

30 Cortisol 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® - Cortisol** Quantitative Test is a Rapid Quantitative assay. The test is intended to use for screening individuals to identify Cortisol 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. Cortisol blood test : <https://www.mountsinai.org/health-library/tests/cortisol-blood-test>
2. R. Gatti, G. Antonelli, M. Prearo, P. Spinella, E. Cappellin, F. Elio, Cortisol assays and diagnostic laboratory procedures in human biological fluids, Clin. Biochem. 42 (2009) 1205–1217, <https://doi.org/10.1016/j.clinbiochem.2009.04.011>.
3. D. Corbalán-Tutau, J.A. Madrid, F. Nicolás, M. Garaulet, Daily profile in two circadian markers "melatonin and cortisol" and associations with metabolic syndrome components, Physiol. Behav. 123 (2014) 231–235, <https://doi.org/10.1016/j.physbeh.2012.06.005>.
4. O. Edwards, J. Galley, R. Courtenay-Evans, J. Hunter, A. Tait, Changes in cortisol metabolism following rifampicin therapy, Lancet 304 (1974) 549–551, [https://doi.org/10.1016/S0140-6736\(74\)92725-1](https://doi.org/10.1016/S0140-6736(74)92725-1).
5. H. Raff, J.W. Findling, A physiologic approach to diagnosis of the Cushing syndrome, Ann. Intern. Med. 138 (2003) 980–991, <https://doi.org/10.7326/0003-4819-138-12-200306170-00010>.
6. A.V. Chobanian, G.L. Bakris, H.R. Black, W.C. Cushman, L.A. Green, J.L. Izzo Jr et al., The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report, JAMA 289 (2003) 2560–2571, <https://doi.org/10.1001/jama.289.19.2560>.
7. J.A. Whitworth, G.J. Mangos, J.J. Kelly, Cushing, cortisol, and cardiovascular disease, Hypertension 36 (2000) 912–916, <https://doi.org/10.1161/hyp.36.5.912>.
8. M. Al'Absi, D. Arnett, Adrenocortical responses to psychological stress and risk for hypertension, Biomed. Pharmacother. 54 (2000) 234–244, [https://doi.org/10.1016/S0753-3322\(00\)80065-7](https://doi.org/10.1016/S0753-3322(00)80065-7).
9. J. Kelly, G. Mangos, P. Williamson, J. Whitworth, Cortisol and hypertension, Clin. Exp. Pharmacol. Physiol. 25 (1998) S51–S56, <https://doi.org/10.1111/j.1440-1681.1998.tb02301.x>.
10. J.A. Whitworth, P.M. Williamson, G. Mangos, J.J. Kelly, Cardiovascular consequences of cortisol excess, Vasc. Health Risk Manag. 1 (2005) 291, <https://doi.org/10.2147/vhrm.2005.1.4.291>. Total Cortisol: Health Encyclopedia: [https://www.umc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=Cortisol\\_total](https://www.umc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=Cortisol_total).

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**SHELF LIFE: 18 Months**

## INDEX OF CE SYMBOLS

|  |  |  |   |  |
|--|--|--|---|--|
|  Consult instructions for use |  IVD For in vitro diagnostic use only |  Use by                           |  REF Catalog No. |  LOT Lot Number |
|  Tests per kit                |  Store between 4-30°C                 |  EC REP Authorized Representative |  Do not reuse    |  Manufacturer   |



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