CE IVD

One Step Test for NT-proBNP/cTnI

(Colloidal Gold)

User Manual Cat.# CG1004

INTENDED USE

One Step Test for NT-proBNP/cTnI (Colloidal Gold) is intended for *in vitro* quantitative determination of N-terminal B-type natriuretic peptide precursor/cardiac Troponin I (NT-proBNP/cTnI) in serum, plasma or whole blood. This test is used as an aid in the clinical diagnosis, prognosis and evaluation of Heart Failure (HF), Acute Myocardial Infarction (AMI) and Acute Coronary Syndrome (ACS).

SUMMARY

N-terminal B-type natriuretic peptide precursor is secreted from the left cardiac ventricle in response to volume and pressure overload. It's an inactive N-terminal fragment that split from BNP prohormone. NT-proBNP can be used to evaluate heart contractile, diastolic dysfunction, and ventricular segmental wall motion coordination. Besides, it has high sensitivity and negative predictive value (>97%). As a gold standard recommended by the European Society of Cardiology, American Heart Association, and American College of Cardiology for the diagnosis and prognosis of heart failure, NT-proBNP is used to find heart failure patient at the early stage, determine HF risk levels, monitor medical efficiency of HF drug, evaluate prognosis of HF patient and to distinguish dyspnea that caused by HF from other diseases. Furthermore, NT-proBNP is a risk assessment indicator for Acute Coronary Syndrome.

Troponin, a molecular complex that is bound to the thin filament (actin) of striated muscle fibers, acts with intracellular calcium to control the interaction of the thin filament with the thick filament (myosin), thus regulating muscle contraction. Troponin consists of three regulatory proteins: T, which connects the troponin complex and tropomyosin (another cardiac muscle regulatory protein); I, which prevents muscle contraction in the absence of calcium; C, which binds calcium. Cardiac Troponin I (MW 22.5 kDa) and the two skeletal muscle isoforms of Troponin I have considerable amino acid sequence homology, but cTnI contains an additional N-terminal sequence and is highly specific for myocardium.

Clinical studies have demonstrated the release of cTnI into the blood stream within hours following AMI or ischemic damage. Elevated levels of cTnI are detectable in blood within 4 to 6 hours after the onset of chest pain, reaching peak concentrations in approximately 8 to 28 hours, and remain elevated for 3 to 10 days following AMI. Due to the high myocardial specificity and the long duration of elevation, cTnI has become an important marker in the diagnosis and evaluation of patients suspected of having an AMI.

PRINCIPLE

Mixed monoclonal antibodies against human NT-proBNP and cTnI were conjugated with colloidal gold and another set of an anti-human NT-proBNP polyclonal antibody and an anti-human cTnI monoclonal antibody were coated on different test lines respectively. After the sample has been applied to the test strip, the gold-labelled anti-human NT-proBNP or cTnI monoclonal antibodies will bind with the NT-proBNP and cTnI in sample respectively and form marked antigen-antibody complexes. These complexes move to the test card detection zone by capillary action. Then marked antigen-antibody complexes will be captured on different test lines by another set of anti-human NT-proBNP polyclonal antibody or anti-human cTnI monoclonal antibody resulting in a purplish red streaks appear on the test lines. The color intensity of each test line increases in proportion to the amount of NT-proBNP or cTnI in sample.

Then insert test card into FIA8000 Quantitative Immunoassay Analyzer (hereinafter referred to as FIA8000), the concentrations of NT-proBNP and cTnI in sample will be determined and displayed on the screen. The value will be stored in FIA8000 and available for downloading. The result can be easily transmitted to the laboratory or hospital information system.

CONTENTS

A kit contains:

1. Getein NT-proBNP/cTnI test card in a sealed pouch with desiccant ····· 25
2. Disposable pipet ····· 25
3. User manual ····· 1
4. SD card ····· 1
5. Whole blood buffer ····· 1

A test card consists of:

A plastic shell and a reagent strip which is composed of a sample pad, a colloidal gold pad, nitrocellulose membrane (coated with gold-labelled anti-human NT-proBNP and cTnI monoclonal antibodies), nitrocellulose membrane with 2 test lines (these two test lines are coated with another anti-human NT-proBNP polyclonal antibody and another anti-human cTnI monoclonal antibody, respectively, and the control line C is coated with rabbit anti-mouse IgG antibody), absorbent paper and liner.

Whole blood buffer composition:

Phosphate buffered saline, proteins, detergent, preservative, stabilizer.

Note: Do not mix or interchange different batches of kits.

APPLICABLE DEVICE

FIA8000 Quantitative Immunoassay Analyzer

STORAGE AND STABILITY

Store the test card at 4~30°C with a valid period of 24 months.
Use the test card within 1 hour once the foil pouch is opened.
Store the whole blood buffer at 0~30°C with a valid period of 24 months.
Store the whole blood buffer at 2~8°C for better results.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. For professional use only.
3. Do not use the kit beyond the expiration date.
4. Do not use the test card if the foil pouch is damaged.
5. Do not open pouches until ready to perform the test.
6. Do not reuse the test card.
7. Do not reuse the pipet.
8. Handle all specimens as potentially infectious. Proper handling and disposal methods should be followed in accordance with local regulations.
9. Carefully read and follow user manual to ensure proper test performance.

SPECIMEN COLLECTION AND PREPARATION

1. This test can be used for ***serum, plasma and whole blood samples. Heparin and sodium citrate*** should be used as the anticoagulant for plasma and whole blood. Samples should be free of hemolysis.
2. Suggest using serum or plasma for better results.
3. Serum or plasma can be used directly. For whole blood sample, one drop of whole blood buffer must be added before testing.
4. If testing will be delayed, serum and plasma samples may be stored up to 1 day at 2~8°C or stored at -20°C for 3 months before testing (whole blood sample may be stored up to 3 days at 2~8°C).
5. Refrigerated or frozen sample should reach room temperature and be homogeneous before testing. Avoid multiple freeze-thaw cycles.
6. Do not use heat-inactivated samples.
7. SAMPLE VOLUME: ***120 µl***.

TEST PROCEDURE

1. Collect specimens according to user manual.

2. Test card, sample and reagent should be brought to room temperature before testing.
3. Confirm SD card lot No. in accordance with test kit lot No.. Perform “QC (SD)” calibration when necessary (Details refer to 8.2.1 of FIA8000 User Manual).
4. On the main interface of FIA8000, press "ENT" button to enter testing interface.
5. Remove the test card from the sealed pouch immediately before use. Label the test card with patient or control identification.
6. Put the test card on a clean table, horizontally placed.
7. Using sample transfer pipette, deliver ***120 µl*** of sample (or 4 drops of sample when using disposable pipet) into the sample port on the test card (for whole blood sample, one drop of whole blood buffer must be added after loading 120 µl sample on the test card).
8. ***Reaction time: 18 minutes.*** Insert the test card into FIA8000 and press "ENT" button after reaction time is elapsed. The result will be shown on the screen and printed automatically.

Notes:
1. It is required to perform “QC (SD)” calibration when using a new batch of kits.
2. It is suggested to calibrate once for one batch of kits.
3. Make sure the test card insertion is correct and complete.

TEST RESULTS

Valid: When a purplish-red band appears at the control area (C), use FIA8000 to analyze the test card and get the result.
Invalid: If no colored band appears in the control area (C), the test result is invalid. The test should be repeated and if the same situation happened again, please stop using this batch of products and contact your supplier.

EXPECTED VALUE

The expected normal value for NT-proBNP was determined by testing samples from 2,500 apparently healthy individuals. The 95th percentile of the concentration for NT-proBNP is 185 pg/ml and the 97.5th percentile of the concentration for NT-proBNP is 300 pg/ml. Because of the apparent difference of the concentration of NT-proBNP among different age groups, the reference value of the NT-proBNP are reported in groups. Details refer to Table 1. NT-proBNP clinical diagnosis value: refer to Roche criterion, details see Table 2.

Table 1 NT-proBNP reference value

Age / Percentile	≤44	45-54	55-64	65-74	≥75	Statistic analysis
95	98.5	130	215	290	530	185
97.5	116	170	270	350	740	300

Table 2 Standard of excluding/diagnosing HF by NT-proBNP

Age	<50	50-75	≥75	Diagnosis of HF
NT-proBNP (pg/ml)	≥450	≥900	≥1800	High probability of HF
	300-450	300-900	300-1800	Low probability of HF, need to combine with other clinical evaluation
	<300	<300	<300	Exclude HF

The expected normal value for cTnI was determined by testing samples from 500 apparently healthy individuals. The 99th percentile of the concentration for cTnI is 0.5 ng/ml. (The probability that value of a normal person below 0.5 ng/ml is 99%.) It is recommended that each laboratory establish its own expected values for the population it serves.

PERFORMANCE CHARACTERISTICS

	NT-proBNP	cTnI
Measuring Range	100~12000 pg/ml	0.5~50.0 ng/ml
Lower Detection Limit	≤ 100 ng/ml	≤ 0.5 ng/ml
Recovery	99%(mean)	95%(mean)
Within-Run Precision	≤10%	
Between-Run Precision	≤15%	

Method Comparison:
The assay was compared with Roche MODULAR ANALYTICS E170 and its matching NT-proBNP test kits, SIEMENS IMMULITE 1000/2000 and its matching cTnI test kits with 200 serum samples (60 positive samples and 140 negative samples).
The correlation coefficient (r) for NT-proBNP is 0.955, the correlation coefficient (r) for cTnI is 0.942.

LIMITATIONS

1. As with all diagnostic tests, a definitive clinical diagnosis should not be made based on the result of a single test. The test results should be interpreted considering all other test results and clinical information such as clinical signs and symptoms.
2. Samples containing interferents may influence the results. The table below listed the maximum allowance of these potential interferents.

Interferent	Hemoglobin	Triglyceride	Bilirubin
Concentration (Max)	10 g/L	10 g/L	0.2 g/L

REFERENCES

1. Mauro Pantaghini; Undefined International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Scientific Division Committee on Standardization of Markers of Cardiac Damage. Clin Chem Lab Med, 1998, 36:887~893.
2. Pfister R, Scholz M, Wielckens K, Erdmann E, Schneider CA. The value of natriuretic peptides NT-pro-BNP and BNP for the assessment of left-ventricular volume and function. A prospective study of 150 patients. Deutsche medizinische Wochenschrift (1946) 2002;127(49):2605.
3. EN ISO 18113-1:2009 *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements.
4. EN ISO 18113-2:2009 *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: *In vitro* diagnostic reagents for professional use (ISO 18113-2: 2009).

DESCRIPTION OF SYMBOLS USED

The following graphical symbols used in or found on One Step Test for NT-proBNP/cTnI (Colloidal Gold) are the most common ones appearing on medical devices and their packaging. They are explained in more details in the European Standard EN 980:2008 and International Standard ISO 15223-1:2007.

Key to symbols used			
	Manufacturer		Expiration date
	Do not reuse		Date of manufacture
	Consult instructions for use	LOT	Batch code
	Temperature limitation	IVD	*In vitro* diagnostic medical device
	Sufficient for	EC REP	Authorized representative in the European Community
	CE mark		Do not use if package is damaged

Thank you for purchasing One Step Test for NT-proBNP/cTnI (Colloidal Gold). Please read this user manual carefully before operating to ensure proper use.

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