ABCDx DUOCheck™

Rapid quantitative test for the assay of amino terminal fragment of the Brain Natriuretic Peptide (NT-proBNP) and Heart-type fatty acid-binding protein (H-FABP) in whole blood, serum or heparinized plasma samples.
For use only in conjunction with the ABCDx BRAINCheck™ App
Ref. 1809891A (20 tests)
Ref. 1809891A-10T (10 tests)

I. INTENDED PURPOSE

ABCDx DUOCheck™ is a rapid test for the detection of human NT-proBNP and H-FABP in whole blood, serum or heparinized plasma samples. When used in conjunction with the ABCDx BRAINCheck™ App software, the ABCDx DUOCheck™ test allows quantitative measurement of the concentration of NT-proBNP and H-FABP in human samples. It is intended to be used as an aid in the management of patients with cerebrovascular disorders. The test is intended for near-patient testing by healthcare professionals.
No medical decision should be taken with the sole measurement of NT-proBNP and/or H-FAPB concentration. Additional clinical examinations are necessary for management of patient with cerebrovascular disorders.

II. PRINCIPLE

a) NT-ProBNP -amino terminal fragment of the Brain Natriuretic Peptide

Natriuretic peptides are hormones with diuretic and vasodilator effects, mainly secreted in the left ventricle as a mechanism to compensate for pressure overload (1). BNP as well NT-proBNP, which are not present in normal myocardium, are the products of proteolytic processing of proBNP molecule for which the concentration increases with vascular events such as heart failure (HF), acute coronary syndromes, cardiomyopathy (2). BNP and NT-proBNP have therefore gained widespread acceptance as important tools for diagnosis and risk stratification in the acute care setting (3,4,5).
Due to the high correlation found between cardiac dysfunction and stroke, NT-proBNP has been considered as a marker of stroke risk. NT-proBNP levels increase importantly within 24–48 hours after ischemic stroke and start to decline thereafter. For this reason, NT-proBNP has been proposed as a useful marker in the identification of patients with cardioembolic stroke as well as in the etiological classification of TIA (Transient Ischemic Attack) and stroke (8,9).

b) H-FABP (FABP3)- Heart-type fatty acid-binding protein

Heart-type fatty acid-binding protein (H-FABP or FABP) is a subtype of fatty-acid binding protein family. It is a small cytoplasmic protein of 15 kDa.
H-FABP can be found in brain, myocardial muscle, endothelial cells, lung, and kidney. It is released from tissue to peripheral blood following ischemic event. Clinically, plasma H-FABP level is known to be a sensitive biomarker for acute myocardial infarction. In stroke events, H-FABP has a rapid increase in concentration with a peak at 3h after symptom onset. Thereafter, the concentrations remained high for 5 days. The early increase of blood concentration and steadiness in time make this protein a potential interesting tool for brain lesion diagnostics (10,11,12).

However, as a sole marker, NT-proBNP or H-FABP concentrations are not sufficient to be used in routine clinical practice because of low sensitivity for stroke diagnosis. The quantification of NT-proBNP and/or H-FABP together using the ABCDx DUOCheck™ may lead to an improvement of patient's management and to assist medical doctors to provide the most appropriate treatment to the patients.

ABCDx DUOCheck™ is a rapid quantitative assay for the detection of NT-proBNP and H-FABP in whole blood, serum or heparinized plasma samples. The test device consists of a plastic housing containing two different sticks for the detection of NT-proBNP and H-FABP.
The method is based on a unique combination of monoclonal dye conjugate and solid phase antibodies to identify NT-proBNP or H-FABP in the test samples with a high degree of specificity.
Depending on the NT-proBNP and H-FABP concentration in the sample, different lines will appear on the test, allowing their quantitative measurement, when used in combination with the ABCDX BRAINCheck™ App.

III. ABCDx DUOCheck™ KIT COMPONENTS

Each kit contains everything needed to perform 20 tests or 10 tests.

- ABCDx DUOCheck™ test devices:	20	10
- Disposable plastic pipettes:	40	20
- NT-proBNP diluent in a dropper bottle:	5 mL	2 x 1.5 mL
- H-FABP diluent in a dropper bottle:	5 mL	2 X 1.5 mL
- Instructions leaflet:	1	1

-Controls (Optional):
Positive controls (NT-proBNP ref. V18000, H-FABP ref. V9800) and Negative control (NT-proBNP ref. V18001, H-FABP ref. V9801): a freeze-dried preparation of a non-infectious compound in diluted human serum, tested and found negative for anti-HIV, anti-HCV antibodies and HBs antigen, containing 0.05 % sodium azide is optionally available as positive and negative controls (1x 0.25 mL). The concentration range is indicated on the vial label.

IV. STORAGE AND STABILITY

1. All ABCDx DUOCheck™ kit components should be stored at room temperature (+4°C to +30°C) in the sealed pouch.
2. Do not freeze the test kit.
3. The ABCDx DUOCheck™ kit is stable until the expiry date stated on the package label.

V. PRECAUTIONS

1. This test is designed for in vitro diagnostic use and professional use only.
2. Read the instructions for use carefully before proceeding the test.
3. Handle all specimens as if containing infectious agents. When the assay procedure is completed, dispose of specimens carefully in dedicated biohazard disposal box.
4. Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
5. Do not eat, drink nor smoke in the area where specimens and kit reagents are handled.
6. Avoid any contact between hands and eyes or nose during specimen collection and testing.
7. Do not use beyond the expiration date which appears on the package label.
8. Do not use a test from a damaged protective wrapper.
9. **When the test is performed with whole blood samples, only fresh samples should be used (< 4 hours).**
10. **The ABCDx DUOCheck™ is to be performed using serum, heparinized plasma or heparinized whole blood samples only.**

VI. SPECIMEN COLLECTION AND PREPARATION

1. ABCDx DUOCheck™ test is to be performed using human whole blood, serum or heparinized plasma samples only. **Do not use haemolyzed samples.**
2. The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid haemolysis).
3. Patients samples should be tested immediately.
4. Specimens containing precipitate may show inconsistent test results. Such specimens should be clarified prior to assaying.
5. If the test is to be run within 48 hours after collection the specimen should be stored in the refrigerator (+2°C to +8°C). If testing is delayed more than 48 hours, the specimen should be frozen. The frozen specimen must be completely thawed, thoroughly mixed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.
6. In case of cloudiness, high viscosity or presence of particulate matter into the serum specimen, it should be diluted with equal volume (V/V) of negative specimen before testing.

VII. ASSAY PROCEDURE

a) Controls testing

For both freeze-dried positive and negative controls for NT-proBNP or H-FABP, reconstitute each vial with 0.25 mL of distilled water.

Wait for 15 minutes after dissolving. Fill the serum dropper with the control and by holding it vertically, dispense 1 drop (25 µL) into the sample well of the cassette and proceed in the same way as for a patient's sample. The expected concentration level (**in pg/mL for NT-proBNP and ng/mL for H-FABP**) is indicated on the vial label and obtained result must match the indicated value. The concentration level can change slightly depending on lot number.

NT-proBNP: The reconstituted vial should be kept between +2°C and +8°C and should be used within 7 days after reconstitution.

H-FABP: The reconstituted vial should be kept between +2°C and +8°C and should be used within 24 hours after reconstitution.

b) Sample testing

Follow the below instructions

1- Allow sample and ABCDx DUOCheck™ test device to come to room temperature prior to testing.
2- Remove the reaction device from its protective wrapper by tearing along the split.
3- It is recommended to test one sample or control at a time.
4- Fill the serum dropper with specimen (serum or plasma or whole blood) and by holding it vertically, dispense 1 drop (25 µL) into each sample well. If whole blood sample is used, dispense 2 drops (50 µL) into the sample well.
5- Note: It is very important to wait for the blood to absorb completely before adding the diluent.
6- Hold the diluent vial vertically and add exactly 4 drops (150 µL) of each diluent into the corresponding sample well (NT-proBNP or H-FABP). Add Diluent A (Black label) to the NT-proBNP spot (Left) and add diluent B (red label) to the H-FABP spot (Right). **Wait for each drop to absorb completely before adding the next drop.**
7- **Read the result with the ABCDx BRAINCheck™ App after 15 minutes and no longer than 20 minutes. The results will be displayed by the App:** NT-proBNP results are expressed in pg/mL. H-FABP results are expressed in ng/mL.

VIII. PERFORMANCES CHARACTERISTICS

1) NT-proBNP

a) Linearity

A study was performed using dilutions of a serum sample standardized to the Biomerieux Mini Vidas Analyser and the dose response obtained with the ABCDx DUOCheck™ test fits a linear regression within the range of 2000 to 6500 pg/mL. The measuring range is 2000 – 6500 pg/mL.

For the NT-proBNP concentration lower than 2000 pg/mL, the result will be shown as "<2000 pg/mL".
For the NT-proBNP concentration higher than 6500 pg/mL, the result will be shown as ">6500 pg/mL

b) Analytical sensitivity

The sensitivity of NT-proBNP with the ABCDx DUOCheck™ test is 2000 pg/mL.
Lower NT-proBNP concentrations will be shown as "<2000 pg/mL".

c) Analytical specificity

The following substances have been tested for cross reaction (Table 1). The data have been communicated by the anti NT-proBNP antibodies supplier.

Substances	Reactivity (%)
NT-proBNP	100%
proBNP	100%
BNP	None

Table 1: Analytical specificity

d) Intra-lot reproducibility

9 replicates each of 3 plasma samples having a NT-proBNP concentration of 2342 pg/mL, 3536 pg/mL and 5244 pg/mL have been performed. The obtained results showed a coefficient of variation (CV) of 34%, 24% and 17% respectively.

e) Accuracy

A clinical study was performed using 25 serum samples analysed with both the VedaLab DUO NT-proBNP+FABP-CHECK-1 test and the ABCDx DUOCheck™ test. Obtained results show a correlation r^2=0,9012 between both methods (figure 1).

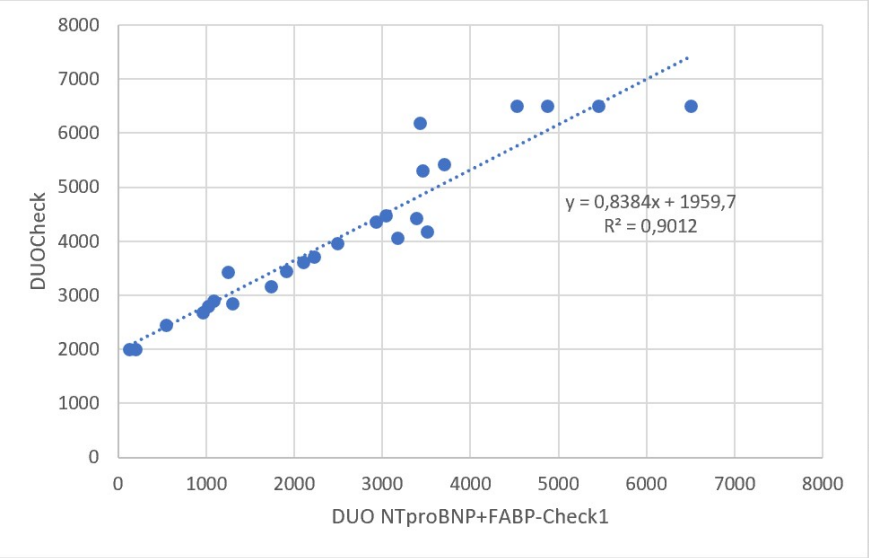


Figure 1

f) Hook effect

High positive serum samples having an NT-proBNP concentration up to 147 ng/mL constantly showed results >6500 pg/mL and did not show any hook effect (13).

g) Reference ranges

It is recommended that each laboratory determines its own reference values. Standard reference values are indicated below (6,7).

< 75 years old: < 125 pg/mL

>75 years old: < 450 pg/mL

h) Interferences

- Bilirubin, triglycerides and haemoglobin have been shown to not interfere with NT-proBNP concentration determination up to concentrations of 10 mg/L, 15 g/L and 5 g/L respectively (13).
- Rheumatoid factor (FR) concentration up to 2,000 IU/mL do not interfere in determination of the NT-proBNP concentration (13).
- Neither HAMA (Human Anti-Mouse Antibodies) of type 1 (antibodies that can be present in healthy patients) nor HAMA of type 2 (appearing in patients after treatment with monoclonal antibodies) interfere with the determination of the NT-proBNP concentration (13).

2) H-FABP

a) Linearity

A study was performed using dilutions of H-FABP purified protein in H-FABP free serum sample and the dose response obtained with the ABCDx DUOCheck™ test fits a linear regression within the range of 9 to 120 ng/mL. The measuring range is 9 - 120 ng/mL.

For H-FABP concentration below 9 ng/mL, the result will be given as "< 9 ng/mL".

For H-FABP concentration over 120 ng/mL, the result will be given as "> 120 ng/mL"

b) Analytical sensitivity

The sensitivity of H-FABP with the ABCDx DUOCheck™ test is 9 ng/mL.

Lower H-FABP concentrations will be shown as "< 9 ng/mL".

c) intra-lot reproducibility

9 replicates each of 3 plasma samples having a H-FABP concentration of 36 ng/mL, 58 ng/mL and 98 ng/mL have been performed. The obtained results showed a coefficient of variation (CV) of 25%, 17% and 19% respectively.

d) Accuracy

A clinical study was performed using 14 serum/plasma samples analysed with both the VedaLab DUO NT-proBNP+FABP-CHECK-1 test and the ABCDx DUOCheck™ test. Obtained results show a correlation r^2=0,9781 between both methods (figure 2).

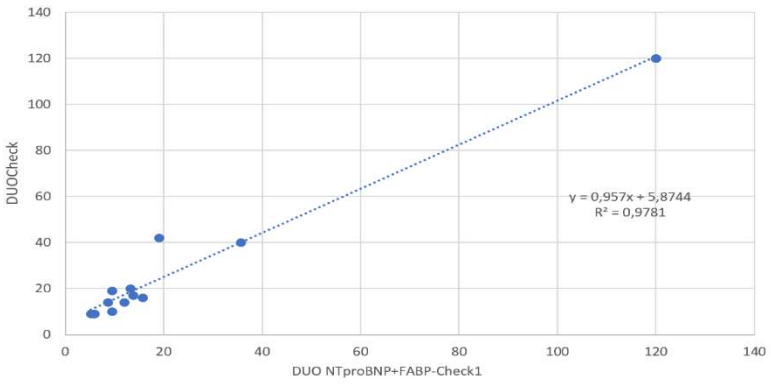


Figure 2

e) Hook effect

No hook effect was observed up to a H-FABP concentration of 10,000 ng/mL (10 µg/mL). The result obtained was consistently "> 120 ng/mL" (13).

f) Reference ranges

It is recommended that each laboratory determines its own reference values. Reference values for H-FABP were determined from 0.5 to 4 ng/mL (14).

g) Interferences

- Bilirubin, triglycerides and haemoglobin have been shown not to interfere with h-FABP concentration determination up to concentrations of 30 mg/L, 15 g/L and 5 g/L respectively (13).
- Rheumatoid factor (RF) concentration up to 475 IU/mL do not interfere in the determination of H-FABP concentration (13).
- HAMA (Human Anti-Mouse Antibodies) of type 2 (appearing in patients after treatment with monoclonal antibodies) does not interfere with the determination of H-FABP concentration. HAMA of type 1 (antibodies that can be present in healthy patients) may interfere with the determination of H-FABP concentration (13).

IX. LIMITATIONS

1. As for any diagnostic procedure, the physician should evaluate the data obtained using this kit in the light of the other clinical information available.
2. If the reading time (15 to 20 minutes) is not strictly respected, wrong results will be obtained.
3. This format of test should not be used for visual reading.
4. As for any diagnostic method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/- 25% should be considered for the final value and for the clinical significance of the result.
5. It is recommended that each laboratory establish its own references ranges based on representative patient population in order to test the validity of the supplied data. Therefore, the given data should only be intended as orientational guidelines.
6. For better results, it is recommended to strictly follow the proceeding temperature recommendations.
7. High levels of RF (Rheumatoid factor) or CRP (C-reactive protein) may create interferences and therefore lead to false positive results. Such cases should be discriminated before testing.
8. The test is designed to eliminate the potential interference of human anti-mouse antibodies (HAMA). However high level of HAMA could show falsely positive results.
9. **Use only fresh whole blood samples (<4 hours) when test is to be performed with whole blood sample.**
10. **Only heparinized samples should be used in case anticoagulants are present.**
11. H-FABP can be elevated in patients with renal insufficiency or *angina pectoris*. In low amounts, H-FABP is also present in skeletal muscle. Therefore, it can be elevated in individuals that performed physically prior to the testing.

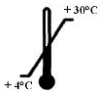
X. BIBLIOGRAPHY

1- **Pandey KN**. Biology of natriuretic peptides and their receptors. Peptides 2005;26: 901-32.

2- **Di Angelantonio E, Chowdury R, Sarwar N, Ray KK, Gobin R, Saleheen D, and al**. B-type natriuretic peptides and cardiovascular risk: systematic review and meta-analysis of 40 prospective studies. Circulation 2009;120: 2177-87

3- **Maisel AS, Krishnaswamy P, Nowak RM, McCordJ Hollander JE, Duc P, and al.** Rapid measurement of the B-type natriuretic peptide in emergency diagnosis of heart failure. N engl J Med 2002;347: 161-7.

4- **Januzzi JL Jr, Camargo CA, Anwarunddin S, Baggish AL, Chen AA, Krauser DG, and al**. The N-Terminal pro-BNP investigation of dyspnea in the mergency department (PRIDE) study. Am J Cardiol 2005;95: 948-54.
5- **G.C.M. Lissen, T. Jaarsma, H.L Hillege, A.A. Voors, D. J. Van Veldhuisen.** A comparison of the prognostic value of BNP versus NT- pro BNP after hospitalisation for heart failure. Neth Heart 2018;26: 486-492
6- **S. Ekelund, K. Manfred.** Choosing cut-offs for NT pro BNP – Acute care testing org – 2011; 1-4
7- **Pastural—Thaunat M., Ecochard R, Boumendjel N., Abdullah E. and al.** Relative changes in NT-proBNP level: An important risk predictor of cardiovascular congestion in Haemodialysis Patients. Nephron Extra 2012; 2: 311-318.
8- **Fonseca AC, Matias JS, E Melo TP, Pires C, Geraldes R, Canhão P, Brito D, Ferro JM**. Time course of NT-proBNP levels after acute ischemic stroke. Acta Neurol Scand. 2013 Oct;128(4):235-40. doi: 10.1111/ane.12112. Epub 2013 Mar 7. PMID: 23464981.
9- **Di Castelnuovo A, Veronesi G, Costanzo S, Zeller T, Schnabel RB, de Curtis A, Salomaa V, Borchini R, Ferrario M, Giampaoli S, Kee F, Söderberg S, Niiranen T, Kuulasmaa K, de Gaetano G, Donati MB, Blankenberg S, Iacoviello L;** BiomarCaRE Investigators. NT-proBNP (N-Terminal Pro-B-Type Natriuretic Peptide) and the Risk of Stroke. Stroke. 2019 Mar;50(3):610-617. doi: 10.1161/STROKEAHA.118.023218. PMID: 30786848.
10- **Wunderlich MT, Hanhoff T, Goertler M, Spener F, Glatz JF, Wallesch CW, Pelsers MM**. Release of brain-type and heart-type fatty acid-binding proteins in serum after acute ischaemic stroke. J Neurol. 2005 Jun;252(6):718-24. doi: 10.1007/s00415-005-0725-z. Epub 2005 Apr 18. PMID: 15834650.
11- **Park SY, Kim MH, Kim OJ, Ahn HJ, Song JY, Jeong JY, Oh SH.** Plasma heart-type fatty acid binding protein level in acute ischemic stroke: comparative analysis with plasma S100B level for diagnosis of stroke and prediction of long-term clinical outcome. Clin Neurol Neurosurg. 2013 Apr;115(4):405-10. doi: 10.1016/j.clineuro.2012.06.004. Epub 2012 Jul 4. PMID: 22766253.
12- **Zimmermann-Ivol CG, Burkhard PR, Le Floch-Rohr J, Allard L, Hochstrasser DF, Sanchez JC.** Fatty acid binding protein as a serum marker for the early diagnosis of stroke: a pilot study. Mol Cell Proteomics. 2004 Jan;3(1):66-72. doi: 10.1074/mcp.M300066-MCP200. Epub 2003 Oct 26. PMID: 14581522.
13- **VedaLab DUO** NT-ProBNP+FABP-Check-1 IFU -MD18000009m0c-2022/05.
14- **Da Molin S, Cappellini F, Falbo R, Signorini S, Brambilla P.** Verification of an immuno-turbidimetric assay for heart-type fatty acid-binding protein (H-FABP) on a clinical chemistry platform and establishment of the upper reference limit, Clin. Biochem. 47 (16–17) (2014) 247–249.

	Read the instructions before use		Do not reuse
	Temperature limitations		Manufactured by VEDALAB – France
	For *in vitro* diagnostic use		CE marked

CH REP	ABCDx SA, Avenue de Sécheron 15, Campus Biotech Innovation Park, 1202 – Geneva, Switzerland		

Revision date : 2022/11

CHANGES DESCRIPTION

Changes type:

- N/A Not Applicable (creation)
- Technical change Addition, revision and/or removal of information related to the product.
- Administrative Implementation of non-technical changes noticeable to the end-user.

Changes type	**Change description**
Administrative	Addition of product reference for 10 tests

Note: Minor typographical, grammar, spelling and formatting changes are not reported in the change details.