ABCDx BRAINCheck™ App

Instructions for Use

Application software for quantitative measurement of the concentration of amino terminal fragment of the Brain Natriuretic Peptide (NT-proBNP) and Heart-type fatty acid-binding protein (H-FABP) in human blood. For use only in conjunction with the DUOCheck™ rapid test.
Ref. LVO000000001

1 Intended use

The ABCDx BRAINCheck™ App is a smartphone application software to be used in conjunction with the rapid test DUOCheck® LFA device for quantitative measurement of the concentration of NT-proBNP and H-FABP in human blood. The device is intended to be used as an aid in the management of patients with cerebrovascular disorders. The device 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 patients with cerebrovascular disorders.
The test is intended to be used only by healthcare professionals, who have been trained on the successful performance of the test.

2 Principle

2.1 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 (6,7).

2.2 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 (8,9,10).

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 BRAINCheck™ may lead to an improvement of patient's management and to assist medical doctors to provide the most appropriate treatment to the patients.

2.3 Method of measurement

The ABCDx BRAINCheck™ App is a smartphone application software intended to be used to scan the rapid test DUOCheck® using a smartphone device. The mobile application turns a smartphone into a test cassette reader using state of the art image processing. First, the application guides the user through the test procedures step by step with an intuitive tutorial. Once the test cassette has completed its incubation, the application built-in timer indicates to the user that the test is ready and that the user can scan the LFA cassette using his smartphone. The application acquires an appropriate image using detection methods, analyses images and interprets it to yield a quantitative result. The main functionality of the mobile application incorporates user interface (UI), launch of the reader, display and storage of data.
The application captures an image of the test DUOCheck® to detect visual markers that result from the immunological reaction between the reagents and the sample. The test device is designed as such that there is a control line demonstrating that the test has properly functioned. The test device shows a test line whose intensity is proportional to the quantity of the biomarker present in the sample, allowing the calculation of the biomarker concentration displayed by the application.

3 Reagent Test Device

The DUOCheck™ manufactured by VedaLab 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 strips 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 (11).

4 Specimen Type

- The DUOCheck™ test is to be performed using human whole blood, serum or heparinized plasma samples. **Do not use haemolyzed samples.**
- The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid haemolysis).
- Patients' samples should be tested immediately (within 4 hours from blood draw).

5 Installation

- The ABCDX BRAINCheck™ App has been validated for use with specific smartphone brands and models. The list of smartphone models supported by ABCDx can be found here. For use of the ABCDX BRAINCheck™ App with a different smartphone model, please contact ABCDx for information (techsupport@abcdx.ch).
- Minimal phone requirements: 5M Pixel phone camera with macro mode and a LED flash.
- Minimal Operating system requirements: Android version 6.1 onwards, IOS version 12 onwards.
- To install the ABCDx BRAINCheck™ software application, contact ABCDx (techsupport@abcdx.ch). Follow the instructions provided by email to connect to the application center, download the application and install it on the smartphone.

6 Warning and Precautions

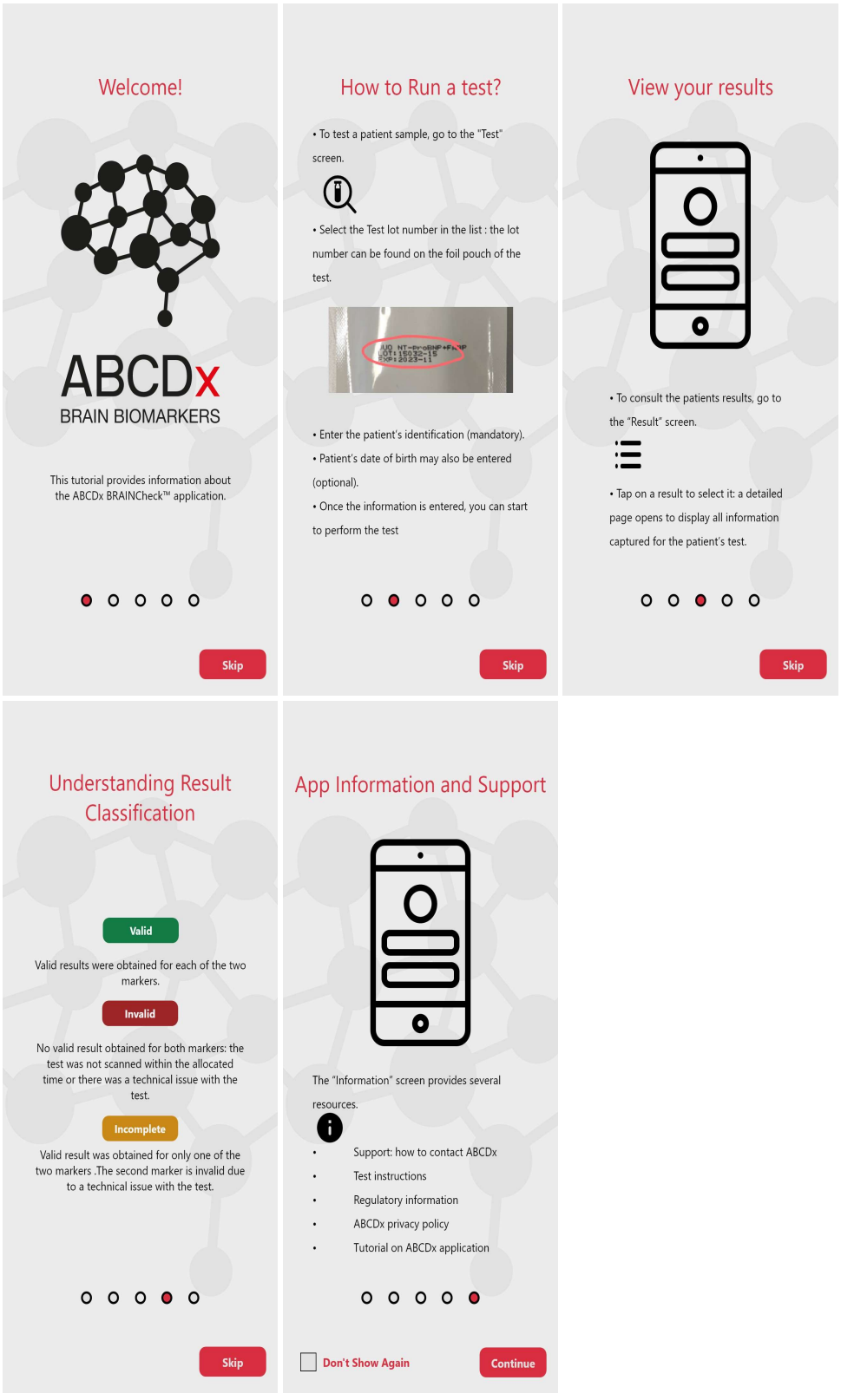
- This test is designed for in vitro diagnostic use and professional use only.
- Read the instructions for use carefully before proceeding the test.
- Handle all specimens as if containing infectious agents. When the assay procedure is completed, dispose of specimens and used test device carefully in dedicated biohazard disposal box.
- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
- Do not eat, drink nor smoke in the area where specimens and kit reagents are handled.
- Avoid any contact between hands and eyes or nose during specimen collection and testing.
- When the test is performed with whole blood samples, only fresh samples should be used (< 4 hours).

7 Operating instructions

The ABCDx BRAINCheck ™ App display on-screen instructions for the user. Specific user instructions are detailed in the following sections.

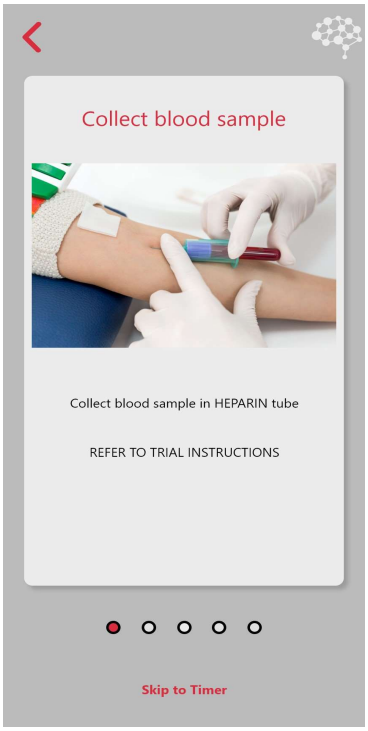
7.1 Tutorial on Application functionalities

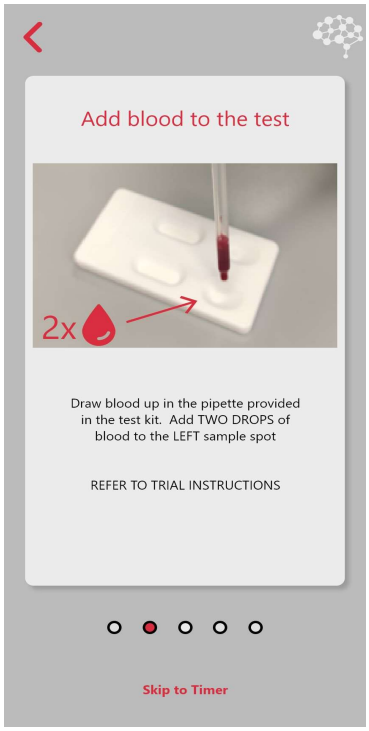
- Upon opening the application, a tutorial describing the application functionalities is displayed. The user may skip the presentation.

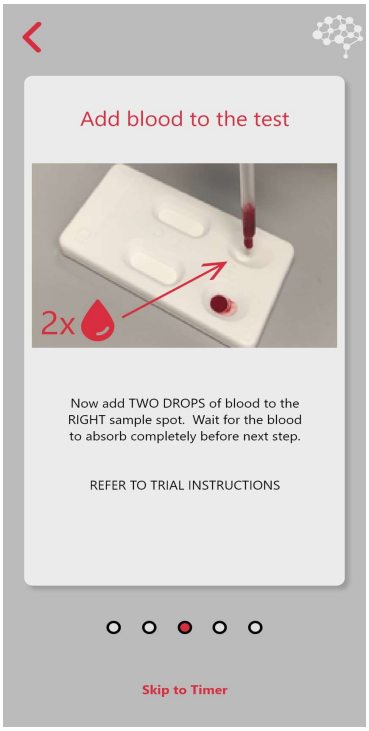


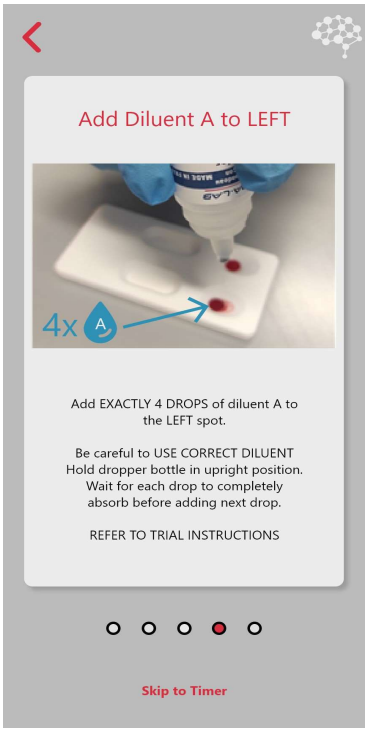
7.2 Test instructions

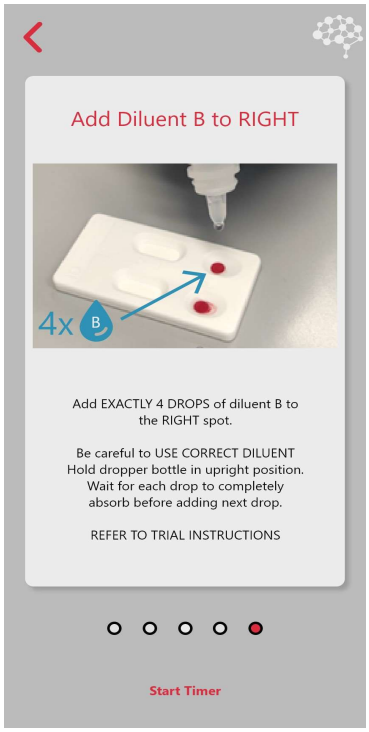
- When the user wishes to start a test, the following tests instructions will be displayed.



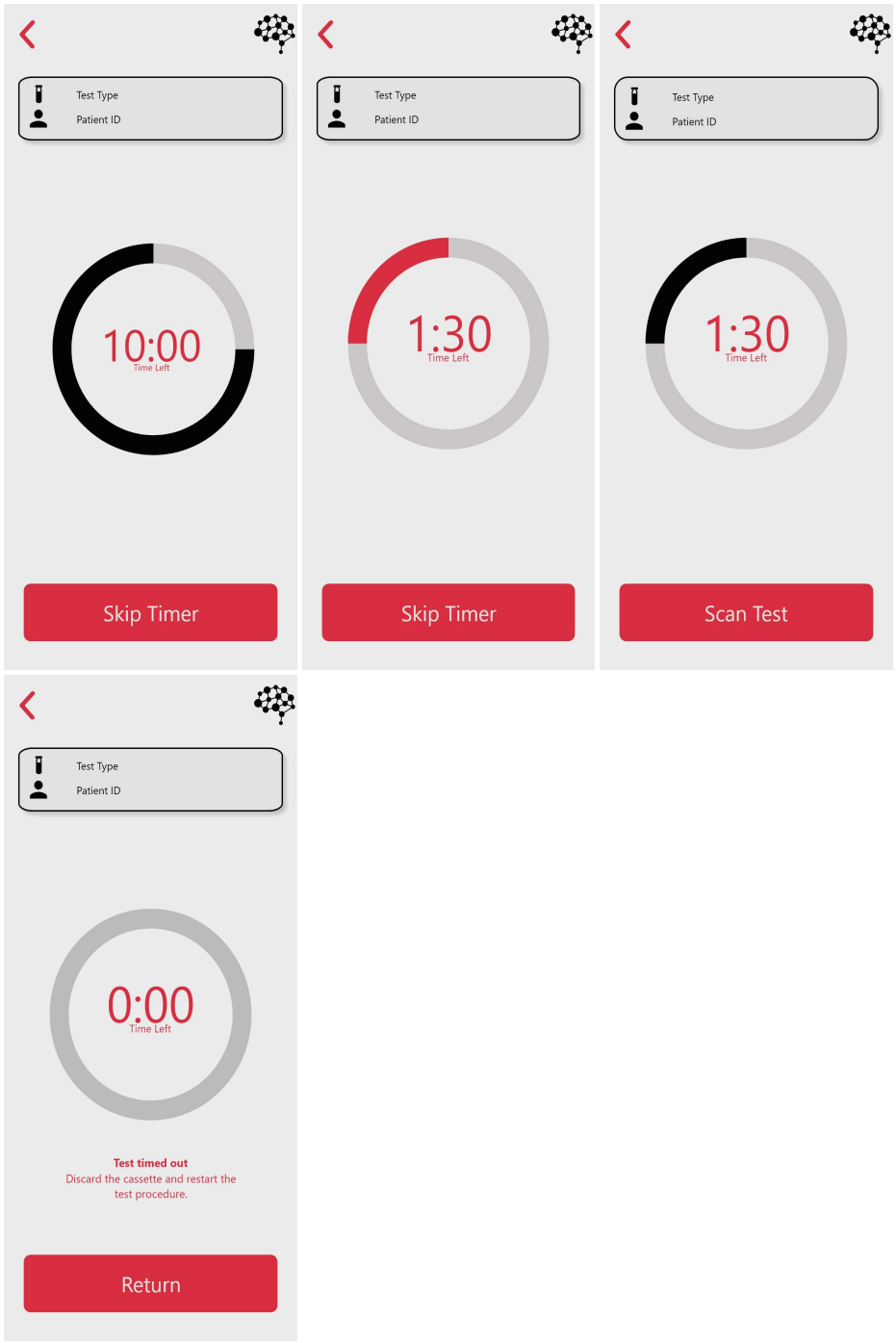








7.3 Incubation and scanning



- The ABCDx BRAINCheck™ App displays a timer that guides the user for the proper incubation time for the DUOCheck™ test incubation of 15 minutes. The timer will indicate that the scanning time is approaching by turning red 2 minutes before the end of the incubation time.
- The “scan test” button will activate at the end of the incubation period of 15 minutes and for a total time of 5 minutes, allowing the user to scan the test during the correct incubation time. A loud sound alarm will also launch after 15 minutes during 30 seconds to attract user’s attention.

- If the test is not scanned between 15 and 20 minutes, the scan button becomes unavailable, and the screen displays a message indicating to the user that the test cassette must be discarded, and another test must be started.
- The user may skip the timer by pressing the "skip timer" button, however **this is not recommended**. If the user decides to skip the timer, the result screen will indicate that the timer has been skipped.
- When the test is ready to scan, the scan test button activates: a grid is displayed on the screen. Place the test cassette to match with the grid screen, moving the phone until the grid is exactly adjusted to the test cassette: the grid will then turn Green, and the test is scanned automatically. Results will then be displayed on screen.

7.4 Reviewing results

- To consult the patients results, go to the "Result" screen. Tap on a result to select it: a detailed page opens to display all information captured for the patient's test.
- NT-proBNP results are displayed in pg/mL. H-FABP results are displayed in ng/mL. The test results are classified as Valid, Invalid or Incomplete:
    - Valid: Valid results were obtained for each of the two markers.
    - Invalid: No valid result obtained for both markers: the test was not scanned within the allocated time or there was a technical issue with the test.
    - Incomplete: Valid result was obtained for only one of the two markers. The second marker is invalid due to a technical issue with the test.
- The results acquired with the BRAINCheck™ App can be consulted on the phone during a maximum period of 30 days. Once this period has elapsed, the results are automatically deleted by the application.

8 About page

The following information is provided on the About page:

- Regulatory Information: the regulatory information is also displayed on the app stores.
- Support
    - "Visit web support": a hyperlink will bring the user directly to the ABCDx webpage, where the ABCDx BRAINCheck™ Instructions For Use and the DUOCheck™ test Instructions For Use can be found.
    - "Email us": will open an email to ABCDx technical support at the following address techsupport@abcdx.ch
- General conditions
    - "Privacy policy": a hyperlink will bring the user to the ABCDx privacy policy on the ABCDx web site.
    - "Terms and conditions": a hyperlink will bring the user directly to the ABCDx terms and conditions document on the ABCDx web site.

9 Limitations of use

- For optimal result at the time of scanning, place the test cassette on a grey or dark background and ensure the lights do not project shadows on the test cassette. Avoid placing the test cassette on shiny or glossy background as the phone camera may be unable to scan the test.
- It is recommended to avoid very dark lighting environment during test scanning.
- It is recommended to avoid moving environments during the test scanning as the time for picture acquisition by the App may be increased. If scanning in a vehicle, allow the scan to be taken while the vehicle is immobile.
- For accuracy of the test results, do not scan a test cassette outside of the recommended incubation time of 15 to 20 minutes.
- For accuracy of test results, follow strictly the test instructions described in section 7.2. Specifically add exactly 2 drops of whole blood and 4 drops of each buffer to the test cassette and wait for each drop of buffer to absorb before adding the next one.
- The ABCDX BRAINCheck™ App has been validated for use with specific smartphone brands and models. The list of smartphone models supported by ABCDx can be found here. For use of the ABCDX BRAINCheck™ App with a different smartphone model, please contact ABCDx for information (techsupport@abcdx.ch).

10 Maintenance and troubleshooting

- Before starting a test, ensure that the smartphone camera lens is clean: if necessary, gently clean the lens with a tissue. No other maintenance is necessary.
- For any question related to the performance of the device, please contact ABCDx using the link on the support page or techsupport@abcdx.ch.

11 Device Performances Characteristics

11.1 NT-proBNP

11.1.1. 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 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”.

11.1.2 Analytical sensitivity

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

11.1.3 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 (11).

Substances	Reactivity (%)

NT-proBNP	100%
proBNP	100%
BNP	None

Table 1: Analytical specificity

11.1.4 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.

11.1.5 Accuracy

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

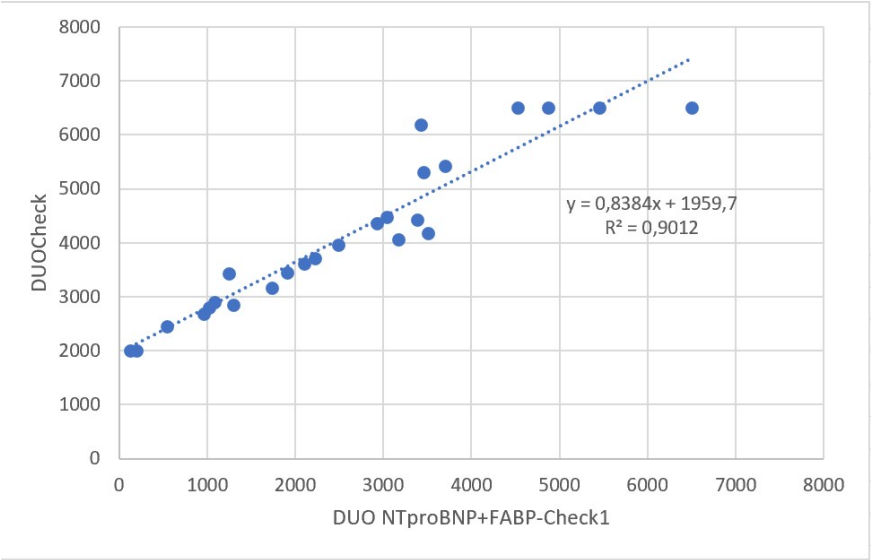


Figure 1

11.1.6 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 (11).

11.1.7 Reference ranges

It is recommended that each laboratory determines its own reference values. Standard reference values are indicated below (5,6).

< 75 years old: < 125 pg/mL

>75 years old: < 450 pg/mL

11.1.8 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 (11).
- Rheumatoid factor (FR) concentration up to 2,000 IU/mL do not interfere in determination of the NT-proBNP concentration (11).
- 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 (11).

11.2 H-FABP

11.2.1. 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 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"

11.2.2 Analytical sensitivity

The sensitivity of H-FABP with the DUOCheck™ test is 9 ng/mL.
Lower H-FABP concentrations will be shown as "< 9 ng/mL".

11.2.3 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.

11.2.4 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 DUOCheck™ test. Obtained results show a correlation r^2 = 0,9781 between both methods (figure 2).

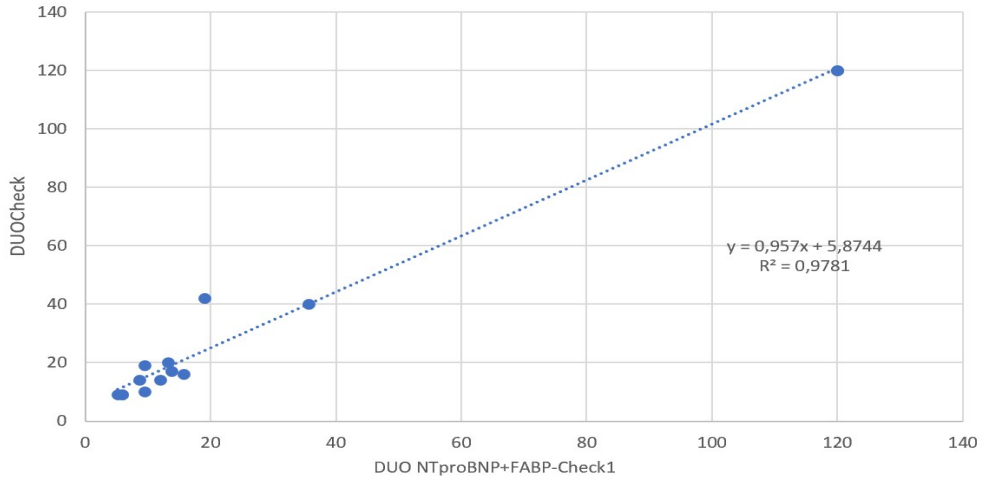


Figure 2

11.2.5 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" (11).

11.2.6 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 (12).

11.2.7 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 (11).
- Rheumatoid factor (RF) concentration up to 475 IU/mL do not interfere in the determination of H-FABP concentration (11).
- 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 (11).

12 Bibliography

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CE	ABCDx BRAINCheck™ App	IVD	In-vitro diagnostic medical device Only for in-vitro purposes
	ABCDx SA, Avenue de Sécheron 15, Campus Biotech Innovation Park, 1202 – Geneva, Switzerland	EC REP	ABCDx Spain SL Avenida San Francisco Javier 9, Edificio Sevilla 2 10a planta, n1, 41018 Sevilla, Spain
	Consult electronic instructions for use		
REF	LVO000000001	#	Version 1.0.1
UDI	(01)07649988079719(8012)V1.0.1		

13 Change Description

Change type	Change description
N/A	Not Applicable (creation)

Legend:

- Technical change: Addition, revision and/or removal of information related to the product.
- Administrative: Implementation of non-technical changes noticeable to the end-user.
- Note: Minor typographical, grammar, spelling and formatting changes are not reported in the change details.