

Team Results Document

AUC Imhoteps

Abobakr Salamah

Ahmed El-Baz

Ahmed Koptan

Ahmed Yamany

Alaa Ahmed

David Salama

Mayada Mazher

Mohamed Shahawy

Nada Moustafa

Yehia Gab-Allah

Prof. Dr. Hassan Azzazy

August 30, 2017

Contents

1. Biosensor System and Assay	4
2. Analytical Performance	6
3. Novelty and Creativity	7
3.1 Already available	7
3.2 New developments	7
4. Translation Potential	8
4.1 Healthcare application potential.....	8
4.2. Industrialization and commercialization potential.....	8
5. Team and Support	10
5.1 Contributions of the team members.....	10
5.3 Sponsors.....	10
6. Final remarks	11
References	12
Appendix 1: Survey	13
Appendix B: Emergency idea: Sakaguchi test for Arginine.....	16
Appendix C: Industrial Translational Potential.....	20

Summary for the SensUs website

In order to sense NT-proBNP in plasma, we designed an assay that utilizes the concept of 'Sandwich immunocomplexes'; where the antigen, NT-proBNP, is sandwiched between two antibodies, one of which is fixed on a solid surface and the other carries a reporter molecule emitting a detectable signal that is measured by a Smartphone-based read-out device. In our prototype, the signal produced is yellow, with increasing intensity as the level of NT-proBNP rises in the patient's plasma. This colored reaction product is placed inside a 3D-printed chamber along with a suitable Smartphone that takes a picture and analyze it to estimate the level of NT-proBNP. The total testing time of the prototype is 25 minutes using a sample size of about a 20 μ l of plasma. The read-out device is successfully miniaturized to a light, hand-held size. Although this prototype is a mere proof-of-concept of the suitability of fast ELISA and mobile phone-based detection strategies for sensing NT-proBNP, it paves the way to our much refined envisioned end product. In it, the patient only needs to apply a drop of blood onto a treated nitrocellulose paper strip and use his Smartphone equipped with a specially developed app to get a result in ten minutes.

1. Biosensor System and Assay

1.1 Assay principle

Our biosensor relies on the well-established sandwich immunoassay principle. To form the sandwich, two antibodies (Abs) are used, each binds to a different epitope on NT-proBNP. The highly-specific capture antibody is attached to a solid surface, then the antigen (Ag), NT-proBNP, is added followed by the addition of a secondary antibody (detection antibody) which binds to it at a different epitope than the capture. The antigen is, thus 'sandwiched' between the two antibodies.

In this method, the Ag-Ab binding affinity determines the sensitivity of the assay. An increased concentration of the Ag requires an increased concentration of the secondary antibody to obtain a higher measured response. The standard curve of a sandwich immunoassay has a positive slope. The detectable signal, denoting the extent of the binding, is produced via a reporter attached to the detection antibody and measured via a suitable readout device [1]

1.2 Assay and biosensor system details

1.2.1 Prototype

In the prototype, we display a rather typical fast ELISA with a smart, mobile phone-based color detector for the readout. Capture antibodies are fixed on 96-well plates to which non-diluted plasma samples (20 ul), biotinylated detection antibodies and Streptavidin-HRP conjugate are added followed by plate sealing and a short (7 minutes) incubation period in room temperature with consistent, gentle shaking. Shaking, via varying methods, helps decrease the lengthy incubation time traditionally needed for ELISA tests [2]. Three rounds of one-minute washing are then carried out followed by the addition of chromogens and a 10-minutes incubation in the dark. A stop solution is added and a yellow color is instantly visible. The resultant fluid is taken out of the well and into a cuvette that fits for insertion inside a custom, 3D-printed, opaque gadget in which a smartphone is placed to take a picture of the colored product for analysis.

As for the detection device, it consists mainly of a mobile phone or more specifically its camera, an Arduino microcontroller, a 1Sheeld mobile phone bluetooth shield for the arduino and a light source for excitation, which is an external monochromatic LED. The assembly is shown in Figure 1. The detection process starts when the LED lights up the detection chamber containing the assay → A picture is taken by the mobile phone's camera and communicated to the 1Sheeld using the mobile phone bluetooth module → The 1Sheeld serially transmits the data to the Arduino through its pins → The Arduino compares the image's RGB values with the preset thresholds and analyses the RGB values versus concentrations curve to determine the correct reading → This result is then transmitted back through the same path through the serial pins, and through the 1Sheeld and back to the mobile phone screen which displays the results for the user.

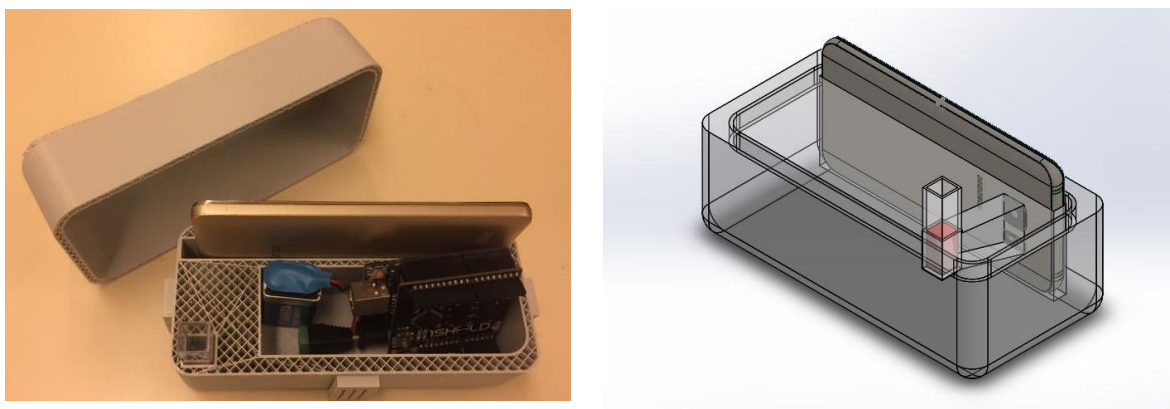


Figure 1: Prototype gadget, following printing (left), designed (right)

1.2.2 Envisioned end product

The envisioned assay comprises the formation of the sandwich immunoassay on a nitrocellulose membrane in a lateral flow assay (LFA) fashion. The test strip would have a sample pad treated to halt blood cells' further movement towards the conjugate pad. This would allow fresh blood to be used directly as the test sample and eliminate the need for plasma collection protocols, and thus decrease the cost of testing and make it more user-friendly for non-professional users. Blood plasma would travel on the test strip to reach the conjugate pad where NT-proBNP would bind to the detection antibodies labeled with R-phycoerythrin (R-PE). A fluorescent signal is believed to be more sensitive in a quantitative test than a colorimetric one [3]. The sandwich would finally form at the test line, where the capture antibodies are fixed. Excitation of R-PE would, then, be done via an LED in a dark, opaque detection room/gadget similar to the one designed for the prototype. A smartphone fit inside the gadget would be used to take a picture of the produced fluorescence through an optical filter. A specially developed mobile application would then be used for photo analysis and display of results. This strategy also allows for cloud sharing of the results between patients and physicians.

Ideally, the final biosensor system would allow the user to enter all parameters believed to affect NT-proBNP blood level, i.e. not only the age, sex [4] and heart disease history of the patient, but, also his/her body mass index (BMI) [5], state of renal function [6] and currently or chronically used medications [7]. Integration of all of the provided information would help determine the significance of each patient's NT-proBNP level and allow physicians to make individualized decisions, tailored for each patient.

2. Analytical Performance

2.1 For prototype

Sample volume	20-40 μ l
Type of sample	Non-diluted plasma
Total test time	25-30 minutes
Detection range	1.27-360 pg/ml of NT-proBNP
Sample handling, loading and overall test simplicity	Current test is not suitable for the use of the general public or untrained personnels
Biosafety	Any fluids resulting from the test steps are biohazardous and need proper disposal

2.2 For envisioned end product

Sample volume	20 μ l
Type of sample	Fresh blood or blood byproduct (Plasma, Serum)
Total test time	10 minutes
Detection range	1-10000 pg/ml of NT-proBNP
Sample handling, loading and overall test simplicity	Suitable for use by the general public
Biosafety	Significantly more safe, only the test strip/cartridge is disposed. There would be no free fluid to be discarded.

3. Novelty and Creativity

3.1 Already available

In regards to the development of biosensors, few research centers in Egypt are equipped with the facilities required. AUC is among the few facilities having previously produced a biosensor. However, there are no automated equipment for the development of an LFA strip, such as a cutter and a dispenser, and readout techniques are bulky spectrophotometers.

In addition, the political instability and security measures constantly in place render the purchase of materials and their clearance a struggle. Chemical and biological materials required for research are almost always imported; and oftentimes, the clearance process for them takes months with unreliable results.

3.2 New developments

The team reached out to medical professionals and the public to collect data about the current and desired solutions for the diagnosis of heart failure, among which was a survey (see Appendix A) and direct interviews. Despite delays in funding, the team thoroughly researched emergency protocols for immediate application. One of which is a chemical idea based on the Sakaguchi test, summarized in Appendix C.

With the small funding that was given, the team successfully simplified the developed ideas to a more affordable application. While there was repeated delays in the security clearance, the team devised creative methods to deal with this stressful situation. Materials that arrived early were tested separately to determine their functionality. The team designed and manufactured a manual dispenser and a paper cutter that were ready to use once the membrane was available [8]. However, the unpredictable nature of the clearance eventually meant that the membrane for the lateral flow assay and its treatment chemicals did not arrive as of the writing of this document.

Despite this, the team developed a prototype that proves the concept of performing a fast ELISA for a fluid-based biosensor system that uses a mobile phone readout. This significantly decreases the size of the readout device and time required for the test in Egypt. We, as a team, consider the perseverance and creative new solutions that were brought up despite the constantly changing circumstances to be a triumph.

4. Translation Potential

4.1 Healthcare application potential

Heart failure (HF) is a challenging international health issue that affects 1-2% of the adult population in the developed countries. The elderly are predominantly affected, with increased prevalence to >10% after 70 years of age [9]. In Egypt, cardiovascular diseases are the leading causes of mortality (46%) with overall burden of 6 K DALYs. In many Middle Eastern countries, our primary target populations, there was an observed increase in the prevalence of HF risk factors, e.g. diabetes mellitus, obesity, and hypertension, with HF in the Middle Eastern populations developing at least 10 years before their Western counterparts [10]. Investigations into the exact current state of HF in Egypt are lacking, thus, we have conducted a survey (Appendix A) to acquire the points of view of health care practitioners regarding heart failure diagnosis. The results can be summarized

Based on the above results, the use of NT-proBNP is limited to clinical practice in the MENA region, most probably due to the availability, cost and time delay of the test. This is despite the importance of NT pro-BNP in the heart of HF diagnostics just after clinical evaluation. It provides comparable level of evidence and indication class to Echocardiography as well as representing a good negative test [9]. MENA cardiologists who participated in our survey listed the accuracy and the low price-per-test as their most favored criteria in any envisioned NT-proBNP testing system, followed by the reasonable price-per-device, a short time-to-result interval and bedside testing feasibility. For practitioners of other specialties, however, shorter time-to-result intervals came second to the accuracy, followed by bedside testing feasibility and ease of use.

Our envisioned end-product, comprising a cheap, single-use LFA strip and a smart detector meets the needs of both the intended patient population and caregivers. The innate rapidity of LFA, along with the sensitivity of sandwich immunoassays cover the most important requirements of both sectors. Our smartphone-based handheld detector offers the intended ease-of-use and portability of the test, making it suitable for bedside and emergency settings as well as in remote, under-privileged and conflicted regions. In addition, the use of a mobile phone for analysis, presentation and data storage insures user-friendliness and offers the option of data sharing between patients and their physicians, allowing for better management, follow-up and more robust medical records.

Our device, however, may present a challenge with its maintenance or trouble-shooting of errors. The detector has built-in light source and specific filters that are essential for proper function, mobile phones that do not support the application would not be suitable for use in our device, and the data-sharing advantage would be severely compromised in areas with impaired connectivity.

4.2. Industrialization and commercialization potential

4.2.1 International Market segmentation

- Egyptian Map (Phase one: 2017-2019) (refer to Appendix C)
- African Map (Phase two: 2018-2020)
- Middle East Map (Phase three: industrial mass production & international market invasion)

A) Suitability of the technologies underlying the biosensor prototype for industrialization and commercialization

Our biosensor readout device depends on a technology that is now in the hands of more or less every single person which has a smartphone. The gadget depends on using the mobile phone to substitute other components that would have, otherwise, been manufactured separately, which brings down the total cost of the readout device and increase the user-friendliness and suitability for the general public.

B) Technology roadmap: Prototype Vs envisioned end product

Please see the full comparison in the Appendix C

C) Miniaturization aspects of the prototype

One of the greatest benefits of our device is that it is in its smallest possible size (Figure 1), which is mainly dependent on the cuvette size along with the mobile phone.

D) The gap between our prototype and the envisioned end product

The greatest gap between our current product and the envisioned one lies in the pricing, since we bought our components and chemicals separately and 3D-printed the device, which would never be the case in the mass production.

Surely there are other differences between the two products that do not include manufacturing techniques. For example, our product depends on an Arduino microcontroller and a 1Sheeld chip to read from the mobile camera and into the 1Sheeld which is a bluetooth module supported by a mobile application to use a color detection module. This will send back to the Arduino controller using the regular serial pins and according to certain thresholds preset earlier. In reality however, this is too expensive to implement on each and every device and it also needs a certain level of user experience. Thus, we would implement a much smaller solution, which would be completely based on the mobile phone of the user. This way, the mobile phone will handle all the processing, imaging, data transfer and results display all at once with a simple touch of the end user.

5. Team and Support

5.1 Contributions of the team members

Person	Type of Support
Abobakr Salama	Collection of ideas and protocols, lab work, helped in purchases, healthcare translational potential
Ahmed Koptan, Mohamed Shahawy	Early discussions, dropped out of the team for personal reasons
Ahmed El-Baz	Came up with the LFA idea, lab work on the emergency plan, helped in purchases
Ahmed Yamany	Participated in early discussions, helped in purchases
Alaa Ahmed	Handled purchases and clearance, participated in lab work on ELISA
David Salama	Designed the manual dispenser, designed the gadget for cuvette reading, wrote the code, handled the 3D printing
Mayada Mazhar	Collection of ideas and protocols, responsible for MNPs, handled business plan, participated in lab work on ELISA,
Nada Moustafa	Team coordinator, collection of ideas and protocols, handled healthcare translational potential, participated in lab work on ELISA
Yehia Gaballah	Helped in the writing of the code and 3D printing, writing of the engineering part of the document

5.2 People who have given support

Person	Type of Support
Dr. Ahmed Abdelwahab	Synthesizing magnetic nanoparticles for us to use.
Dr. Ahmed Elewa	Developing the emergency plan and provided mentorship
Dr. Tarek Abdelmonem, Dr Mohamed Wafaey	Medical professionals whom we interviewed for information regarding biosensors
Dr. Mohamed Serry	Providing early guidance in the possible development of a microfluidic chip
Dr. Mona Elharmeel	Giving advice on how to create a business plan
Sara Mohsen	A PhD student who provided us with materials and chemicals since we were short on funding
Amgad Ouf	A research assistant who provided advice and various chemicals since we were short on funding
Menna Elfar, Menna Ghouraba, Ahmed Elshehaby	Master's students who provided advice on various lab work

5.3 Sponsors

Organization	Contribution
American University in Cairo	\$3000

6. Final remarks

For nondisclosed bureaucratic reasons of the university, funding for the project only came in May 2017. The amount that was given from the university was \$3000, which was not enough to cover the expenses. Team members paid for many chemicals and manufacturing themselves in the hope of being compensated later. In addition, due to the political instability and security issues mentioned in section.3, the purchase and clearance of materials took two more months, finally pushing the team to perform all lab work within a period of one and a half month before the competition.

The shipment from HyTest including antibodies, antigen and plasma, on which is based most of the lab work was not kept in its proper temperature conditions while waiting in the Egyptian airport for clearance. When they were finally cleared in July 29, the team repeatedly tested them by using ELISA, which gave dubious results indicating possible denaturation. The team had already purchased a kit in case of emergency and the antibodies from this kit are the ones used in the prototype.

As mentioned in section.3, the lateral flow membrane did not arrive as of the writing of this document. That is why the prototype and the envisioned product seem to be very far apart in terms of concept.

The team is immensely honored to be a part of this prestigious competition and wishes to thank all those who participated in the organization of this event. Thank you for all your hard work.

References

- [1] K. L. Cox, V. Devanarayan, A. Kriauciunas, J. Manetta, C. Montrose and S. Sittampalam, "Immunoassay Methods," *Assay Guidance Manual*, 2012.
- [2] J. Li, P. Zrazhevsky and X. Gao, "Eliminating Size-Associated Diffusion Constraints for Rapid On-Surface Bioassays with Nanoparticle Probes," *Small*, vol. 12, no. 8, pp. 1035-1043, 2016.
- [3] J. Gibbs, "Selecting the Detection System - Colorimetric Fluorescent, Luminescent Methods," ELISA Technical Bulletin, Corning, Kennebunk, ME.
- [4] I. Raymond, B. A. Groenning, P. R. Hildebrandt, J. C. Nilsson, M. Baumann, J. Trawinski and F. Pedersen, "The influence of age, sex and other variables on the plasma level of N-terminal pro brain natriuretic peptide in a large sample of the general population," *Heart*, vol. 89, no. 7, pp. 745-751, 2003.
- [5] D. G. Krauser, D. Lloyd-Jones, C. U. Chae, R. Cameron, S. Anwaruddin, A. L. Baggish, A. Chen, R. Tung and J. Januzzi, "Effect of body mass index on natriuretic peptide levels in patients with acute congestive heart failure: a ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) substudy.," *Am Heart*, vol. 149, no. 4, pp. 744-750, 2005.
- [6] M. Schou, F. Gustafsson, C. Kistorp, P. Corell, A. Kjaer and P. Hildebrandt, "Effects of body mass index and age on N-terminal pro brain natriuretic peptide are associated with glomerular filtration rate in chronic heart failure patients.," *Clinical Chemistry*, vol. 53, no. 11, pp. 1928-1935, 2007.
- [7] C. Balion, P. Santaguida, R. McKelvie, S. Hill, M. McQueen, A. Worster and P. Raina, "Physiological, pathological, pharmacological, biochemical and hematological factors affecting BNP and NT-proBNP.," *Clinical Biochemistry*, vol. 41, no. 4, pp. 231-239, 2008.
- [8] S. Choi, J.-H. Lee, J.-S. Choi and H.-I. Jung, "Economical and rapid manufacturing of lateral flow immunosensor using fountain pens and gold colloidal solution," *Royal Society of Chemistry*, vol. 7, p. 1834–1842, 2014.
- [9] P. Ponikowski, A. A. Voors, S. D. Anker, H. Bueno, J. G. Cleland, A. J. Coats, V. Falk, J. R. Gonzalez-Juanatey, V.-P. Harjola and E. A. Jankowska, "2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)," *European Heart Journal*, vol. 37, no. 27, pp. 2129-2200, 2016.
- [10] M. Q. Al-Shamiri, "Heart Failure in the Middle East," *Current Cardiology Reviews*, vol. 9, no. 2, p. 174–178, 2013.
- [11] "Modification of the Sakaguchi reaction: Spectrophotometric determination of arginine in proteins without previous hydrolysis," *Archives in Biochemistry and Biophysics*, vol. 117, no. 3, pp. 534-540, 1966.

Appendix 1: Survey

Questions

Heart failure diagnosis survey for health care practitioners

This serves as a survey to study how Healthcare givers all over the world view heart failure, its current tests in the market and how they envision a better product

** Required*

Q1: What is your Specialty? *

- Cardiologist
- ER Physician
- Internist
- GP
- Intensivist
- Clinical Pathologist
- Pediatrician
- Other:

Q2: In which region do you practice? *

- Middle East and/or Africa
- Europe
- Americas
- Asia
- Oceania
- Other:

Q3: From your experience, on a scale from 1 to 10, how often do you encounter heart failure patients? *

Q4: From your experience, on a scale from 1 to 10, how difficult do you think the heart failure diagnosis is? *

Q5: From your experience, What are/is the confusing differential diagnoses? (you can choose more than one answer) *

- Chest Infection
- Chronic Obstructive Lung Disease
- Non Cardiogenic Pulmonary Edema
- Renal Failure
- Liver cell Failure
- Hypovolemic Shock
- Anaphylactic Shock

- Other:

Q6: What are the most common investigations you use for confirmation of heart failure diagnosis? (you can choose more than one answer) *

- Echocardiography
- ECG
- Laboratory tests
- CT
- CMR
- Invasive Hemodynamic Monitoring
- Other:

Q7: Do you use NT-Pro BNP tests for confirmation of your diagnosis? *

- Yes
- No

Q8: What percentage of patients do you use NT-Pro BNP test for? *

- 80% - 100%
- 60% - 80%
- 40% - 60%
- 20% - 40%
- Less than 20%

Q9: What type of NT-Pro BNP tests do you use? *

- Lab tests
- Bedside tests

Q10: Why do you think NT-Pro BNP tests are important? (you can choose more than one answer) *

- For its Accuracy
- To exclude other diseases with similar symptoms
- Because of its Prognostic Value
- Other:

Q11: On a scale from 1 to 5 (1 being of little importance and 5 being extremely important), What are the importance of these factors in this envisioned product? *

- Time to result
- Accuracy
- Bedside test
- Price/device
- Price/test
- Ease of use
- Interface friendliness
- Online sharing of data

Q12: Do you think including your mobile phone camera as a part of the test would make it easier and more friendly? *

- Yes
- No
- Maybe

Q13: What is the maximum time you would tolerate between obtaining the sample and reading the results?

- 20-30 Minutes
- 10-20 Minutes
- 10-5 Minutes

Answers

Total number of participants	99
Prevailing specialties	* Cardiology: n=50 * Intensive care: n=12 * General practitioners (GPs): n=13
Geographical distribution of participating cardiologists	* Middle East/Africa: n=33 * Elsewhere: n=17
Frequency of HF encounter (On a scale from 1 to 10)	* Cardiologists: 7.28 * GPs: 5.33
Difficulty of heart failure diagnosis for Middle Eastern/African participating physicians (On a scale from 1 to 10)	* Cardiologists: 6.91 * Non-cardiologists: 5.24
Sorting of most confusing differential diagnoses based on the experience of Middle Eastern/African physicians	Non-cardiogenic pulmonary edema Chronic obstructive pulmonary disease Chest infections
Middle eastern/ African physicians' investigations of choice	96% of physicians listed Echocardiography as an investigation they frequently rely on.
Use of NT-proBNP testing in HF diagnosis in Middle East/ Africa	Only 33% of participants stated their use of NT-proBNP as a diagnostic test, 14.7% of which were non-cardiologists.
Percentage of patients for which NT-proBNP test is requested	42% of physicians who use NT-proBNP test for diagnosis order it for 80-100% of their patients, while 24% of the using physicians order it for less than 20% of their patients.
Maximum time tolerated between sample collection and obtaining the results	* 20-30 minutes, for 56.1%, * 10-20 minutes, for 23.5% and, * 5-10 minutes, for 20.4% of participating physicians.
Possible advantages of using Smartphone camera as part of the detector (friendliness and ease of use)	39.4% of participating physicians believe that a Smartphone camera-based detector would have those advantages.

Appendix B: Emergency idea: Sakaguchi test for Arginine

1. Biosensor System and Assay

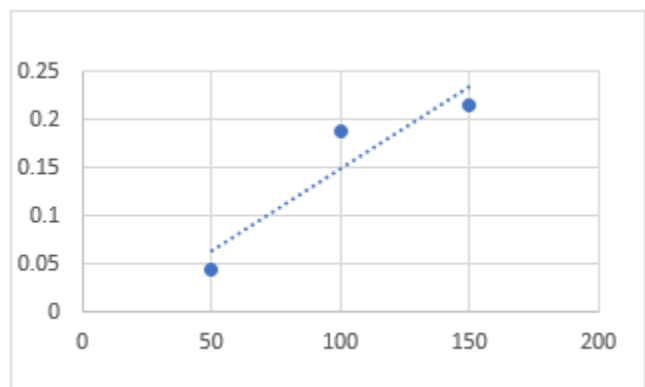
According to the amino acid sequence of the NT-pro BNP biomarker, the amino acid Arginine (R) was located at seven residues with higher molar ratio in the poly-peptide chain. As shown in Table 1, the R molar ratio is 12.86579924. Thus, our assay relies on the fact that R reacts with α -naphthol and sodium hypobromite /chlorite as an oxidizing agent, to form red complex as a positive result. The reaction named Sakaguchi reaction had been used for decades as a chemical method for qualitative more than quantitative determination of any amino acid containing guanidium group [11]. In other words, it is a test for R.

Table 1: Molar ratio of the 18 amino acids sequenced in the NT-Pro BNP.

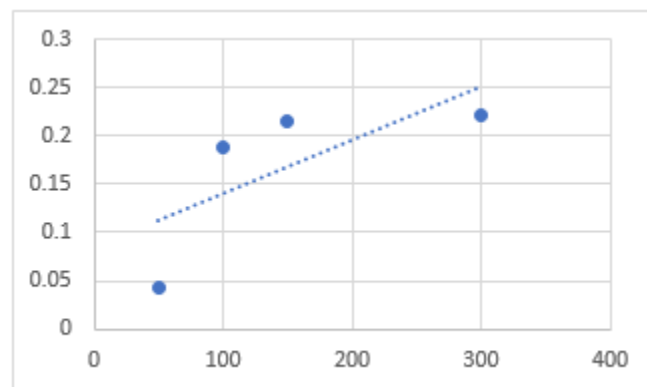
Amino Acid Symbol	Amino acid name	Amino acid M.Wt	No of each amino acid in protein sequence	Total weight of amino acid in protein (g)	Amino acid molar ratio
R	Arginine	174.2	7	1219.4	12.86579924
H	Histidine	155.1546	4	620.6184	6.455473015
P	Proline	115.13	5	575.65	5.714537591
L	Leucine	131.17	10	1311.7	13.31646699
G	Glycine	75.0666	7	525.4662	4.70043162
S	Serine	105.09	8	840.72	8.198152229
A	Alanine	89.09	3	267.27	2.509501559
D	Aspartic acid	133.11	1	133.11	1.354474255
E	Glutamic acid	147.13	8	1177.04	12.15555629
T	Threonine	119.1192	5	595.596	5.949237821
Q	Glutamine	146.14	6	876.84	9.046772534
N	Asparagine	132.1179	1	132.1179	1.342800431
K	Lysine	146.19	3	438.57	4.525151284
V	Valine	117.151	4	468.604	4.666752737
W	Tryptophan	204.225	1	204.225	2.191268944
I	Isoleucine	131.1729	1	131.1729	1.331680822
M	Methionine	149.21	1	149.21	1.543919443
Y	Tyrosine	181.19	1	181.19	1.920221125
total No of amino acids			76		
No of peptide linkage			75		
No of water molecules condensate in peptide link formation			75		
total mass of water condensate in protein synthesis			1350		
Molar ratio of water			15.88515546		
molar mass of protine (g)				8498.5004	99.78819793
molar mass of protine (D)				8457.4	

The precise quantification of R in the sample and R molar ratio in NT-pro BNP can lead to discovering a linear relationship between Sakaguchi adduct and NT-pro BNP concentration as a chemical analytical method for protein determination. The solid phase separation technique and alkaline hydrolysis of the protein in the sample are two involved techniques in our assay. Applying all reagents will be in two steps through simple syringe system, the first one includes solid phase, sodium hydroxide (NaOH) and alpha-naphthol; While, the second includes sodium hypobromite (NaOBr) then Urea.

2. Analytical Performance

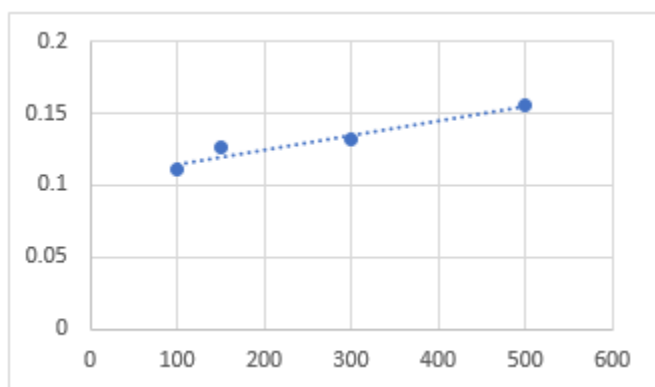


A

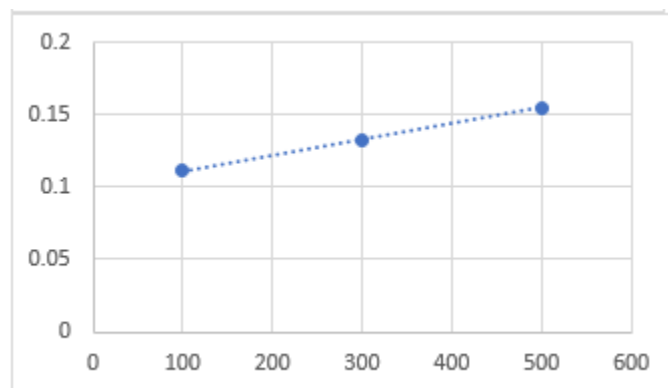


B

Figure 2 R concentrations (50,100,150 pg./ml) versus Sakaguchi adduct abs. with the spectrophotometer* with correlation coefficient $R=0.92849$ [A]. R concentrations (50,100,150,300 pg./ml) versus Sakaguchi adduct abs. with the spectrophotometer with correlation coefficient $R=0.7190$ [B] both at 500 nm. [Instrument Properties]: Instrument Type: UV-1800 Series, Measuring Mode: Absorbance, Slit Width: 1.0 nm, Light Source Change Wavelength: 340.0 nm, S/R Exchange

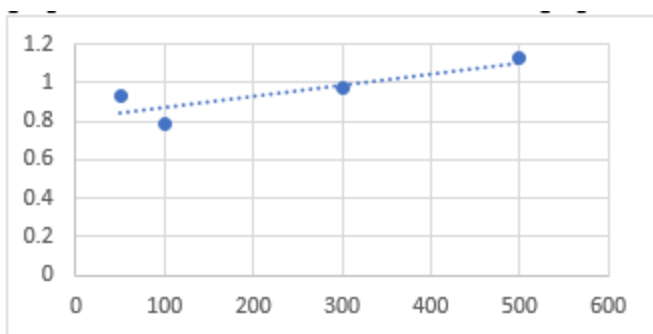


A

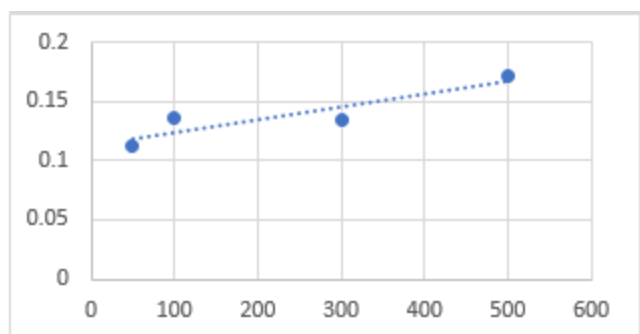


B

Figure 3: R concentrations (100,150,300,500 Pg./ml) versus Sakaguchi adduct blue signal absorbance with the mobile detector* with correlation coefficient $R=0.9682$ [A]. R concentrations (100,300,500 Pg./ml) versus Sakaguchi adduct abs. with the mobile detector with correlation coefficient $R=0.9995$ [B]. *instrument description: Portable, green colored background, iPhone7 and colorometer application.

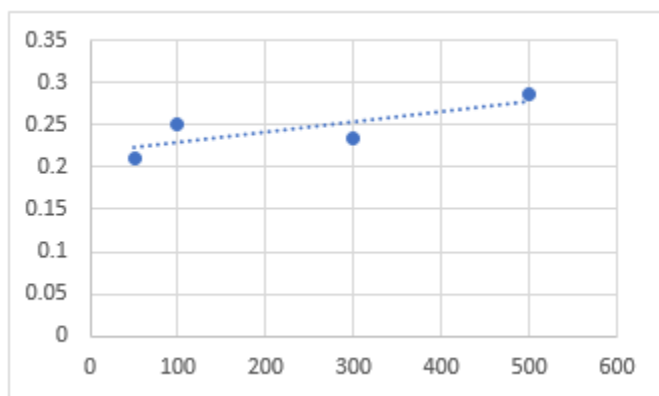


A

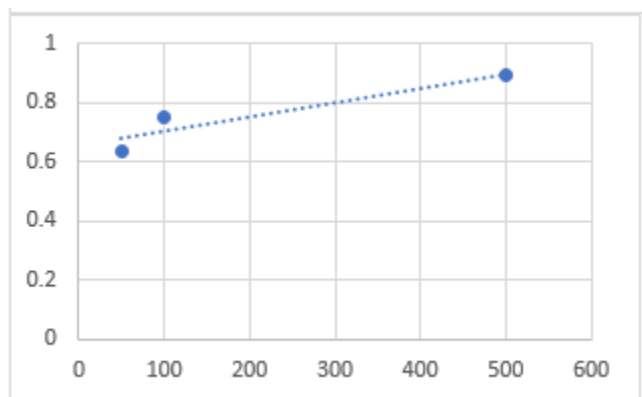


B

Figure 4: R concentrations (50,100,300,500 Pg./ml) versus Sakaguchi adduct* abs. at 338 nm with the spectrophotometer with correlation coefficient $R=0.8616$ [A]. R concentrations (50,100,150,300 Pg./ml) versus Sakaguchi adduct abs. with the spectrophotometer at 500 nm with correlation coefficient $R= 0.9082$ [B]. *The reagents for this experiment kept for three weeks in the refrigerator except for Urea solution.



A



B

Figure 5: R concentrations (50,100,300,500 pg./ml) versus Sakaguchi adduct** abs. of blue signal with the green background mobile detector* with correlation coefficient $R= 0.7909$ [A]. R concentrations (50,100,500 pg./ml) versus Sakaguchi adduct abs. of blue signal with the orange background with the mobile detector with correlation coefficient $R= 0.9319$ [B]. *instrument description: Portable, color changeable backgrounds, iPhone7 and colorometer application. **The reagents for this experiment kept for three weeks in the refrigerator except for Urea solution.

3. New Developments

To match the expectations of the physicians about non-expensive point of care testing in short time helping in taking a decision with acute heart failure patient, we developed a new biosensor with a very little cost (less than 1 \$ for the test), reproducible (the reagents can be stored for weeks in the refrigerator), fast (the reaction takes about 15 min.), with very simple materials and worldwide available detection system (Mobile phone and plastic gadget). Also, the usage of the biosensor will include few steps to help in such medical decision by the physician.

4. Sponsors

- A) Inspire Pharmaceutical Company
- B) Avance Research Center

Appendix C: Industrial Translational Potential

International Market segmentation:

Phase 1: The Egyptian Map

Health Economic consideration:

After floating of the Egyptian pound versus the USD, the Egyptian society is facing a disastrous economic situation. This increased the challenges in the medical and healthcare market and altered cheap, relatively low-tech, yet effective solutions to be highly in demand. With this in mind, we invented our prototype to be as cheap as can be while performing its function.

Health Social Considerations:

To deliver a diagnostic product to the Egyptian market, we have to not only take into consideration the international standards and technologies, but also, work to produce a socially suitable device. Thus, we opted for a mobile-phone based detector to bypass the possible difficulty of handling less-familiar sophisticated technologies by the general population, which is part of our intended target group.

Advantages of envisioned prototype in Egyptian market:

- Time effective (Total test time=10 minutes)
- Suitable for bedside testing
- Low price-per-strip (5 LE), and price-per-device (400 LE), when commercialized
- Friendly device that is suitable for use in community pharmacies, emergency and operation rooms.
- Accurate and effective

Our designed envisioned end product is, thus, suitable for large scale marketing in countries that share the same criteria as Egypt in terms of their economic situation, healthcare segment and public segment demands. We estimate that our product would be suitable for the majority of both the African and the Middle-Eastern markets, although, careful pre-commercialization studies involving experts in these markets should be conducted first.

Technology roadmap: Prototype Vs. envisioned end product

A. Prototype:

Scientific Description:

Product	Prototype	Envisioned End product
1 - Type of Assay	ELISA (Fluid based)	Lateral flow Assay (Nitrocellulose strip-based)
2 - Type of signal	Colorimetric signal	Fluorometric signal

3 - Detection process	Smartphone attached to an Arduino with the use of 1sheeld	Smartphone with a specially developed app
4 - Detection limit	360 pg/ml NT-proBNP	10000 pg/ml NT-ProBNP
5 - Time estimated for test sample during testing round	25 minutes	10 minutes
6 - Market feasibility and suitability	Not suitable for immediate commercialization	Suitable for commercialization
7 - Biosafety	Less due to the need to safely discard the resultant reaction fluids	More user friendly and safe (No free fluids, cartridge is more safe and easy to dispose of)
8 - Point of care	Needs a microfluidic design to be more suitable for POC testing	Designed to be a POC test
9 - Commercial price	Manufacturing of the detection gadget costs around 300 USD	In mass production, it should be not more than 30 USD

Suitability of the biosensor concepts for high-volume manufacturing:

In our biosensor, high-volume manufacturing will be one of the strongest edges since it will cut the costs of the items, and automating the processes we did by hand will also cut the time needed to prepare each test chip or cartridge. Programming of our own custom software to make it available later on for all users will prove very efficient in terms of price for mass production on the long term.

Prototype gadget and envisioned product gadget cost in LE or USD

Prototype price:

Gadget => 2000 LE

Arduino => 200 LE

1Sheeld => 900 LE

Total => 4100 LE which is equivalent to approximately 232 USD

Competitor analysis, comparison of the biosensor prototype to products that are already available on the market:

Most famous competitors' products are:

- 1) Roche cobas h 232 POC system
- 2) Samsung LABGEO IB10
- 3) Abbott iStat

Our envisioned product is smaller than all 3 products since it can be held in one hand. It also uses the mobile phone easily instead of adding overhead hardware that is already in the hands of almost every user or his/her caregiver.

Moreover, the maximum time our device uses to get results is 10 minutes which is within the average numbers between the all devices but definitely less than the maximum number offered by all 3 devices (12 minutes in the Roche device, 20 minutes in the Samsung device and 10 minutes in the Abbott device).

Business Model for Envisioned LFA product:

1- Customer value proposition:

We propose a POC (point of care) test that is portable and user friendly for the Egyptian, African and middle eastern markets. The envisioned prototype is capable of facing the economic challenges of these markets.

2- Profit formula:

Loss = Cost price (C.P.) – Selling Price (S.P.)

- Cost price in mass production for (Labeling kits + LFA membranes + Antibodies + protein A) \$
- Cost price for mass production of (Gadget + Filters)
- Selling price for each strip and gadget in \$

Cost price corresponds to the GDP growth in Egypt, which grew 4.3% in 2016 compared to the previous year. This rate is 1-tenth of one percent less than the 4.4% published in 2015.

Egypt GDP Annual Per capita Evolution:

Date	GDP per capita	Annual Change
2016	3,685\$	-1.3%

3- Key resources:

- Physical: point of sales system
 - Networks: with community pharmacies, Hospitals. (Strips), cardiology clinics
 - Networks for gadgets: Samsung, Nokia, Mobile shops, Amazon, community pharmacies, Hospitals, cardiology clinics.
- Intellectual: We will attain the copyrights of our developed mobile phone application. This application will allow for integration of a medical decision support system and cloud sharing of results between patients and their physicians will maintaining information confidentiality and user security.
- Financial:
 - Cash funds
 - Lines of credit
 - Guarantees

- Human :
 - Creative scientists
 - Skilled Engineering team
 - Marketing and product development team
 - Intensive sales resources

4- Key processes:

We took into consideration the suitability of the envisioned product and how it would be advantageous in our targeted markets, with minimal cost, and high accuracy and sensitivity.

- Location:
 - Market segmentation phases will span three regions: Egypt, Africa and the middle East .
 - Physical : In community pharmacies, Hospitals, Emergency rooms, Cardiology clinics
 - Online : Cloud services (Amazon, Allianz)
- Equipment needed for mass production of envisioned LFA strip:
- Automatic Dispenser
- Automatic Cutter
- 3-Smart Phones
- Manpower : co-workers who are involved in the mass production process in all branches