Talanta 224 (2021) 121860

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simultaneous express immunoassay of multiple cardiac biomarkers with an automatic platform in human plasma

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ARTICLE INFO

Keywords:
Magnetic beads
Fluorescent microscopy
Immunoassay
Human plasma proteins
Cardiac biomarkers
Multiplex detection

ABSTRACT

C-reactive protein, cystatin C, myoglobin, and D-dimer represent the inflammatory or thromboembolic status of the patient and play important roles in early diagnostics of acute myocardial infarction. Each protein can indicate some health problems, but their simultaneous detection can be crucial for differential diagnostics. The express analysis of these proteins in a small drop of plasma was developed using magnetic beads. The suggested method is based on immunomagnetic extraction of the target analyte from plasma samples and its simultaneous labelling by fluorescent dye. Reaction time was optimized for quantification of cardiac biomarkers in the spike solutions and human plasma samples. In this paper, we developed a one-protein detection technique for each cardiac biomarker and united it to a four-protein facility using an automatic platform. The proposed technique requires only 17 μL of the human plasma and takes 14 min for four-protein measuring. The suggested technique covers concentration difference by more than two orders of magnitude and demonstrates analytical applicability by measurements of human plasma samples of 16 volunteers.

1. Introduction

A wide range of diseases demonstrates similar symptoms and requires differential diagnostics. Since there are many causes of chest pain, which can originate from the heart, lungs, aorta, gastrointestinal tract, it is necessary to identify the origin of pain. One of the most informative analyses is a blood analysis for specific markers (cardiovascular, inflammatory, kidney, cancer, etc.). Rapid blood tests available for individual application or express analysis in a hospital can make preliminary diagnostics more differential and effective.

Blood markers for infection, kidney and heart disease are needed to be detected. Early and fast diagnostics of such biomarkers can dramatically decrease possible complications and simplify treatment. Four human plasma proteins – C-reactive protein (CRP), cystatin C (Cys-C), myoglobin (Myo), and D-dimer (D-Dm) – represent the inflammatory or thromboembolic status of the patient and play the role of cardiac markers [1,2].

Myoglobin is a cytoplasmic protein of skeletal muscle and myocardium with a molecular weight of 17.9 kDa. Physiological concentration of Myo in the blood is from 6 to 85 ng mL^{-1} [3]. The excess of Myo allows evaluation of muscle tissue destruction, the dynamics of the process and the effectiveness of treatment. Durable Myo excess in blood indicates its ongoing release from muscle tissue, since its half-elimination from the blood (through kidneys) is about 9 min [4]. Cystatin-C is a low molecular (13.4 kDa) protein. The increase in Cys-C level in blood can indicate mild renal impairment and the cardiovascular dysfunction of old patients [5]. The physiological level of Cys-C in blood plasma is in the range from 800 to 1200 ng mL^{-1}. CRP is a pentraxin protein with a molecular weight of 25 kDa which takes part in inflammatory and innate immunological processes. Physiological CRP concentration in blood plasma is about 1000 ng mL^{-1}, but can increase dramatically at acute-phase inflammation to 500 μg mL^{-1} [6,7]. Clinical studies have shown that high values of CRP (4.0–6.0 μg mL^{-1}) are associated with a risk of cardiovascular events [8]. D-Dm is a fibrin degradation product of 180 kDa molecular weight which level increases in blood after fibrinolysis (clot degradation). In clinical practice, D-Dm

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https://doi.org/10.1016/j.talanta.2020.121860
Received 10 September 2020; Received in revised form 28 October 2020; Accepted 2 November 2020
Available online 11 November 2020

is used for coagulation and fibrinolysis control [9]. Physiological range of D-Dm is from 0 to 250 ng mL^{-1} [10]. Increase in D-Dm concentration indicates coagulation activation (disseminated intravascular coagulation, deep vein thrombosis, pulmonary thromboembolism, massive tissue damage or surgery, cardiac distress, neoplastic disease). Both coagulation and fibrinolysis lead to D-Dm increase, unlike other similar markers.

Each protein can indicate serious health problems, but their simultaneous detection can be crucial for the entire picture. Fast and accurate simultaneous determination of these four biomarkers might simplify differential diagnostics and treatment strategy.

There are many papers devoted to cardiac biomarkers express detection in microvolume of the sample. Authors [11] describe immunosensor for D-Dm detection in a model system with a fast response time of 20 s. However, this time does not include washing and regeneration of the sensor. The paper [12] describes the electrochemical biosensor for Myo determination in 1 μL of undiluted plasma for 30 min. This immunosensor works in a wide range of Myo concentrations including healthy donors' values and patients with acute myocardial infarction. Nevertheless, works discussing the simultaneous determination of several biomarkers are of particular interest.

Multiplex detection can be executed by dual labelling [13]. Sandwich immunoassay with antibodies labelled with europium (III) Eu^{3+} and samarium (III) Sm^{3+}chelates was employed for Myo and cardiac troponin T (cTnT) measurement followed by fluorescence detection using time-resolved fluorometry. Simultaneous determination of CRP and D-Dm in human blood plasma is carried out by white light reflectance spectroscopy with preliminary running calibrators and biotinylated antibodies [14]. Each step takes 20 min, thus the entire dual-analyte assay lasts approximately 45 min.

The authors [15] announce the detection of multiple cardiac markers for less than 30 min but only in a model system. Creatine kinase MB (CK-MB), CRP, D-Dm and pregnancy-associated plasma protein A (PAPP-A) have been measured simultaneously in a buffer with an integrated acoustic platform. An immunochromatographic test also can show impressive results [16]. The authors have described an immunochromatographic test for the rapid quantification of three biomarkers (troponin I, fatty acid binding protein, and CRP) in serum just in 10 min. The integrated chip [17] translates the binding events on the sensor surface into a readable electrical signal allowing detection of three cardiac biomarkers, troponin T (cTnT), creatine kinase MM (CK-MM) and CK-MB, simultaneously. The integrated chip can attain a low detection limit of 1 pg mL^{-1} for the three cardiac biomarkers from 2 μL blood in 45 min.

Despite the rapid development of accurate detection methods, separation and concentration of the target analyte remain an urgent task. Magnetic beads (MB) can be considered as an advantageous substrate for extraction and concentration of an analyte. MB were applied for the first time in 1977 for solid phase separation [18]. First MB were conjugated not only to specific antibodies but also to streptavidin and DNA fragments for hybridization, isolation and direct detection of specific DNA and mRNA molecules [19]. Currently, conjugates of MB with antibodies are widely used in analytical chemistry both for the separation of complex mixtures and for the specific detection of various compounds [20–23]. Application of MB has revolutionized cardiac biomarkers assay by overcoming the problem of time limitation typical for heterogeneous immunoreaction, decreasing the volume of the sample and concentrating analyte at the detection area. Combination of MB with different detection techniques leads to undoubted advantages like time reduction, sample volume decrease, or LOD lowering for simultaneous cardiac biomarkers detection [24,25]. Surface-enhanced Raman spectroscopy has its advantage for multiplex detection due to narrowness of Raman peaks [26]. Authors used two different types of SERS nano-tags - malachite green isothiocyanate (MGITC) and X-rhodamine-5-(and-6)-isothiocyanate (XRITC) - for simultaneous quantification of cTnI and CK-MB in patient serum for less than 15 min.

In the present paper, the express analysis of four plasma proteins on magnetic beads in combination with micro fluorescence assay was developed. The suggested technique was validated with human plasma samples measurement. One of the most important parameters of express detection – reaction time – was optimized to obtain a four-protein facility using an automatic platform. The test results of the express analysis of four cardiac biomarkers from 7 volunteers executed on the developed automatic facility correlate with values obtained from the independent INVITRO laboratory. The proposed technique requires only 17 μL of plasma and 14 min for four-protein measuring and can be used as an express clinical method for complex blood analysis.

2. Material and methods

Reagents and chemicals. Recombinant proteins: myoglobin, C-reactive protein, D-dimer, cystatin C, as well as monoclonal antibodies: anti-Myo antibodies (clone 4E2cc, 7C3cc and HRP-conjugated 7C3cc), anti-CRP antibodies (clone C2cc, C6cc and HRP-conjugated C6cc), anti-D-Dm antibodies (clone DD189cc, DD255cc and HRP-conjugated DD255cc), anti-Cys-C antibodies (clone Cyst24cc, Cyst19cc and HRP-conjugated Cyst19cc) were purchased from "HyTest" (Russia).

Commercial NH_2-conjugated magnetic beads were purchased from "Sileks" (Russia). Fluorophore BDP 558/568 NHS ester was purchased from "Lumiprobe" (USA). Bovine serum albumin (BSA) and Tween-20 were purchased from "Sigma-Aldrich" (USA). Dialysis was performed using dialysis bags (D9277) purchased from "Sigma-Aldrich" (USA). ELISA was performed by standard technique with self-coated 96-well microplate. All other reagents (salts, acids, etc) were of analytical grade. Ultrapure water (18.2 MΩ cm) used in this work was obtained from Milli-Q water purification system (Darmstadt, Germany).

Covalent immobilization of antibodies at MB with an amine (-NH_2) surface functional group. MB's surface was functionalized with monoclonal antibodies by covalent binding to glutaraldehyde according to the manufacturer's protocol ("Sileks", Russia). A 1.75 mL mixture of 0.025 M phosphate buffer, 22% glutaraldehyde and 6 mg mL^{-1} MB at the final concentration was incubated for 3 h at room temperature with constant stirring. Then MB were triple washed with 0.025 M phosphate buffer solution (pH 7.4). After that, 1 mL of antibody solution (anti-Myo (clone 4E2cc)/anti-CRP (clone C2cc)/anti-D-Dm (clone DD189cc)/anti-Cys-C (clone Cyst24cc)) in 0.025 M PBS, pH 7.4 with 5 mM of ascorbic acid was added to the suspension of MB to a final concentration of 0.5 mg mL^{-1}. The obtained mixture was incubated with stirring for 2 h at room temperature. After incubation 0.5% BSA and 20 mM methionine at final concentration was added to the suspension. This mixture was incubated with stirring overnight at room temperature. The unbound antibodies and other excess reagents were washed-off from the suspension by multiple washing with 0.05 mM PBS, pH 7.4 with 0.15 M of NaCl. The washed MB were resuspended in 1 mL of PBS with 0.1% BSA to obtain the stock suspension of MB 10 mg mL^{-1}. To prevent bacterial contamination 20 μL of 1% sodium azide were added to the finished suspension.

Verification of MB modification by monoclonal antibodies was performed by non-competitive heterogeneous ELISA on magnetic beads as a solid phase.

Depleted plasma preparation. Depleted plasma is human blood plasma free from the target protein. For depleted plasma preparation MB modified with monoclonal antibodies specific to the corresponding protein were added to the plasma in a ratio of 1:10. This mixture was incubated with stirring for 2 h at room temperature. Molecules of the target analyte in plasma were captured on MB and separated from the mixture by the external magnetic field of the permanent magnet.

Depleted plasma was used in the work as a zero point (without target analyte) on the calibration curves and for spiked sample preparation. Depleted plasma application allows considering non-specific interactions that contribute to the background signal. The absence of the target protein in depleted plasma was confirmed by ELISA at λ_{450} (see

Fig. S1).

Spike solutions preparation. Depleted blood plasma of healthy donors was used as a matrix for spiked samples preparation. Known amounts of stock concentrations of analytes (Myo, Cys-C, CRP and D-Dm) in PBST were spiked to the depleted plasma in a ratio 1:9 to avoid significant dilution of the sample. The concentration of the obtained spiked solutions was confirmed by ELISA (see Fig. S1).

Synthesis of BDP-antibodies conjugates. BDP-antibodies conjugates were synthesized in the following way: 200 μL of an antibody (clones 7C3cc, C6cc, DD255cc, and Cyst19cc) solution (0.1 mmol) in 50 mM potassium phosphate buffer, pH 7.4, containing 0.1 M sodium chloride (PBS) was mixed with a solution of N-hydroxysuccinimide ester of BDP 558/568 (0.5 mmol) in DMSO and incubated for 2 h in dark at room temperature with continuous stirring. Excess unreacted low molecular weight reagents were removed by dialysis against PBS. The concentration of antibodies in the conjugates was determined spectrophotometrically with bovine anti-mice immunoglobulin-HRP conjugate on antigen coated microplates at λ_{450}. The molar ratio of the fluorescent label to an antibody in the conjugates was 1.2–1.5.

ELISA. Solutions of anti-Myo, anti-D-Dm, anti-CRP, or anti-Cys-C antibodies were prepared in a coating buffer (PBS) in concentration 10 μg mL^{-1}. An aliquot 100 μL of solution per well was added in 96-well microplate and left at 4 °C overnight. The coated plates were washed 3 times with PBS and 0.05% Tween-20 (PBST) and blocked with 1% BSA in PBS for 2 h at room temperature. Direct one-step ELISA was performed in conventional format with the selection of conditions for the studied analytes. After incubation of the plasma solution and HRP-conjugated antibodies, the wells were washed 5 times with 350 μL PBST, followed by addition of 100 μL TMB per well. The enzymatic reaction was stopped by addition of 100 μL of 0.2 M H_2SO_4 and the absorption was measured at λ_{450}. The chosen conditions for ELISA are presented in Table 1.

Biomarkers analysis. Biomarkers analysis represents immunoassay on MB. The volume of plasma required for the analysis depends on the average analyte concentration in plasma and molecular weight of the protein (Table 2). The aliquot of a sample was mixed both with MB labelled with specific antibodies and with fluorescent conjugate according to Table 2. An aliquot 3 μL of ten-fold diluted suspension of MB was used for each analyte measurement. The incubation was carried out with stirring at 37 °C. Three different incubation times were tested to obtain optimal interaction. The sample was washed after incubation 5 times with PBST using a permanent magnet and then transferred to a microcuvette for the following signal detection. The final sample consists of 3 μL of the solid phase (magnetic beads) and 5 μL of PBST to prevent sample drying during the measurement. MB with the sample were immobilized with permanent magnet and fluorescence measurement was executed from the beads' surface.

Fluorescent measurements were performed using spectrometer innoRam (BWTech) with laser 532 nm (maximum capacity 40 mW) equipped with video microscope BAC102. Objective PL L 10/0.40 focuses the laser beam to the spot of 210 μm width. The laser power intensity has been optimized for each analyte and presented in Table 2.

Independent commercial laboratory (INVITRO lab) values were used as a reference for measurements in the donors' plasma. All experiments were carried out in 5 replicates. Mean and confidence intervals were calculated.

The automated bioanalytical platform for biomarker simultaneous quantification. The automated bioanalytical platform was produced in the laboratory for simultaneous quantification of Myo, Cys-C, CRP, and D-Dm in blood plasma.

Such a platform consists of a chip with reagents, a manipulator and micropumps Cavro® XCalibur Pump (XC) (Tecan Trading AG, Switzerland), and fluorescence detector system with a laser wavelength of 532 nm. All actions of the manipulator, the pumps, and the fluorescence detector were controlled by a customized script. The total time for the procedure including sample injection, mixing, incubation, washing, and signal detection was 14 min.

The automated bioanalytical platform (Fig. 1*a*,*b*) works as follows. The chip with reagents (Fig. 1 element 1) is installed to the platform of the manipulator (Fig. 1 element 2) and the action script is started. Each analyte has an individual well with MB labelled with specific antibodies. Four individual wells of the chip are connected to the washing pump system (Fig. 1 element 3) for analyte pretreatment.

The tip (Fig. 1 element 4) collects blood plasma and carries it to the analyte's wells. The volumes of plasma and fluorescent conjugate are presented in Table 2. For automatic measurements, the analytes are separated into two groups of high (CRP and Cys-C) and low (Myo and D-Dm) concentration in blood. Plasma sample for Myo and D-Dm detection is twice diluted with fluorescent conjugates, and for CRP and Cys-C detection is ten-times diluted. Such a type of dilution allows considering the concentration difference by two orders of magnitude. When the sample is mixed with MB and fluorescent conjugate, the incubation time starts. After the incubation time, the sample is washed three times using a permanent magnet (NdFeB) and the fluorescence signal is obtained from the surface of MB.

Table 1
The conditions for one-step sandwich-ELISA.

Conditions	Analyte			
	Myo	Cys-C	CRP	D-Dm
Sample volume (plasma)	10 μL	10 μL of the sample, diluted 20 times	10 μL of the sample, diluted 10 times	20 μL
The volume of HRP-conjugated antibodies	200 μL	200 μL	200 μL	180 μL
HRP-conjugated antibodies concentration	25 ng mL^{-1}	23.5 ng mL^{-1}	35 ng mL^{-1}	140 ng mL^{-1}
The incubation time of the sample	1 h			
The incubation time with TMB solution	7 min	7 min	10 min	15 min

Table 2
Conditions for one-step MB immunoassay and detection.

Conditions	Analyte			
	Myo	Cys-C	CRP	D-Dm
Sample volume (plasma)	5 μL	1 μL	1 μL	10 μL
The volume of the fluorescent conjugate solution	5 μL	8 μL	8 μL	10 μL
Fluorescent conjugate concentration	10 μg mL^{-1}	8 μg mL^{-1}	8 μg mL^{-1}	10 μg mL^{-1}
The incubation time	3, 5, 10 min	3, 5, 10 min	3, 5, 10 min	5, 10, 15 min
Laser power intensity	20 mW	4 mW	4 mW	30 mW

3. Results and discussion

Technique development. Biomarkers analysis represents one-step immunoassay on MB. Spike solution with a known verified concentration of each analyte is mixed and incubated with functionalized MB and fluorescence conjugate simultaneously. After sandwich formation and its washing, the analyte concentration is in proportion to the number of fluorescent dye molecules. Fluorescence spectrum of BDP dye is presented on Fig. 2. Thus, the maximum intensity of fluorescent spectra plays the role of the analytical signal in the developed technique.

Fig. 3 shows the calibration curves for studied analytes at different incubation times – 10 min (*black*), 5 min (*red*), 3 min (*blue*) for Myo (Fig. 3*a*), Cys-C (Fig. 3*b*), CRP (Figs. 3*c*) and 15 min (*black*), 10 min (*red*), 5 min (*blue*) for D-Dm (Fig. 3*d*). Spiked samples used for these calibration curves were additionally checked by ELISA (Fig. 1S) that confirms

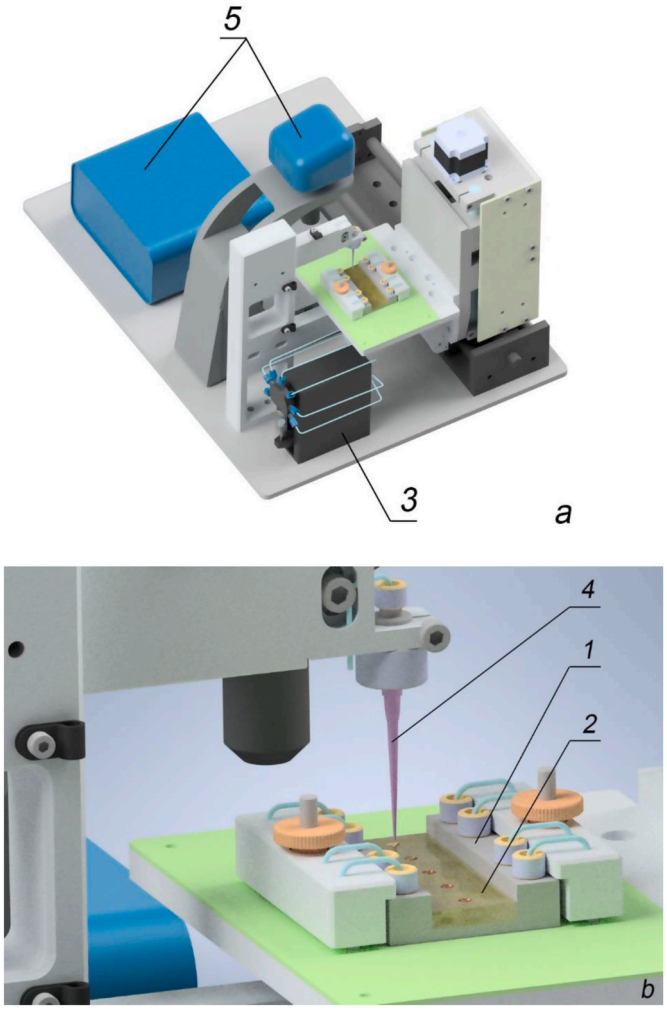

Fig. 1. The operation scheme of the automated bioanalytical platform for simultaneous quantification which consists of (1) the chip with reagents, (2) the platform of the manipulator, (3) washing pump system, (4) tip, (5) fluorescence detector system.

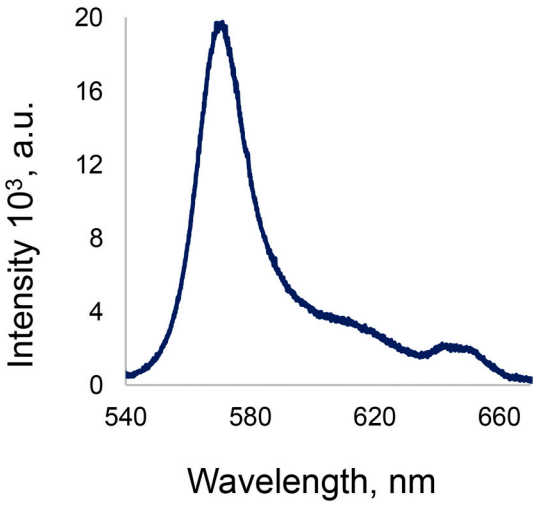

Fig. 2. The fluorescence spectrum of the BDP dye.

high specificity of antibodies.

The concentration range for each analyte covers healthy donors' values: 0–100 ng mL^{-1} for Myo, 0–2000 ng mL^{-1} for Cys-C, 0–1000 ng mL^{-1} for CRP and 0–500 ng mL^{-1} for D-Dm.

Signal intensity represents a linear function versus concentration for all four cardiac biomarkers (Fig. 3). The slope of the calibration curve increases with the time of incubation for Myo, Cys-C, and remains almost constant for CRP and D-Dm. For further experiments (including validation with donors' plasma and automatization) it is necessary to choose one suitable incubation time. For Myo and Cys-C the longer incubation time provides better sensitivity (Fig. 3*a,b*). We are looking for a compromise between the duration of incubation and detection sensitivity. Since we want to get better sensitivity for the less time, it is reasonable to choose 5 min as an appropriate incubation time for further experiments with Myo and Cys-C.

According to the 5-min calibration curve, the calculated LOD of Myo in plasma is 5.7 ng mL^{-1} (316 pM) and for Cys-C is 143 ng mL^{-1} (10.7 nM).

In contrast with Myo and Cys-C, the slope of the CRP calibration curve does not change with different incubation time (Fig. 3*c*). This fact means that we are in conditions of saturation even at 3 min incubation - every possible molecule of CRP is bound to an antibody. This is obviously due to the high affinity of the antibodies and the rapid formation of the complex. Since 3 min incubation provides adequate binding, it was chosen as the optimal time for CRP. The calculated LOD for CRP in plasma is 67 ng mL^{-1} (2.7 nM).

D-Dm is a comparatively large protein with the molecular weight of 180 kDa. It means that compared to three other proteins, D-Dm has larger diffusion limitations when binding to antibodies. Nevertheless, the developed technique allows D-Dm quantification within its physiological range with satisfactory accuracy at 10 min incubation time. The LOD of D-Dm is calculated as 61 ng mL^{-1} (0.34 nM).

Thus, the immunomagnetic fluorescent assay has proven to be an excellent tool for rapid cardiac biomarkers quantification in a small amount of the sample.

Validation of technique. To validate the developed immunoassay, quantification of analyte concentration was carried out at optimal incubation time in the blood plasma of 16 healthy donors. Plasma samples were selected to fit in calibration curves (Fig. 3) according to the INVITRO results.

Calculated results were compared to the independent measurements provided by reference commercial laboratory INVITRO lab for the same donors (Table 3 and Fig. 2S). Analyte concentrations measured by the developed immunomagnetic assay are in good agreement with data obtained by INVITRO lab. All experiments were carried out in 5 replicates. Mean and confidence intervals were calculated.

The obtained results emphasize the applicability of optimal conditions for the cardiac biomarkers concentration measurement in human plasma samples. These conditions could be used for further adaptation of the immunomagnetic assay for the automatic platform for simultaneous quantification of four analytes. The optimization of the parameters of the immunomagnetic assay is the key stage for the following automatization of the measurement process.

Simultaneous quantification by the automatic bioanalytical platform. Automatization can simplify multiplex detection by reducing the time for routine procedures like washing and transfer of reagents.

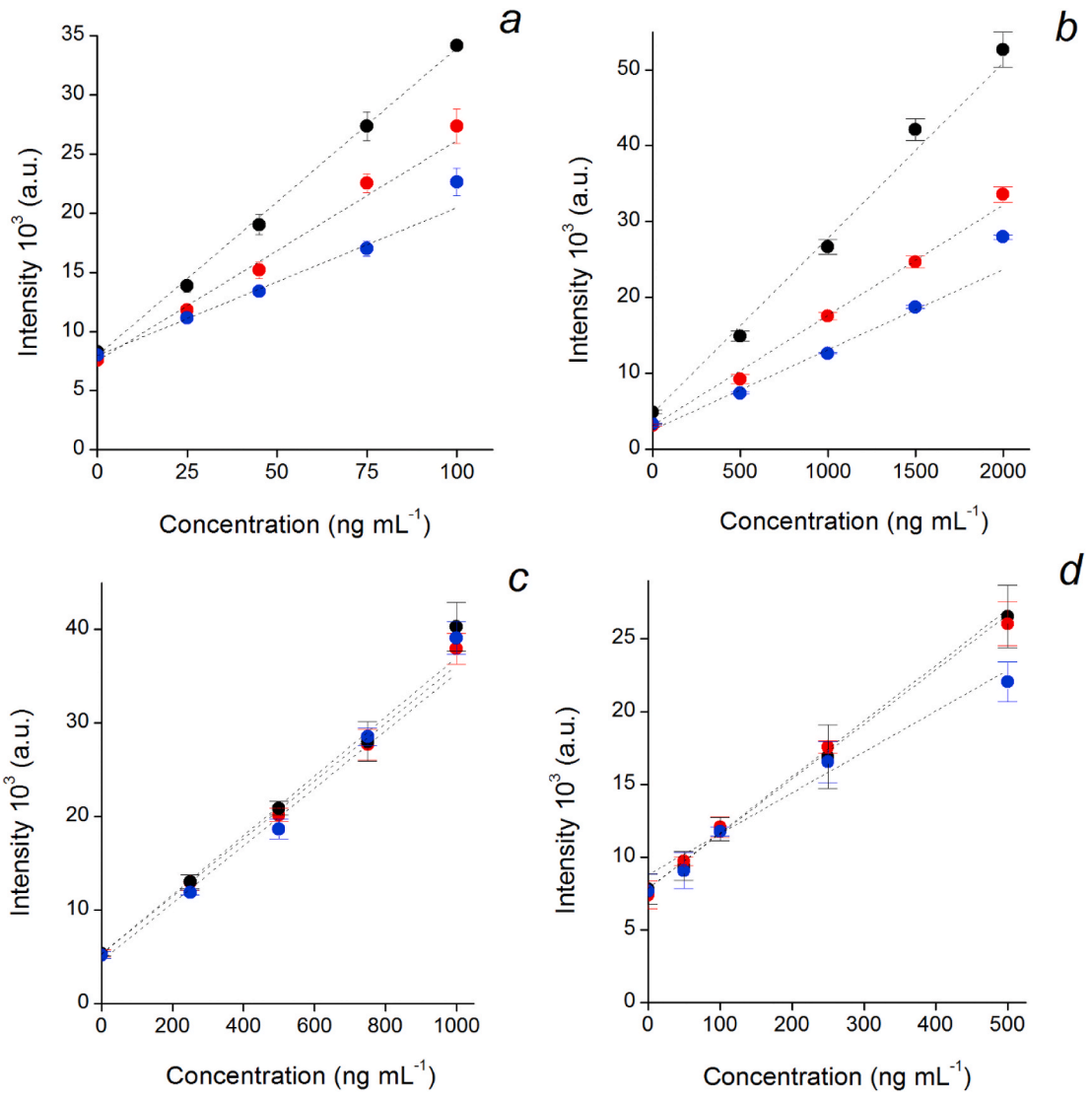

Fig. 3. Calibration curves for *a*) Myo, *b*) Cys-C *c*) CRP and *d*) D-Dm spiked samples at different incubation time: 10 min (*black*), 5 min (*red*), 3 min (*blue*) for myoglobin, cystatin-C, CRP and 15 min (*black*), 10 min (*red*), 5 min (*blue*) for D-dimer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Concentration quantification of cardiac biomarkers in the blood plasma of healthy donors.

Myoglobin, ng mL^{-1}			Cystatin-C, ng mL^{-1}			CRP, ng mL^{-1}			D-Dimer, ng mL^{-1}		
Donor	INVITRO	MB assay	Donor	INVITRO	MB assay	Donor	INVITRO	MB assay	Donor	INVITRO	MB assay
D1	18	18 ± 1	D6	760	753 ± 33	D1	200	194 ± 7	D12	21	28 ± 23
D2	33	29 ± 3	D2	810	791 ± 33	D10	500	466 ± 20	D14	76	70 ± 31
D3	42	38 ± 3	D7	1150	1127 ± 32	D11	600	601 ± 24	D15	270	287 ± 105
D4	56	52 ± 3	D8	1450	1440 ± 57	D12	900	922 ± 33	D16	374	433 ± 38
D5	61	60 ± 3	D9	2110	2224 ± 102	D13	1000	998 ± 35	D13	530	554 ± 29

Also, automatization can dramatically decrease the analysis time due to carrying out several parallel processes.

Simultaneous quantification of four cardiac biomarkers in the blood plasma of seven donors was performed with the developed automated bioanalytical platform to demonstrate the possibilities and advantages of automatization (Table 4). Considering the standard error, the results correspond to INVITRO values. Automatization makes possible simultaneous measurement of Myo, Cys-C, CRP, and D-Dm in 17 μL of blood plasma within 14 min. It is also worth noting that this technique provides an accurate independent measurement of each analyte even in presence of a high concentration of other analytes. For example, donor A5 has high CRP value that lies out of range (OOR) for fluorescent measurements. Despite this fact, three other analytes are accurately quantified without any interference from high CRP value.

Table 4
Simultaneous quantification of cardiac biomarkers in the blood plasma of healthy donors using the developed automated bioanalytical platform.

Donor	Myoglobin, ng mL^{-1}		Cystatin-C, ng mL^{-1}		CRP, ng mL^{-1}		D-Dimer, ng mL^{-1}	
	INVITRO	Auto	INVITRO	Auto	INVITRO	Auto	INVITRO	Auto
A1	15	21 ± 7	770	806 ± 50	700	671 ± 60	215	200 ± 30
A2	17	14 ± 7	790	772 ± 50	300	329 ± 60	65	70 ± 30
A3	25	20 ± 7	900	910 ± 50	500	405 ± 60	<21	8 ± 30
A4	33	40 ± 7	810	1012 ± 50	200	230 ± 60	134	100 ± 30
A5	46	44 ± 7	1650	1689 ± 50	8700	OOR[a]	206	190 ± 30
A6	58	51 ± 7	1770	1815 ± 50	800	730 ± 60	230	330 ± 30
A7	61	62 ± 7	1420	1433 ± 50	200	264 ± 60	29	34 ± 30

[a] OOR – the value of the analyte lies out of measurement range.

4. Conclusion

The micro fluorescent immunoassay has been developed for the express measurement of four cardiac biomarkers – C-reactive protein, cystatin C, myoglobin, and D-dimer, using magnetic beads. The proposed technique covers concentration difference by more than two orders of magnitude – from 18 to 2220 ng mL^{-1}. Reaction time was optimized for quantification of cardiac biomarkers in the spike solutions and human plasma samples. The suggested technique was validated with measurements of human plasma samples of 16 volunteers.

Automatization has simplified multiplex detection and dramatically decreased the analysis time. The automatic platform has been developed for the simultaneous analysis of cardiac biomarkers in 17 μL of the sample for 14 min. The test results of the express analysis of four cardiac biomarkers from 7 volunteers executed on the developed automatic facility correlate with INVITRO laboratory measurement. The suggested prototype can be developed into a point-of-care device for express blood analysis and differential diagnostics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was performed under the Russian Federation State Assignment no AAAA-A19-119071890024-8 and AAAA-A19-119110790066-5. The authors are grateful to the Troitsk Research and Development Center (Russia) for engineering support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121860.

Credit author statement

NLN, ONS, and TSK – fluorescence experimental setup and results discussion; ADV, LVY, and AEB – ELISA experimental setup and results discussion; NAB – fluorescent conjugate synthesis; SVY – computer modelling; AVE and INK – research management and scientific consulting.

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