Int J Clin Exp Med 2024;17(3):32-40
www.ijcem.com /ISSN:1940-5901/IJCEM0152552

Original Article

Development of a time-resolved fluorescent immunochromatographic assay for the quantitative detection of cardiovascular disease by MPO and Lp-PLA2

Shuang-Zhe Zhang[1,2*], Jian-Shu Zheng[1*], Shu-Hong Luo[1,3], Bu-Zhuo Xu[1], Xiao-Yan Liu[1], Ben-Yue Zhang[3], Hao Tang[1,3], Yi-Ming Weng[1], Xiao-Mao Yin[5], Xue-Dong Song[1], Ruo-Pan Huang[1,2,3,4]

[1]Guangzhou Co., Ltd., 79 Ruihe Road, Huangpu District, Guangzhou 510535, Guangdong, China; [2]South China Biochip Research Center, 79 Ruihe Road, Huangpu District, Guangzhou 510535, Guangdong, China; [3]RayBiotech Life Inc., Peachtree Corners, GA 30092, USA; [4]The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, Guangdong, China; [5]Clinical Laboratory, Guangzhou Red Cross Hospital, No. 396, Tongfu Middle Road, Haizhu District, Guangzhou 510220, Guangdong, China. [*]Co-first authors.

Received July 31, 2023; Accepted January 22, 2024; Epub March 15, 2024; Published March 30, 2024

Abstract: Introduction: Several studies have identified a link between inflammatory biomarkers in the blood and cardiovascular disease, highlighting their utility in diagnosis and prognosis. Lp-PLA2, combined with MPO, a commonly used marker of vascular disease, for dual detection, can improve the specificity and greatly reduce the false positive rate. Methods: We developed an MPO/Lp-PLA2 dual detection test strip utilizing time-resolved fluorescent (TRF) technology to detect cardiovascular disease. Results: This test is highly sensitive and specific, allowing for rapid diagnosis (15 min) of MPO and Lp-PLA2 simultaneously, without the need for any large-scale instrumentation. The MPO/Lp-PLA2 dual detection test strip has been validated using antigen standard curves and calibrated using clinical serum samples. The correlation coefficient (R^2) of MPO and Lp-PLA2 is 0.9739 and 0.9658, respectively. Conclusion: This rapid dual detection system outlined here has promising potential for the diagnosis and prognosis of cardiovascular disease.

Keywords: MPO/Lp-PLA2 dual detection, time-resolved fluorescent (TRF), cardiovascular disease, rapid diagnosis

Introduction

Increasingly, more people are suffering from various vascular diseases such as atherosclerosis, coronary heart disease, cerebrovascular diseases (CVDs) or aneurysms [1]. Vascular diseases exhibit a comparatively elevated incidence of morbidity, disability, and mortality. With the increase in life expectancy and changes in the socioeconomic environment in many countries, more individuals, especially the younger generations, are more vulnerable to developing vascular diseases [2-4]. The pathogenesis of vascular disease is intricate, and inflammation plays an extremely important role in its occurrence and development. Inflammation can manage infection and tissue damage to maintain homeostasis, but if left unresolved, inflammation can cause vascular endothelial dysfunction, initiating the development of vascular disease [5, 6]. Due to this connection, the analysis of proteins associated with inflammatory signaling pathways has emerged as a dependable approach for diagnosing vascular diseases. Myeloperoxidase (MPO), an enzyme secreted by leukocytes (neutrophils and monocytes), catalyzes the formation of various active oxidants. In addition to participating in defense reactions, MPO also plays an important role in oxidation [7]. MPO is closely related to the catalytic reaction of nitric oxide (NO), abnormal lipid metabolism, and acute coronary syndrome. NO is an important regulator of the normal physiological function of the heart and cerebrovascular system, and it is also a substrate of MPO [8]. Numerous *in vitro*

https://doi.org/10.62347/XNIJ8727

studies and animal trials have confirmed that MPO catalyzes the consumption of NO and inhibits its biological activity. Meanwhile, MPO facilitates the nitrosation of NO, which further damages vascular endothelial function [9]. Consequently, MPO stands as a valuable and autonomous marker for assessing vascular endothelial dysfunction [10].

Lp-PLA2 is primarily secreted by macrophages and circulates in the blood in complex with low-density lipoprotein (LDL) and high-density lipoprotein (HDL) [11]. Since vascular disease is often accompanied by an inflammatory response, high concentrations of Lp-PLA2 are present in the blood. Lp-PLA2 and its associated lipoprotein complexes play a role in modifying lipoproteins, which can contribute to the development of atherosclerosis and inflammation within blood vessel walls. Many studies [12-14], using animal models and human clinical trials, confirm the strong correlation between Lp-PLA2 and vascular diseases.

Time-resolved fluorescent immunochromatographic assay (TRFIA) is a detection method which combines immunochromatography with time-resolved fluorescence technology. In this technique, Nanoparticles containing lanthanide elements, often Europium (Eu^{3+}), are utilized as markers. These lanthanide elements are chosen for their distinct characteristics, including a long fluorescence lifetime and a large Stokes shift. These features effectively eliminate interference from non-specific background fluorescence through two resolution techniques: wavelength and time. The concentration of the target substance is determined by analyzing the intensity of the fluorescent signal, allowing for precise quantification [15].

It is widely believed the sensitivity and accuracy of the dual detection of MPO and Lp-PLA2 are higher than that of the single detection, and a large number of studies [16-19] have shown that combined detection is helpful for the auxiliary diagnosis of cardiovascular disease. A dual detection method of MPO and Lp-PLA2 was initially established. The carboxyl-rich surface fluorescent microspheres were covalently bound to the amino groups of the unconjugated antibodies. The coupled immunomicrospheres were sprayed on the immunomicrosphere pad, which had three lines: namely, the anti-MPO line, the anti-Lp-PLA2 line, and the polyclonal anti-rabbit IgG line. When the sample on the sample pad reached the immunomicrosphere pad, the MPO and Lp-PLA2 antigens in the sample were then specifically bound to their respective labeled antibodies, forming the “antigen-labeled antibody-fluorescent microspheres” complex I. Complex I continued to move forward to the detection line containing immobilized corresponding antibodies, forming the “coated antibody-antigen-labeled antibody-microsphere” complex II. Rabbit IgG polyclonal antibody immunomicrospheres were combined with goat anti-rabbit IgG monoclonal antibodies immobilized on the control line. The fluorescence immunochromatography result interpretation recorder was used to measure the fluorescence signal of complex II in the detection line. Since the intensity of the fluorescence signal was positively correlated with the concentration of MPO and Lp-PLA2 in the sample, the detection results were substituted into the corresponding standard curve to obtain the concentrations of MPO and Lp-PLA2 in the detection sample.

Although a commonly used marker of vascular disease, MPO is often affected by individual variations and other diseases, leading to interference. Incorporating Lp-PLA2 for dual detection can enhance specificity and significantly reduce the occurrence of false positives. Herein, we established a time-resolved fluorescence immunochromatography dual detection method for MPO and Lp-PLA2. This method offers a technical approach for diagnosing cardiovascular disease, assessing prognosis, and predicting treatment outcomes. It is sensitive, specific, efficient, and does not require professional technicians or expensive equipment to be successful.

Materials and methods

Materials

MPO monoclonal antibodies (cat# 4M43-16E3 and cat# 4M43-18B7), Lp-PLA2 monoclonal antibodies (cat# 4LA7cc-PL46cc and cat# 4LA7cc-PL42cc), recombinant MPO antigen (cat# AG0007) and recombinant Lp-PLA2 antigen (cat# CSB-DP113B) were purchased from RayBiotech, Inc. Goat anti-rabbit IgG (cat# P200301) and rabbit IgG (N160701) were purchased from Boyin Biotech Ltd., Hangzhou, China. Nitrocellulose membranes (cat# VIV-

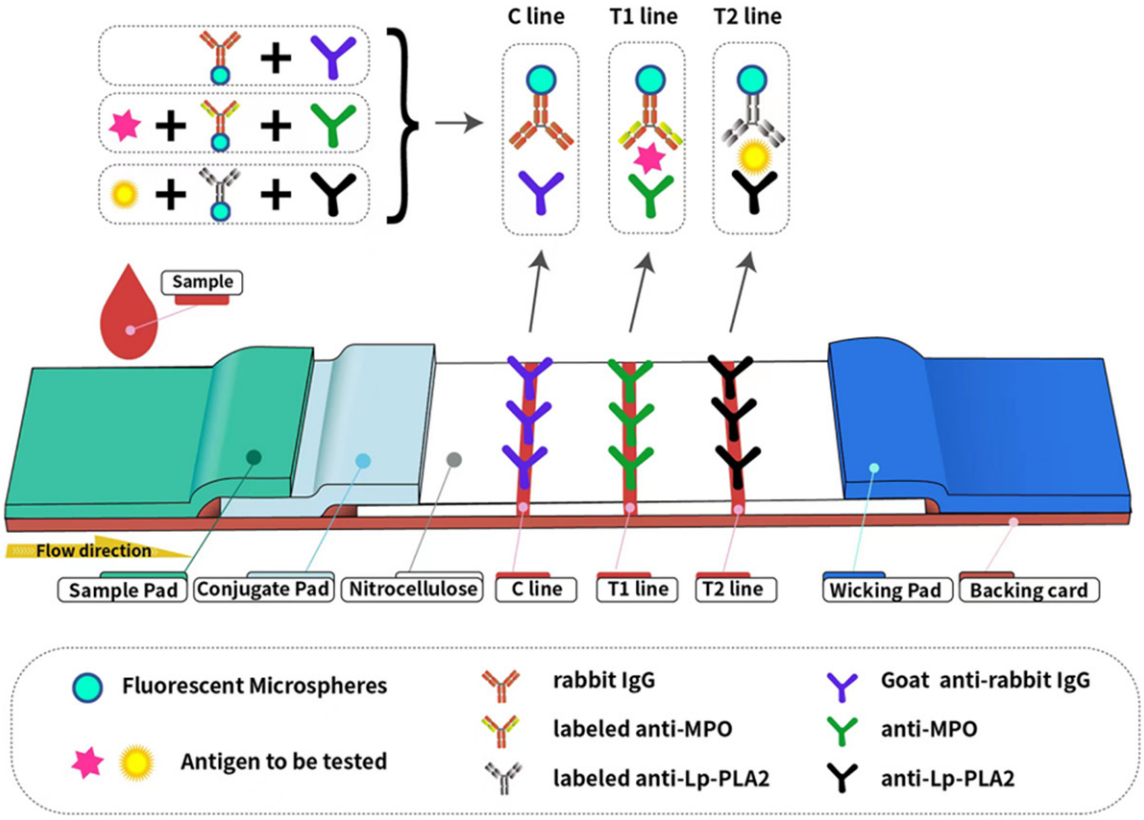

Figure 1. Schematic diagram of dual detection test strips. Test strips include backing card, nitrocellulose membrane, fiberglass membrane (sample pad and conjugate pad) and absorbent paper (wicking pad). The nitrocellulose membrane was coated with goat anti-rabbit polyclonal antibody as the quality control line (C line), MPO monoclonal antibody as the detection line 1 (T1 line), and Lp-PLA2 monoclonal antibody as the detection line 2 (T2 line). The fiberglass membrane is divided into a sample pad and a conjugate pad, and the conjugate pad is sprayed with time-resolved fluorescent microsphere-labeled MPO monoclonal antibody complexes and Lp-PLA2 monoclonal antibody complexes.

12025100R) were from Life Sciences Pall Vivid 120. Latex particles (Cat# FCEU003) were from Bangs Laboratories.

Dual detection TRF assay design of MPO and Lp-PLA2

A single channel TRF reader (Guangzhou Labsim Biotech Co., Ltd., model # AFS-1000) was used for detection according to previously established methods [20]. The dual detection method for MPO and Lp-PLA2 consisted of three stages. Stage 1: antibody-microspheres were prepared by covalently linking microspheres with reactive carboxyl groups on the surface to antibodies under the catalysis of 1-Ethyl-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS). The antibody-microspheres were washed and restored. Stage 2: the sample pad was blocked with blocking agents and detection antibodies and calibration antibodies were immobilized at discrete locations on nitrocellulose membranes (the detection zone and calibration zone, respectively). Stage 3: the sample pad, nitrocellulose membrane, and wicking pad were assembled into a plastic casing to create a test cassette (**Figure 1**).

Procedures for MPO and Lp-PLA2 measurements

The TRF reader was turned on for 5 min before use. The sample was diluted appropriately, and 100 μL was deposited onto the sample zone of the cassette. The cassette was placed at room temperature for 15 minutes, after which it was read immediately with the AFS-1000 reader.

The fluorescence values of the C and T lines were recorded and interpolated using a standard curve.

Linearity and dynamic range study

A series of concentrations of Lp-PLA2 - 0, 10, 20, 50, 100, 300, 600, and 1200 U/L and MPO - 0, 93.75, 187.5, 375, 750, 1500, and 3000 pmol/L were prepared. For each concentration, five test cassettes from two different lots were evaluated. The signal for each test cassette was collected three times.

Determination of the lowest detection limit

Dual detection strips were tested with matrix serum in 20 replicates. C line, T1 line, T2 line fluorescence values were recorded and the mean ($\overline{X}$) and standard deviation (SD) of the respective T/C were calculated. The value of X+2SD was substituted into the equation established by the matrix serum and the lowest point of the linear range to obtain the respective lowest detection limit.

Determination of the stability of the reagents

The following concentrations of MPO were selected for the standard, 167, 100, 33, 10, 3.33, 1 pmol/L. Various concentrations of MPO were measured at room temperature over a 4-week period. This was compared to measurements obtained at 50°C over 4 weeks. Each concentration was measured in technical triplicate. The same procedures were used to determine concentrations of Lp-PLA2. The standard concentrations used for Lp-PLA2 are 50, 30, 10, 3, 1, 0.3 and 0.1 U/L.

Clinical samples

Serum samples selected need to be complete with information, including age, sex, and clinical diagnosis. Samples of hemolysis, lipidemia and renal dysfunction were excluded. Based on the above criteria, 230 serum samples were collected from the Affiliated Cancer Hospital and the Guangzhou Medical University. Prior to collection of patient information and serum samples, written informed consent was obtained from each participant. The institutional ethics committee of Guangzhou Medical University approved this study. Samples were collected from healthy individuals and from those with cardiovascular disease. For MPO validation, there were 60 patients with coronary heart disease, 11 cases of acute myocarditis, 10 cases of hypertension, 13 cases of coronary atherosclerotic heart disease, and 13 healthy people. For Lp-PLA2 validation, there were 73 patients with coronary heart disease, 8 cases of acute myocarditis, 9 cases of heart failure, 20 cases of hypertension, and 13 healthy people. A total of 230 patients were included, including 143 males and 87 females. The average age is 62.93 years, with the youngest age being 3 years old and the oldest being 96 years old. Before testing, the samples were diluted 30-fold.

Statistical analysis

For statistical analyses, the GraphPad Prism 7 software package was used. Linear regression analyses were used to assess correlation. The transformation of the original data is a one-log or double-logarithmic transformation depending on the fit. When fitting data, simple linear regression is sufficient.

Results

Establishment of standard curve of MPO and Lp-PLA2

A set of linear standards with concentrations 10, 20, 50, 100, 300, 600, and 1200 U/L were obtained by serial dilution of the standard, Lp-PLA2: 1200 U/L. Similarly, concentrations of 93.75, 187.5, 375, 750, 1500, and 3000 pmol/L were obtained by serial dilution of the standard, MPO: 3000 pmol/L.

The dual detection test strips were assembled, and the standards were deposited onto the sample pad. After 15 min, they were immediately detected by the fluorescence immunoassay analyzer. Each standard concentration was tested 4 times. The fluorescence values for lines C, T1 and T2 were recorded (**Tables 1** and **2**). The log(X)-log(Y) mathematical model was used where the log of the standard concentrations was plotted on the X-axis and the log of the average T/C values were plotted on the Y-axis (**Figure 2B** and **2D**). A linear equation was fitted, establishing a standard curve. A basic plot was also created using the concentration of the standard in the X-axis and the T/C value in the Y-axis (**Figure 2A** and **2C**). The

Table 1. T/C signals measured using 4 cassettes for a series of Lp-PLA2 concentrations

Lp-PLA2 (U/L)	1200	600	300	100	50	20	10
T/C	0.766	0.378	0.179	0.047	0.022	0.011	0.008
	0.777	0.415	0.184	0.050	0.023	0.011	0.007
	0.845	0.386	0.165	0.058	0.020	0.010	0.007
	0.835	0.407	0.168	0.049	0.022	0.011	0.008
AVG	0.806	0.397	0.174	0.051	0.022	0.011	0.008
SD	0.040	0.018	0.009	0.005	0.001	0.000	0.001
CV	5.00%	4.43%	5.09%	9.49%	5.55%	1.89%	8.92%

Table 2. T/C signals measured using 4 cassettes for a series of MPO concentrations

MPO (pmol/L)	3000	1500	750	375	187.5	93.75
T/C	2.019	1.194	0.646	0.293	0.157	0.097
	1.980	1.038	0.586	0.332	0.142	0.086
	1.818	1.186	0.610	0.304	0.149	0.086
	1.887	1.035	0.550	0.303	0.139	0.088
AVG	1.926	1.113	0.598	0.308	0.147	0.089
SD	0.091	0.089	0.041	0.017	0.008	0.005
CV	4.72%	7.97%	6.78%	5.39%	5.39%	6.09%

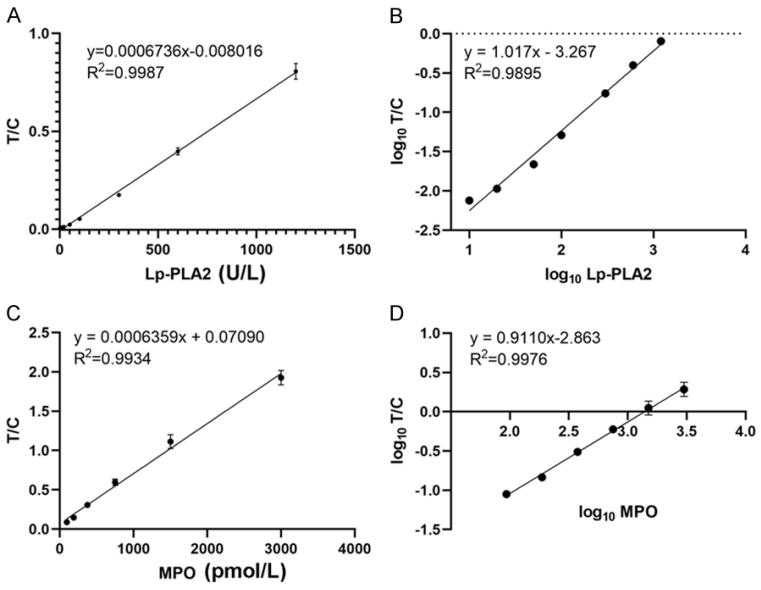

Figure 2. Linearity assessment of MPO and Lp-PLA2. A. Linear fitting and correlation coefficient (R^2) of T/C value and Lp-PLA2 concentration. B. Linear fitting and correlation coefficient (R^2) of $\log_{10}$ T/C value and $\log_{10}$ Lp-PLA2 concentration. C. Linear fitting and correlation coefficient (R^2) of T/C value and MPO concentration. D. Linear fitting and correlation coefficient (R^2) of $\log_{10}$ T/C value and $\log_{10}$ MPO concentration. Error bars represent standard deviation (SD) for quadruplicate readings.

results indicated that on the test strip Lp-PLA2 has good linearity (R^2=0.9987) in the range of 10-1200 pmol/L and MPO has good linearity (R^2=0.9934) in the range of 93.75-3000 pmol/L, which is suitable for determining common concentration ranges in the human body.

The sensitivity of the test strip was determined by calculating the lowest detection limit from the fitted equation. The lowest detection limits for MPO and Lp-PLA2 were 1.68 and 9.16, respectively.

The specificity of MPO and Lp-PLA2 was determined by mixing common interfering substances and comparing the results to samples without those interfering substances. MPO and Lp-PLA2 were mixed with the common interfering substances - bilirubin, cholesterol, triglycerides, hemoglobin, HAMA, and RF factors. MPO and Lp-PLA2 test results were unaffected in the presence of the following concentrations of substances: bilirubin ≤ 2 mg/mL, cholesterol ≤ 20 mg/mL, triglyceride ≤ 40 mg/mL, hemoglobin ≤ 30 mg/mL, HAMA ≤ 300 ng/mL, and RF factor ≤ 200 IU/mL. Therefore, the constructed dual-detection test strip method has good sensitivity and specificity for testing blood samples under common conditions.

Linearity and accuracy of MPO and Lp-PLA2 dual detection

There is a strong correlation between the measured concentration and the actual concentration of MPO and Lp-PLA2. The correlation coefficients (R^2) of MPO and Lp-PLA2 are 0.9972 and 0.9979, respectively (**Figure 3A** and **3B**).

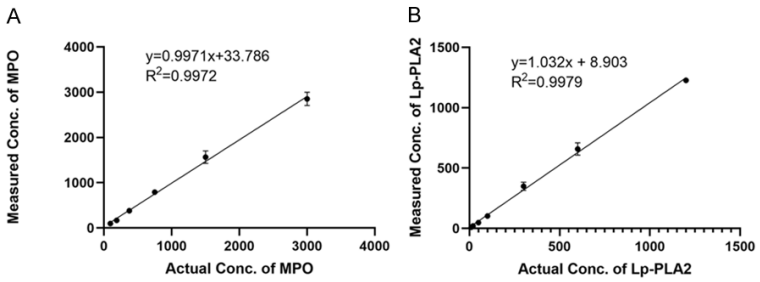

Figure 3. Correlation between measured MPO and Lp-PLA2 concentrations and actual concentrations. A. MPO. B. Lp-PLA2. Error bars represent standard deviation (SD) for three replicates.

Table 3. Comparison of average measured MPO concentrations with the actual MPO concentrations

Actual concentration of MPO (pmol/L)	750	375	187.5
Measured concentration of MPO (pmol/L)	754.540	325.530	162.770
	758.070	349.720	180.820
	744.420	338.610	173.130
Avg. concentration of MPO (pmol/L)	752.343	337.953	172.240
CV	0.94%	3.58%	5.26%
Accuracy	0.31%	-9.88%	-8.14%

Table 4. Comparison of average measured Lp-PLA2 concentrations with the actual Lp-PLA2 concentrations

Actual concentration of LP-PLA2 (U/L)	600	300	100
Measured concentration of LP-PLA2 (U/L)	713.28	329.51	99.66
	616.42	271.83	109.22
	648.69	313.89	98.97
Avg. concentration of LP-PLA2 (U/L)	659.460	305.078	102.615
CV	7.48%	9.78%	5.58%
Accuracy	9.91%	1.69%	2.61%

The standard MPO concentrations of 750, 375, and 187.5 pmol/L, and standard Lp-PLA2 concentrations of 600, 300, and 100 U/L were measured three times using the TRF reader. The deviation from the actual concentration was determined to be less than 6% for MPO (**Table 3**) and less than 10% for Lp-PLA2 (**Table 4**). Thus, MPO/Lp-PLA2 standards of known concentration correlated well with measured values. The triplicate readings indicate the RayBiotech dual detection strip had good accuracy with < 10% deviation.

Repeatability and stability of MPO and Lp-PLA2 dual detection

Next, we evaluated the repeatability of the dual detection strip. Testing ten replicates of 700 and 350 pmol/L of MPO and ten replicates of 600 and 300 U/L of Lp-PLA2, indicated CVs less than 9% (**Table 5**), which demonstrated good reproducibility of dual-detection measurement data.

Stability was determined by comparing data when the test paper was heated to 50°C vs when it was at room temperature (18-25°C). The T/C value obtained under heated conditions was comparable to those obtained at room temperature over 1-4 weeks. Test results did not differ with varying concentrations of MPO and Lp-PLA2 under those conditions.

The results of MPO (**Figure 4A**) and Lp-PLA2 (**Figure 4B**) indicated that the reaction curve is consistent with the reagents stored at room temperature, and the relative deviation of the T/C values of the high, medium and low levels of fluorescence signals is not more than 15%. Based on this data, we concluded that the MPO/Lp-PLA2 reagents can be stored at 2-30°C for 12 months.

Evaluation of clinical samples

In addition, the measured value of MPO in the dual detection of 107 human blood samples using RayBiotech test strips was compared with the MPO single detection product of Relia Bioengineering (Shanghai) Co., Ltd. which is already on the market. A series of concentration results measured for these two products had a high correlation (R^2=0.9739) (**Figure 5A**). The measured value of Lp-PLA2 in 123 human blood samples using RayBiotech test strips was compared with the Lp-PLA2 single detection product of Fosun Diagnostic Technology (Shanghai) Co., Ltd. which is already on the market. Lp-PLA2 concentration measurements for both products correlated well over a range of concentrations (R^2=0.9658) (**Figure 5B**). The results above demonstrate the utility of Raybiotech's MPO/Lp-PLA2 dual-detection TRF test strips for analyzing clinical blood sam-

Table 5. Determination of *MPO and Lp-PLA2 dual detection* repeatability

MPO	Repeatability		Lp-PLA2	Repeatability	
Actual conc. (pmol/L)	750	375	Actual conc. (U/L)	600	300
Measured conc. (pmol/L)	714.16	344.99	Measured conc. (U/L)	615.74	319.27
	726.99	395.65		699.17	297.25
	705.64	376.62		674.26	288.45
	722.95	345.8		638.69	275.76
	766.55	337.25		676.53	286.34
	763.41	324.02		585.16	293.98
	765.76	374.95		623.75	297.33
	773.79	343.29		693.39	320.67
	736.22	302.06		679.19	308.76
	744.34	395.8		527.72	311.21
AVG	741.981	354.043	AVG	641.360	299.901
SD	24.405	30.863	SD	54.615	14.755
CV	3.29%	8.72%	CV	8.52%	4.92%

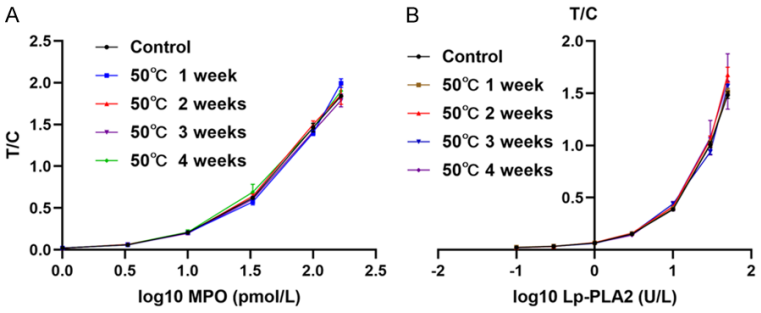

Figure 4. Stability of reagents and the MPO/Lp-PLA2 dual-detection test strip. Error bars represent standard deviation (SD) for three replicates.

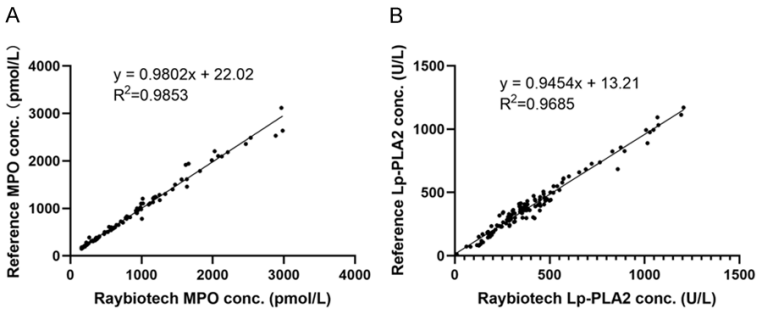

Figure 5. Comparison of clinical blood samples between RayBiotech TRF test strips and other companies. A. Comparison of MPO measurements. B. Comparison of Lp-PLA2 measurements. Serum was diluted 30-fold.

ples, showing a strong correlation with measurements from market-approved products.

Discussion

This study combines the measurement of MPO and Lp-PLA2 for rapid detection (15 min) of cardiovascular disease. Pre-diagnosis can now be quick and accurate in the early stage of cardiovascular disease, allowing for routine examination while saving precious time for patients. This study developed and evaluated the performance of dual-detection test strips, using sensitivity, specificity, stability, and repeatability. Finally, a large number of clinical blood samples were used to evaluate the correlation between the results of the RayBiotech test strip and other commercially available single test strips (MPO: Relia Bioengineering, Lp-PLA2: Fosun Diagnostic Technology). The results have excellent performance, can meet the quantitative determination of MPO and Lp-PLA2 under common conditions, measure the two biomarkers at one time to evaluate the occurrence and development of vascular diseases, and are comparable to the single-detection MPO and single-detection Lp-PLA2 test strips currently sold in the market.

Immunochromatographic test strips have the characteristics of lightness, speed, specificity, and sensitivity, and are very suitable for instant,

on-site detection. Remarkable achievements have been made in the field of antigen, antibody and hapten detection. In the future, the advancement of test strip detection technology should prioritize preserving its traditional strengths while incorporating novel materials and design approaches to create sensitive and quantitative methods for detecting multiple analytes. Digital test strip detection can be harnessed in the Internet+ era, allowing large-scale groups to conduct testing and gather extensive data for establishing an early warning and evaluation platform. RayBiotech's dual detection time-resolved fluorescent immunochromatographic assay for MPO/Lp-PLA2 can provide a quantitative assessment of vascular disease in patients, thereby decreasing the occurrence of false positives associated with MPO single detection. This holds significant promise for various clinical applications.

Acknowledgements

This work was supported by the following grants: RayBiotech innovative research fund, Guangzhou Innovation Leadership Team (CXLJTD-201602), Guangdong Province Key Technologies R&D Program for "Precision Medicine and Stem Cells" (2019B020231002, 2019B020227004), and Basic Research Plan of Guangzhou People's Livelihood Science and Technology Project (202002020021).

Disclosure of conflict of interest

Shuang-Zhe Zhang, Shu-Hong Luo, Bu-Zhuo Xu, Xiao-Yan Liu, Ben-Yue Zhang, Jian-Shu Zheng, Hao Tang, Yi-Ming Weng, Xue-Dong Song, Ruo-Pan Huang are employees of RayBiotech and have a financial stake in RayBiotech.

Address correspondence to: Ruo-Pan Huang, RayBiotech Life Inc., Peachtree Corners, GA 30092, USA. Tel: 1-770-729-2992; E-mail: rhuang@raybiotech.com; Xue-Dong Song, Guangzhou Co., Ltd., 79 Ruihe Road, Huangpu District, Guangzhou 510535, Guangdong, China. E-mail: ajesong99@gmail.com; Xiao-Mao Yin, Clinical Laboratory, Guangzhou Red Cross Hospital, No. 396, Tongfu Middle Road, Haizhu District, Guangzhou 510220, Guangdong, China. E-mail: yinxiaomaoandy@sina.com

References

[1] Huang F, Wang K and Shen J. Lipoprotein-associated phospholipase A2: the story continues. Med Res Rev 2020; 40: 79-134.

[2] Maas AHEM, Rosano G, Cifkova R, Chieffo A, van Dijken D, Hamoda H, Kunadian V, Laan E, Lambrinoudaki I, Maclaran K, Panay N, Stevenson JC, van Trotsenburg M and Collins P. Cardiovascular health after menopause transition, pregnancy disorders, and other gynaecologic conditions: a consensus document from European cardiologists, gynaecologists, and endocrinologists. Eur Heart J 2021; 42: 967-984.

[3] Navar-Boggan AM, Peterson ED, D'Agostino RB Sr, Neely B, Sniderman AD and Pencina MJ. Hyperlipidemia in early adulthood increases long-term risk of coronary heart disease. Circulation 2015; 131: 451-458.

[4] Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, Davis AM, Thompson T, Connell V, Wallace J, Naylor C, Crawford J, Love DR, Hallam L, White J, Lawrence C, Lynch M, Morgan N, James P, du Sart D, Puranik R, Langlois N, Vohra J, Winship I, Atherton J, McGaughran J, Skinner JR and Semsarian C. A prospective study of sudden cardiac death among children and young adults. N Engl J Med 2016; 374: 2441-2452.

[5] Buckley CD, Gilroy DW and Serhan CN. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 2014; 40: 315-327.

[6] Schumann J. Does plasma membrane lipid composition impact the miRNA-mediated regulation of vascular inflammation? Med Hypotheses 2016; 88: 57-59.

[7] Ndrepepa G. Myeloperoxidase - a bridge linking inflammation and oxidative stress with cardiovascular disease. Clin Chim Acta 2019; 493: 36-51.

[8] Lakshmi VM, Nauseef WM and Zenser TV. Myeloperoxidase potentiates nitric oxide-mediated nitrosation. J Biol Chem 2005; 280: 1746-1753.

[9] Abu-Soud HM and Hazen SL. Nitric oxide is a physiological substrate for mammalian peroxidases. J Biol Chem 2000; 275: 37524-37532.

[10] Vita JA, Brennan ML, Gokce N, Mann SA, Goormastic M, Shishehbor MH, Penn MS, Keaney JF Jr and Hazen SL. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation 2004; 110: 1134-1139.

[11] Tellis CC and Tselepis AD. Pathophysiological role and clinical significance of lipoprotein-associated phospholipase A(2) (Lp-PLA(2)) bound to LDL and HDL. Curr Pharm Des 2014; 20: 6256-6269.

[12] Munzel T and Gori T. Lipoprotein-associated phospholipase A(2), a marker of vascular inflammation and systemic vulnerability. Eur Heart J 2009; 30: 2829-2831.

[13] Siddiqui MK, Kennedy G, Carr F, Doney ASF, Pearson ER, Morris AD, Johnson T, McLaughlin

MM, Williams RE and Palmer CNA. Lp-PLA(2) activity is associated with increased risk of diabetic retinopathy: a longitudinal disease progression study. Diabetologia 2018; 61: 1344-1353.

[14] Acosta S, Taimour S, Gottsater A, Persson M, Engstrom G, Melander O, Zarrouk M, Nilsson PM and Smith JG. Lp-PLA2 activity and mass for prediction of incident abdominal aortic aneurysms: a prospective longitudinal cohort study. Atherosclerosis 2017; 262: 14-18.

[15] Wu F, Xu Y, Xu T, Wang Y and Han S. Time-resolved fluorescence immunoassay of thyroxine in serum: Immobilized antigen approach. Anal Biochem 1999; 276: 171-176.

[16] Li L, Li F and Xiao T. Correlation between the serum levels of RANTES with MPO, Lp-PLA2 and GRACE risk score in patients with coronary heart disease. Chinese Journal of Gerontology 2016; 36: 585-587.

[17] Wright CB, Moon Y, Paik MC, Brown TR, Rabbani L, Yoshita M, DeCarli C, Sacco R and Elkind MS. Inflammatory biomarkers of vascular risk as correlates of leukoariosis. Stroke 2009; 40: 3466-3471.

[18] Uydu HA, Bostan M, Yilmaz A, Demir A, Atak M, Satiroglu O, Temiz A, Cicek Y, Erdogan T, Cetin M and Canga A. Comparision of inflammatory biomarkers for detection of coronary stenosis in patients with stable coronary artery disease. Eur Rev Med Pharmacol Sci 2013; 17: 112-118.

[19] Li J, Cao T, Wei Y, Zhang N, Zhou Z, Wang Z, Li J, Zhang Y, Wang S, Wang P, Cheng N, Ye L, Li M, Yu Y, Ding C, Tan Z, Zhan B, He Q, Bao H, Wu Y, Liu L, Li J, Xu X, Cheng X and Huang X. A review of novel cardiac biomarkers in acute or chronic cardiovascular diseases: the role of soluble ST2 (sST2), lipoprotein-associated phospholipase A2 (Lp-PLA2), myeloperoxidase (MPO), and procalcitonin (PCT). Dis Markers 2021; 2021: 6258865.

[20] Xu B, Tang H, Weng Y, Jones VS, Luo S, Cho CY, Lin Y, Fang J, Song X and Huang RP. Development and evaluation of time-resolved fluorescent immunochromatographic assay for quantitative detection of SARS-CoV-2 spike antigen. J Clin Lab Anal 2022; 36: e24513.