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Original Article

Detection of heart-type fatty acid binding protein in serum by a rapid quantitative time-resolved fluorescence immunochromatographic assay

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Abstract: Heart-type fatty acid binding protein (H-FABP) has been previously utilized as a biomarker for the diagnosis, treatment, and prognosis of myocardial damage such as acute myocardial infarction (AMI). However, current assays lack the combination of high detection sensitivity, accuracy, and speed. In this study we developed a sensitive, rapid, and low-cost time-resolved fluorescence lateral flow immunochromatographic assay (TRF-LFA) for the quantification of H-FABP in human serum. Unlike conventional lateral flow assay devices, the developed TRF-LFA kits have the control zone upstream of the test zone, a layout that has been found to provide a more consistent internal calibration. The assay performance metrics (limit of detection, linearity, accuracy, precision, and specificity) were evaluated and a clinical sample comparison to a commercial chemiluminescence-based analyzer was conducted. The kits have a limit of detection (LoD) of 0.7 ng/ml, a linearity range from 0.7 ng/ml to 60 ng/ml, good precision (CV < 12%), excellent accuracy (CV < 6.5%) and high specificity, and good correlation with a commercial analyzer (R^2 > 0.98). The results showed a 96.83% positive correlation with a 95% confidence interval from 92.50% to 100% and a 94.87% negative correlation with a 95% confidence interval of 87.95% to 100%. The H-FABP kits developed here have comparable performance with a commercial chemiluminescence-based analyzer and are cheaper, smaller and the results are provided faster. Thus, it should find wide applications in point-of-care and emergency room settings.

Keywords: Heart-type fatty acid binding protein, time-resolved fluorescence, lateral flow immunochromatographic assay, myocardial ischemic injury

Introduction

Heart-type fatty acid binding protein (H-FABP) is composed of 132 amino acids with a molecular weight of 15 kDa. It is predominantly expressed in heart tissue with very low concentrations in other tissues [1, 2]. H-FABP is involved in active fatty acid metabolism where it transports fatty acids from the cell membrane to mitochondria for oxidation [3]. It also participates in bypass signal transduction. For example, through fatty acid signal translocation to the peroxisome, H-FABP indirectly regulates gene expression through the proliferation activation receptor. It is believed that when myocardial ischemia causes local long-chain fatty acid accumulation, myocardial H-FABP has a protective effect. In addition to its role in cardiac lipid transport, H-FABP has been suggested to play potential roles in cardiomyocyte differentiation, proliferation, and apoptosis [4]. Under physiological conditions, the concentrations of H-FABP are very low in the blood and urine of healthy people. The H-FABP concentration in plasma increases with age.

H-FABP is a biomarker for early detection of myocardial damage such as acute myocardial infarction (AMI), acute coronary syndrome, and heart failure [1, 5-11]. After the occurrence of

myocardial ischemic injury, H-FABP can be found in the blood as early as 1-3 hours after the onset of chest pain and has higher myocardial tissue specificity than myoglobin [3]. Its concentration reaches peak levels at 6-8 hours post onset of chest pain and then returns to normal within 4-30 hours [1, 8-11]. When combined, the measurement of both troponin and H-FABP showed increased sensitivity for the identification of myocardial infarction and acute coronary syndrome in patients presenting with chest pain at 3-6 hours following chest pain onset with a negative predictive value of 98%, which increased diagnostic accuracy [9, 12, 13].

In addition to being a useful biomarker for diagnosis, H-FABP also has prognostic value. Alongside D-dimer, NT-proBNP, and peak troponin T, H-FABP was the only cardiac biomarker that proved to be a statistically significant predictor of death or myocardial infarction (MI) at one year. This prognostic information was independent of troponin T, electrocardiogram, and clinical examination [13].

In recent years, H-FABP's significance for the clinical diagnosis, treatment, and prognosis of acute myocardial infarction has been increasing. There are a number of technologies that have been developed and reported for detection of H-FABP in clinical practice including, enzyme-linked immunoassays (ELISA) [14], electrochemiluminescence (ELICA) [15], capacitive immunosensors [16], latex turbidimetric immunoassays [15], amperometric enzyme immunosensors [17], lateral flow immunochromatographic assays [18, 19], chemiluminescence immunoassays [20], and fluorescence chromatographic immunoassays [21]. Although the detection sensitivity, accuracy, and speed of these technologies have improved over time, no single method has all of the desired performance characteristics. For example, ELISA is slow. While ELICA provides detection with high specificity, sensitivity, and accuracy, it is expensive and not portable. Conventional fluorescence immunochromatographic assays have the advantages of being simple, cheap, fast, and portable; however, they suffer from relatively low sensitivity.

Compared with a traditional fluorescence detection method, time-resolved fluorescence (TRF) detection has many advantages, including lower background and a higher signal/noise ratio, resulting in potentially increased detection sensitivity [22]. Conventional fluorescence detection techniques use the difference between the wavelength of fluorescence signal and background noises (e.g., scattered light of excitation photons, autofluorescence of sample matrices) collected simultaneously to separate the signal from background. The TRF detection technique, on the other hand, utilizes specific molecules which allow detection of the emitted light to occur after excitation, which takes advantage of the difference between the long fluorescence lifetime and the short fluorescence lifetime typical of non-specific background noises for signal separation. It has been well established that the TRF detection technique can in general achieve up to two magnitude higher signal/noise ratios than the conventional fluorescence technique. In addition, unlike conventional fluorescence detection, TRF detection may not need expensive bandpass optical filters for signal-noise separation and can simply use low-cost electronic components. It is feasible to construct a cheaper and smaller, portable TRF reader to measure TRF signals if fluorescence probes with a long lifetime can be used.

This study describes a detailed performance evaluation of a rapid quantitative time-resolved fluorescence immunochromatography assay for the accurate detection of heart-type fatty acid binding protein (H-FABP) with a low LoD and high specificity. The validation of the assay results with a commercial chemiluminescence-based reagent kit is also presented in this report. The results have demonstrated that the developed H-FABP TRF immunochromatographic assay provides comparable detection performance with commercial chemiluminescence-based reagents tested on an automatic chemiluminescence analyzer.

Materials and methods

Materials

Recombinant H-FABP antigen was purchased from Shanghai Linc-Bio Science Co. LTD, Shanghai, China. Cholesterol, bilirubin, triglyceride, human anti-mouse antibody (HAMA) and rheumatoid factor (RF factor) were obtained from the National Institute of Food and Drug Standards of China. The commercial chemilu-

minescence immunoassay test kits were purchased from Snibe Diagnostic Co., Ltd (SNIBE), Shenzhen, China.

Preparation and calibration of H-FABP antigen

Recombinant H-FABP antigen from Shanghai Linc-Bio Science Co. LTD has an SDS-PAGE purity of > 95%. The antigen was found to have high immuno-activity by sandwich ELISA method (data not shown). Antigen samples of various concentrations were prepared by diluting the antigen with 20% bovine serum albumin and were measured three times to obtain an average concentration using H-FABP detection kits (Immune Turbidimetric Method, Registration Certificate #: National Food and Drug Administration (Jin) 2014 No. 2402142) from British Landau Laboratory Diagnostics Co., Ltd. The concentrations of the standard antigen samples were also confirmed by Guangzhou KingMed Diagnostics Group Co., Ltd, Guangzhou, China.

H-FABP test kits

The Raybio H-FABP test kit consists of a disposable test cassette and a re-usable Raybio TRF01 reader from Raybiotech Inc. (Guangzhou, China). The commercial H-FABP test kit consists of a disposable test reagent kit and an automated chemiluminescence analyzer from Shenzhen New Industry Biomedical Engineering Co., Ltd, (Guangdong Machinery Note 20182400517). The commercial clinically proven chemiluminescence-based test reagent kits were used to compare with Raybio H-FABP test kits.

General procedure for H-FABP measurements

For measurement using the Raybio H-FABP test kit, the Raybio TRF01 reader was turned on and stabilized for 5 minutes before use. An ID card associated with each lot of cassettes was inserted and activated. An appropriate amount of sample was diluted with a serum diluent. For plasma and serum samples, 100 µl of diluted sample was applied to the sample zone of the test cassette. The test cassettes were inserted into the TRF01 reader cassette holder for measurement.

For measurement using the commercial H-FABP test kit, the manufacturer's protocol was followed using the commercial H-FABP test reagents and the automatic chemiluminescence analyzer.

Linearity and dynamic range study

A series of diluted H-FABP antigen samples with concentrations of 0 ng/ml, 1.0 ng/ml, 2.7 ng/ml, 8.0 ng/ml, 27 ng/ml, and 60 ng/ml were prepared. For each prepared H-FABP antigen concentration, five cassettes from each of three lots of test cassettes from the Raybio H-FABP test kit were measured with the Raybio TRF01 reader. The signal for each test cassette was collected three times.

Specificity study

To evaluate potential interference of cholesterol, a series of samples were prepared containing 60 ng/ml H-FABP antigen and cholesterol at 10 mg/ml, 20 mg/ml, and 30 mg/ml. To evaluate potential interference of bilirubin, a series of samples were prepared containing 60 ng/ml H-FABP antigen and bilirubin at 1.0 mg/ml, 2.0 mg/ml, and 3.0 mg/ml. To evaluate potential interference of triglycerides, a series of samples were prepared containing 60 ng/ml H-FABP antigen and triglyceride at 30 mg/ml, 40 mg/ml, and 50 mg/ml. To test potential interference of rheumatoid factor (RF), a series of serum samples spiked with 60 ng/ml H-FABP antigen were prepared. Each contained different RF concentrations ranging from 100 IU/ml, 200 IU/ml, to 250 IU/ml. Each of these samples were measured 3 times with the Raybio H-FABP test kit.

To evaluate the impact of HAMA, a series of serum samples spiked with 60 ng/ml H-FABP antigen were prepared. Each contained different HAMA concentrations ranging from 75 ng/ml, 150 ng/ml, to 300 ng/ml. The H-FABP concentrations in each sample were measured 3 times with the Raybio H-FABP test kit.

Preparation of clinical samples

A total of 102 clinical samples (anonymous samples) were obtained from the Third Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China). The protocols for sample collection followed governmental ethical guidelines and respected each individual's right to confidentiality. The venous blood samples were

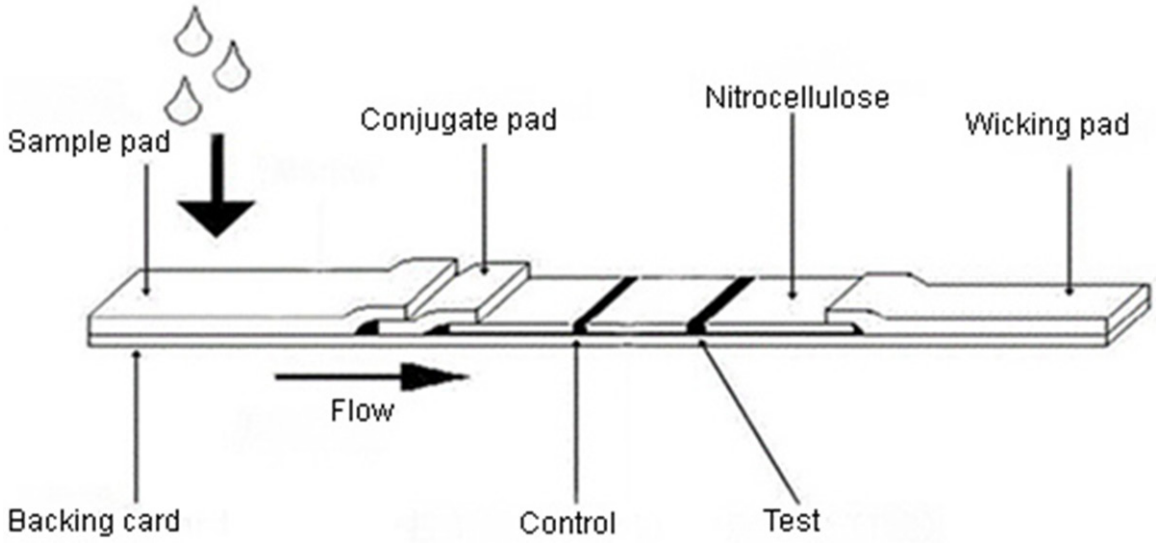

Figure 1. Schematic representation of the Raybio H-FABP cassette test strip.

taken by medical staff using a disposable syringe from a vein in the patient's arm. Whole blood samples were transferred to blood collection tubes with anticoagulants containing heparin, EDTA salt, and sodium citrate. The whole blood samples were centrifuged as soon as possible after blood collection to avoid hemolysis. The serum and plasma portions were separated, transferred to sterilized vials, and labeled for use. The serum and plasma samples were stored at -20°C when testing occurred more than 48 hours after collection.

Inclusion criteria for positive samples were cases that were clinically diagnosed to have coronary heart disease, heart failure, acute myocarditis, hypertension, and chest tightness. The inclusion criteria for negative samples were cases with normal heart function. Exclusion criteria are those with unclear clinical diagnosis, with insufficient specimens for testing, with severe hemolysis, with lipemia and jaundice.

Statistical analyses

The average values and standard deviations for each sample replicate included in the specificity study and limit of detection study were calculated using SPSS 20.0 software from International Business Machine (IBM). Paired sample correlation coefficient and Kappa coefficient analyses of the clinical sample comparison between the Raybio H-FABP test kit and the commercial test kit was also calculated using linear regression analysis with SPSS software. A p-value > 0.05 was considered significant for all analyses.

Results

Development of the Raybio H-FABP test kit

The Raybio H-FABP test kit consists of two major components: a consumable test kit and a time-resolved fluorescence reader (Raybio TRF01). The consumable test kits include a cassette and diluent. The technique utilized to develop the Raybio H-FABP test cassette has been widely described in the literature [22]. The schematic of the Raybio H-FABP cassette consisting of a test strip in a plastic cassette is shown in **Figure 1**. The Raybio H-FABP cassette uses europium-particle-based conjugates to provide a fluorescence signal with a long lifetime (about 500 microseconds). The europium particles have a maximum fluorescence peak at 615 nm and a maximum absorption peak at around 375 nm with a Stoke shift of 240 nm. An anti-H-FABP monoclonal antibody was immobilized in the test zone of the test strip. Europium particles were covalently attached to another anti-H-FABP monoclonal antibody and deposited on the conjugate pad. The anti-H-FABP monoclonal antibody in the test zone captures the europium conjugates in the presence of H-FABP in a sample. The control zone of the test strip was immobilized with a goat anti-chicken IgY antibody. Europium particles were

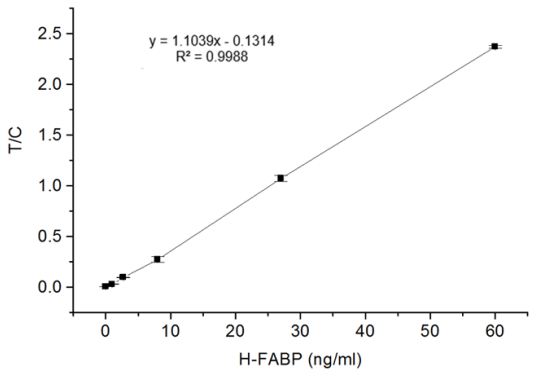

Figure 2. TRF signal (T/C) as a function of H-FABP concentrations. Error bars represent standard deviation for triplicate readings. Each sample was measured by three different cassettes from the same lot and the measured signal was averaged and standard deviations were calculated.

covalently attached to an anti-chicken IgY antibody which are also co-deposited on the conjugate pad. The goat anti-chicken IgY antibody in the control zone captures europium particles which attach to an anti-chicken IgY antibody in the control zone when the liquid sample flows through the control zone.

Linearity and dynamic range

Three different lots of Raybio H-FABP test cassettes were investigated to determine the test kit linearity and dynamic range. All 3 lots showed high linearity between the measured signals and the known H-FABP concentrations over a range from 0 to 60 ng/ml. **Figure 2** shows the TRF signals as a function of concentration for 1 of the 3 lots as an example. The other two lots showed a similar linear relationship between H-FABP concentration and TRF signals. The R^2 values for the 3 cassette lots were found to be 0.9988, 0.9993 and 0.9991, respectively. The standard coefficient of variation (CV) across the 3 lots was 6.2%.

Limit of detection for the assay

A series of diluted samples spiked with different concentrations of H-FABP were measured with the three different lots of the Raybio H-FABP test kit. Each diluted sample was measured with twenty test kits from each lot. The experimental data were averaged, and the standard deviation was calculated. The lowest H-FABP concentration for the diluted samples with an average experimental TRF signal equivalent to 2x the standard deviation of a H-FABP-free diluent blank is taken as limit of detection (LoD). The LoD for the three lots of test kits were 0.72 ng/ml, 0.68 ng/ml, and 0.73 ng/ml, respectively. Thus, the LoD of the Raybio H-FABP test kit was determined to be 0.73 ng/ml.

Assay precision

A series of serum samples were spiked with H-FABP to have the concentrations of 27 ng/ml and 8.0 ng/ml. These samples were used to assess the precision of the Raybio H-FABP test kit. Test kits from three lots were evaluated and each sample was measured with ten test kits from each lot. The CV across the three lots tested was 4.85%, 11.86%, and 5.59%, respectively for the 27 ng/ml samples, and 10.98%, 5.61% and 11.51%, respectively for the 8 ng/ml samples. Overall, the CV across the three lots of the test kits tested was < 12%, which is well within acceptable limits for the type of assay.

Assay accuracy

Three serum samples were spiked with H-FABP to have concentrations of 27 ng/ml, 8 ng/ml, and 2.7 ng/ml. These samples were used to evaluate the accuracy of the Raybio H-FABP test kit. Test kits from three lots were evaluated and each sample was measured in triplicate with each of the three test kit lots. The relative deviation for the concentration (B) was calculated by using the equation $B = (MT)/T \times 100\%$, where M is the average measured concentration and T is the true concentration of the sample. For the three lots, the results showed a relative deviation of 3.49%, 4.81% and 6.24%, respectively. Thus, the Raybio H-FABP test kit is capable of accurately quantifying the H-FABP concentration in serum samples.

Assay specificity

Potential interference from both small molecules and macromolecules was evaluated using H-FABP samples containing cholesterol, bilirubin, or triglycerides to test the impact of those small molecules on the assay specificity. The results of four samples for each small molecules or macromolecules were averaged and standard deviations were calculated. The

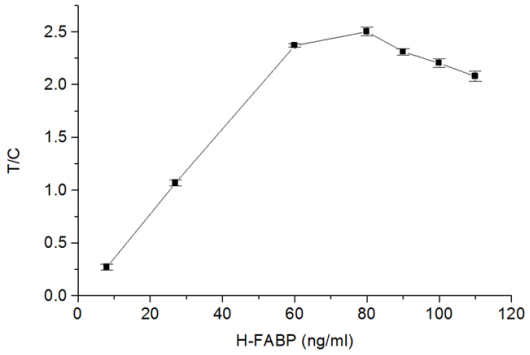

Figure 3. TRF signals vs. H-FABP concentrations in H-FABP antigen samples. Each sample was measured by three different cassettes from the same lot and the measured signal was averaged and standard deviations were calculated.

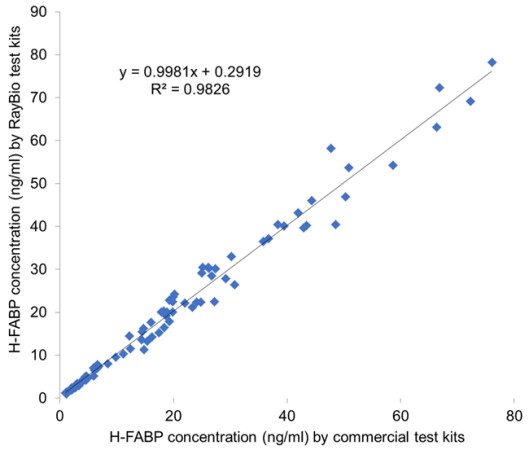

Figure 4. Correlation of the test results by Raybio H-FABP test kit vs commercial test kit.

results demonstrated that samples containing < 20 mg/ml cholesterol, < 2 mg/ml bilirubin, and < 40 mg/ml triglyceride had no impact on the test result with a relative deviation of < 15%.

H-FABP samples containing different concentrations of HAMA or RF factor were prepared to assess the impact of macromolecules on the assay specificity. The results showed that samples containing < 300 ng/ml HAMA and < 200 IU/ml RF factor had no impact on the test result with a relative deviation of < 15%.

Assay Hook effect

The Raybio H-FABP test kit utilizes a sandwich immunoassay principle that could potentially present a Hook effect and result in false negatives when the H-FABP concentration is high. To evaluate the Hook effect of the test kit, a series of H-FABP antigen samples with concentrations of 8.0 ng/ml, 27 ng/ml, 60 ng/ml, 80 ng/ml, 90 ng/ml, 100 ng/ml, and 110 ng/ml were prepared. Each sample was measured in triplicate using three test kits from the same lot. The average signals as a function of H-FABP concentration are illustrated in **Figure 3**. A Hook effect starts to occur when the concentration of H-FABP reaches > 80 ng/ml.

Table 1. Summary of test results of the Raybio H-FABP test kit and the commercial test kit

		Commercial Kit		total
		Positive	Negative	
Raybio Kit	Positive	61	2	63
	Negative	2	37	39
total		63	39	102

Evaluation of clinical samples

To validate the Raybio H-FABP test kit, commercial chemiluminescence immunoassay test kits from Snibe Diagnostic Co., Ltd were used for comparison with 102 clinical serum samples. The 102 serum samples were labeled randomly. The H-FABP concentrations of 39 negative serum samples and 63 positive serum samples were measured with both the commercial test kit and the Raybio H-FABP test kit at the same time. A regression equation of Y = 0.9981X + 0.2919 was obtained with a correlation coefficient of 0.9826 from the correlation of the results from the commercial test kit and the Raybio H-FABP test kit (**Figure 4**). T/C signals measured by Raybiotech kits are +0.29 higher than T/C signals measured by the commercial kits for the same samples. Based on the regression equation, the relative standard deviation was calculated to be 3.98% (relative to the reference value of 6.29 ng/ml). There was a significant correlation ($p < 0.01$) between the measured concentrations of the Raybio H-FABP test kit and the commercial test kit.

A concentration of 6.29 ng/ml of H-FABP was used as a reference value for the Raybio H-FABP test kit and 6.00 ng/ml for the commercial test kit. **Table 1** summarizes the diagnoses of the patient samples using the Raybio H-FABP test

Table 2. Details of four samples with false positives or false negative results compared with the clinical diagnosis using the Raybio H-FABP test kit or the commercial test kit

Sample #	gender	age	clinical diagnosis	Test result (ng/ml)	
				Commercial kits	Raybio kits
38	male	78	Coronary Heart Disease	5.97 (-)	7.01 (+)
46	male	70	Heart failure/pneumonia	6.00 (+/-)	5.23 (-)
68	male	43	Coronary Heart Disease	5.95 (-)	7.02 (+)
96	Female	85	Heart failure/pneumonia	6.15 (+)	6.24 (-)

(+): positive result for the Raybio H-FABP test kit or the commercial kit. (-): negative result for the Raybio H-FABP test kit or the commercial kit. (+/-): inconclusive result for the commercial kit.

kit and the commercial test kit in comparison with the clinical diagnosis. Among the 102 samples, there were 2 false negative results with the Raybio H-FABP test kit while there were 2 false negatives and 1 inconclusive result with the commercial kit (**Table 2**). The measured concentrations of H-FABP for the four falsely resulted samples are very close to the reference value of 6.29 ng/ml, which may explain the false positive or false negative results.

The results show a 96.83% positive correlation with a 95% confidence interval from 92.50% to 100% and a 94.87% negative correlation with a 95% confidence interval of 87.95% to 100%. The overall correlation for both positives and negatives was calculated to be 96.08% with a 95% confidence interval from 92.31% to 99.85%.

SPSS 20.0 software from International Business Machine (IBM) was used to analyze the data to obtain paired sample correlation coefficient (**Table 3**). Linear regression analysis method was used. The *p*-value = 0.270, which is > 0.05, indicating no significant difference between the two sets of data from the two test kits.

SPSS software was also used to calculate the Kappa coefficient based on the measured values for all 102 samples across both test kits. The Symmetric Measures are shown in **Table 4**. The high Kappa value of 0.917 suggests the results from the Raybio H-FABP test kit and the commercial test kit have very good agreement.

Discussion

In almost all lateral flow immunochromatographic assay devices reported in the literature, the control (or calibration) zones were generally placed downstream of the sample flow following the detection zone. This makes sense since most of the reported devices are for the qualitative detection of analytes. The control zone helps users determine if an adequate amount of sample has been applied to allow flow through the whole device for an accurate reading. However, our research has found that placing the control zone (calibration zone) upstream of the detection zone provides more consistent and reliable results when the control zone signals are needed for internal signal calibration for the detection zone. This is important for analyte quantification and is why the layout of the Raybio H-FABP kit reported here is different from previously developed lateral flow immunochromatographic assay cassettes. One limitation of this layout is that the control zone will not be able to assure users of adequate sample loading, introducing a potential source of error and inaccuracy.

The time-resolved fluorescence detection technique was used in the developed Raybio H-FABP test kit. Unlike the conventional wavelength resolved fluorescence detection technique which uses continuous light for exciting fluorescence probes, the time-resolved fluorescence detection technique uses pulsed light for exciting fluorescence probes. In general, the fluorescence signal is much lower than the excitation light for both conventional and time-resolved fluorescence detection techniques. The excitation spectra of most viable fluorescence probes have significant overlap with the fluorescence spectra with a relatively small Stoke shift. Those two factors make complete separation of a relatively weak fluorescence signal from the intense excitation light almost impossible by wavelength difference through optical filters. The incomplete elimination of excitation background is the main reason for the limited detection sensitivity of conventional fluorescence detection techniques. In addition, other broad background light is also negatively added to background noise which results in low signal/noise ratios. However, more efficient elimination of excitation background light and broad background noise can be possible by delayed

Table 3. t-test results for the paired samples

Pairwise difference							
Mean	Standard deviation	Standard error of the mean	95% confidence interval of difference		t	Degree of freedom	Sig. (2-sided)
			Lower Limit	Upper Limit			
-0.25931	2.36064	0.23374	-0.72299	0.20436	-1.109	101	0.270

Table 4. Symmetric Measures in the output of Cohen's Kappa statistical analysis

		Value	Asymp. Std Error[a]	Approx T[b]	Approx Sig.
Measure of agreement	Kappa	.917	.041	9.261	.000
# of valid cases		102			

a. Not assuming the null hypothesis. b. Using the asymptotic standard error assuming the null hypothesis. Asymp. Std. Error: Asymptotic standard error. Approx Sig: Approximate significance. Approx T: Approximate T.

measurement of fluorescence signals if the fluorescence signal has a much longer lifetime than the excitation light and background light. Almost all the photons and scattered photons generated from lasers, LEDs, and lamps have very short lifetimes of < 50 ns. Alternatively, the fluorescence lifetime of some europium chelates and palladium chelates can reach 500,000 ns. This makes europium or palladium chelates or their particles ideal for time-resolved fluorescence detection and the ability to achieve high signal/noise ratios for high detection sensitivity. Furthermore, europium and palladium chelates and particles containing those chelates have a large Stoke shift of > 150 nm, which further facilitates effective wavelength-based optical separation of fluorescence signals from excitation and background noises by cheap cut-off optical filters.

The integration of two different signal separation techniques into one detection platform makes the Raybio H-FABP test kit particularly sensitive and reliable because it cannot only eliminate the excitation background to improve sensitivity but also remove the autofluorescence from sample matrices to achieve more accurate results. The limit of detection for the test kit is predominantly limited by the binding strength of the antibody pairs rather than the detection platform. Further improvement could be achieved in the limit of detection and specificity of the assay if better antibody pairs could be developed.

The Raybio H-FABP test kit integrates the time-resolved fluorescence detection technique and the lateral flow immunoassay technique into one detection platform. The cost to build the reader is less (in general, < $500 per reader) than the cost to construct a conventional fluorescence reader (in general, > $1000). This is because very expensive band-pass optical filters are needed for the conventional fluorescence detection technique to effectively reject background noises while cheap cut-off optical filters are good enough for the time-resolved fluorescence detection technique because time-resolved detection can further eliminate the noise passed through the cut-off optical filters. The cost for time-resolved fluorescence detection is very low due to the use of cheap on-the-shelf electronics widely available.

The Raybio H-FABP test kit not only has a low LoD, high accuracy, good precision, and high specificity, but also costs less to manufacture compared to currently available assays, because of the integration of two low-cost detection techniques into one detection platform.

Conclusions

In this study, a comprehensive performance assessment of the Raybio H-FABP test kit compared with a conventional fluorescence detection kit was conducted. The Raybio H-FABP test kit exhibited a LoD of 0.72 ng/ml, which was significantly lower than the clinically relevant cut-off concentration of 7.0 ng/ml for H-FABP. It also showed a decent linear detection dynamic range from 0 to 60 ng/ml. The test kit showed high detection accuracy (relative deviation of < 7%) and precision (relative standard deviation of less than 12%) for the various concentrations measured. The Raybio H-FABP test kit also demonstrated high specificity with minimal interference from both small molecules

and macromolecules at clinically significant concentrations. The performance results of the Raybio H-FABP test kit showed excellent correlation for 102 random and blinded clinical samples with the results of a commercial chemiluminescence-based test kit. These data suggest the Raybio H-FABP test kit can potentially be used similarly to the commercial test kit for early detection of myocardial damage. In comparison to the commercial chemiluminescence-based test kit, the Raybio H-FABP test kit is cheaper, smaller, and can provide results faster. Thus, it can also have potential applications in point-of-care and emergency room settings.

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Disclosure of conflict of interest

None.

Abbreviations

TRF-LFA, time-resolved fluorescence lateral flow immunochromatographic assay; H-FABP, Heart-type fatty acid binding protein; LoD, limit of detection; AMI, acute myocardial infarction; ELISA, enzyme-linked immunoassay; ELICA, electrochemiluminescence; TRF, time-resolved fluorescence; EDTA, Ethylenediaminetetraacetic acid; Asymp. Std. Error, Asymptotic standard error; Approx. Sig., Approximate significance; Approx. T, Approximate T.

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