

---

## Supplementary materials

### **A Point-of-Care Test based on Selenium Nanoparticles for Heart-Type Fatty Acid-Binding Proteins in Human Serum and Blood**

Lanju Wang <sup>1,2</sup>

Mengli Wu <sup>2</sup>

Jingjing Ma <sup>2</sup>

Ziwei Ma <sup>2</sup>

Jiahui Liang <sup>2</sup>

Ningya Tao <sup>2,4</sup>

Yangguang Ren <sup>3</sup>

Shujun Shao <sup>1</sup>

Xin Qi <sup>1</sup>

Zhizeng Wang <sup>2</sup>

<sup>1</sup>Department of Blood Transfusion, the Affiliated Tumor Hospital of Zhengzhou University, Zhengzhou, 450008, Henan, China; <sup>2</sup>Joint National Laboratory for Antibody Drug Engineering, Clinical Laboratory of the First Affiliated Hospital, School of Medicine, Henan University, Kaifeng 475004, China; <sup>3</sup>Breast and Thyroid Surgery, Huaihe Hospital of Henan University, Kaifeng 475004, China; <sup>4</sup>Pingyu Health School, Zhumadian, 463400, China.

Lanju Wang, Mengli Wu and Jingjing Ma contributed equally to this work.

Correspondence: Xin Qi and Zhizeng Wang

Xin Qi: Department of Blood Transfusion, the Affiliated Tumor Hospital of Zhengzhou University, Zhengzhou, 450008, China; Zhizeng Wang: Joint National Laboratory for Antibody Drug Engineering, Clinical Laboratory of the First Affiliated Hospital, School of Medicine, Henan University, Kaifeng, 475004, Henan, China.

Tel: +86 15038239612; +86 15093628687

E-mail: zlyyqixin4215@zzu.edu.cn; wzhzeng@126.com

---

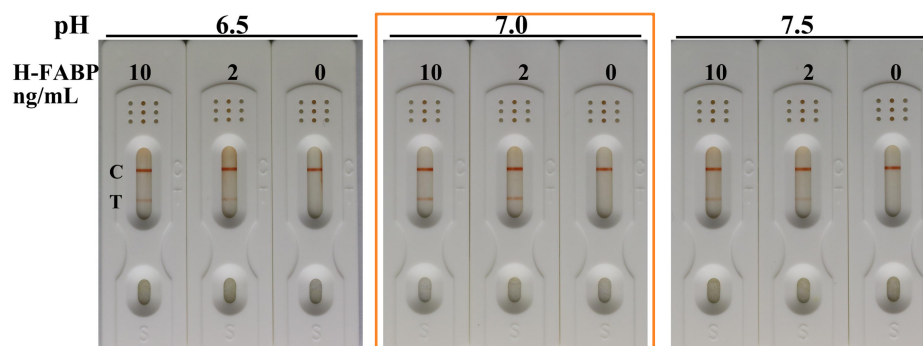
## Contents

1	pH selected results of conjugating conditions for Figure 3	S3
2	Concentration of anti-H-FABP-mAb1 conjugate with selenium nanoparticle for Figure 3D	S4
3	Selected results of coating concentration of test line	S5
4	Result of specificity of the H-FABP test kit for Figure 5A	S6
5	Result of stability of the H-FABP test kit for Figure 5B	S7
6	Basic information of contributors for Figure 6 and Table 1	S8
7	Basic information of samples and H-FABP content of sample for Figure 6	S10
8	Detection results of 16 plasma samples by this kit for Figure 6A and Figure 6B	S13
9	Detection results of 30 blood samples by this kit for Figure 6C and Figure 6D	S14

---

## 1. pH selected results of conjugating conditions for Figure 3C

Optimal pH result of the selenium nanoparticles kit for H-FABP detection was determined by different concentrations ( Figure 1 ) for Figure 3C of manuscript.



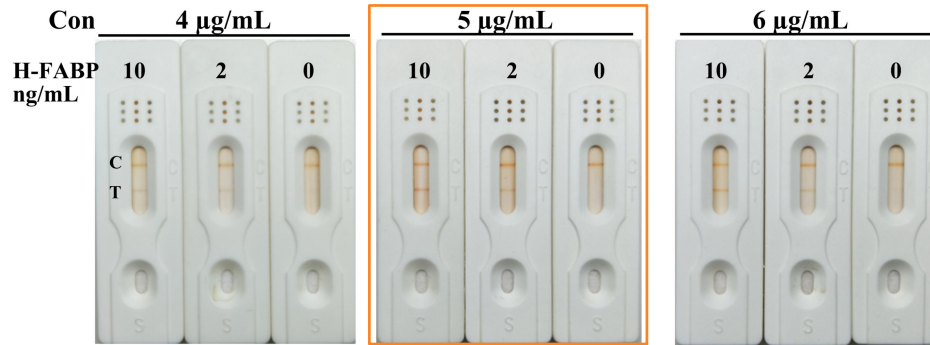
**Supplemental Figure 1 Optimal pH of the H-FABP kit. Detection results at different pH values by H-FABP kits based on selenium nanoparticle.**

H-FABP: Heart-type fatty acid binding protein; C: Control line; T: Test line; Orange box: Best color rendering results.

---

## 2. Concentration of anti-H-FABP-mAb1 conjugate with selenium nanoparticle for Figure 3D

Optimal concentration result of the anti-H-FABP-mAb1 conjugate with selenium nanoparticle was determined by different concentrations ( Figure 2 ) for Figure 3D of manuscript.

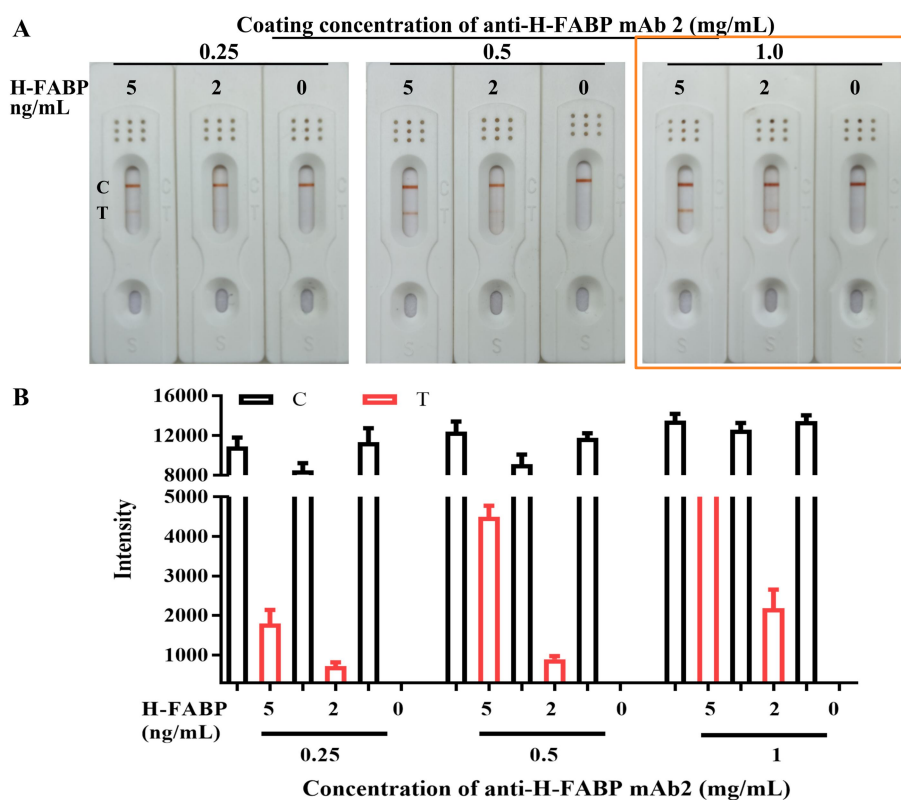


**Supplemental Figure 2 Optimal labelling concentration of the H-FABP kit. Detection results at different concentrations by H-FABP kits based on selenium nanoparticle.**

H-FABP: Heart-type fatty acid binding protein; Con: Concentration; C: Control line; T: Test line;  
Orange box: Best color rendering results.

### 3. Selected results of coating concentration of test line

Different concentrations (0.25, 0.5 and 1.0 mg/mL) of anti-H-FABP mAb 2 was sprayed on test line, according to conjugating condition by supplemental Figure 1 and supplemental Figure 2. There were no false positive results for any of the three conditions, however, under the condition of 0.25 and 0.5 mg/mL, the orange development of the test line is weak (Figure 3A, 3B ) by the naked eye and GraphPad Prism, which may affect the sensitivity of the kit. Figure 3B is a summary produced by GraphPad Prism of the interpretation results of 3 different persons by ImageJ. Therefore, 1.0 mg/mL was selected as the coating concentration of anti-H-FABP mAb 2.

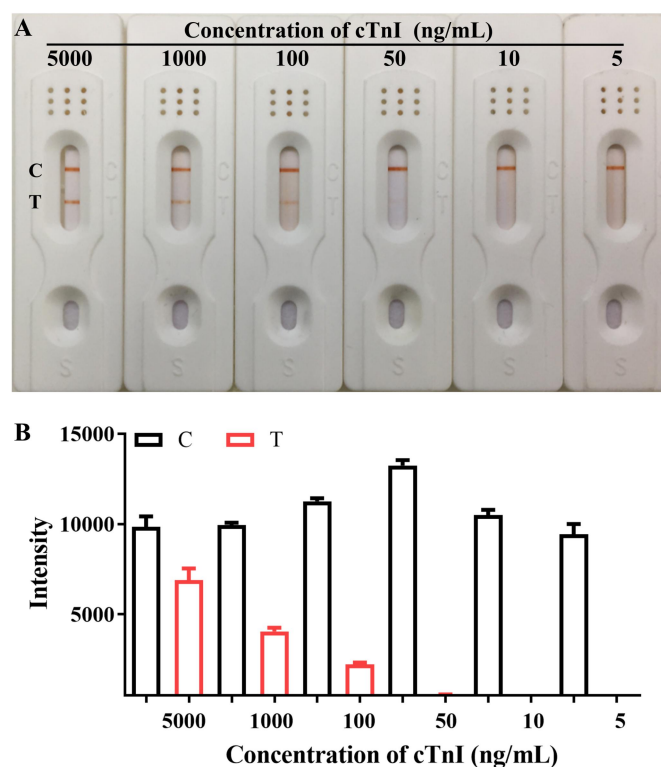


**Supplemental Figure 3 Detection results for H-FABP with different coating concentration of anti-H-FABP mAb 2.**

H-FABP: Heart-type fatty acid binding protein; C: Control line; T: Test line. Orange box: Result for sensitivity detection.

#### 4. Detection results of specificity for cTnI by H-FABP kit

Different concentration (5000, 1000, 100, 50, 10, 5 ng/mL) of cTnI were tested by this H-FABP kit, we found that cross reaction was appeared with high concentration (cTnI  $\geq$  50 ng/mL) of cTnI (Figure 4A, 4B) by the naked eye and GraphPad Prism. Figure 4B is a summary produced by GraphPad Prism of the interpretation results of 3 different persons by ImageJ. This concentration of cTnI is much higher than the clinical reference value according to cTnI to determine acute myocardial infarction (AMI), and does not affect the interpretation of results of the kit for AMI.



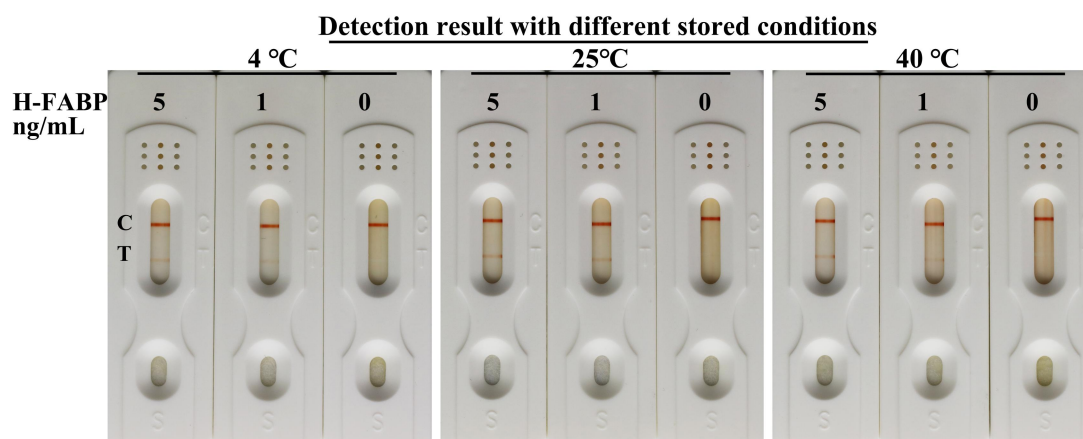
**Supplemental Figure 4 Detection results for cTnI of H-FABP kit based on selenium nanoparticle.**

H-FABP: Heart-type fatty acid binding protein; C: Control line; T: Test line. cTnI: Cardiac troponin I.

---

## 5. Result of stability of the H-FABP test kit for Figure 5B

Stability test result of the H-FABP kit was determined by different storage conditions (4°C, 25°C and 40°C) by naked eyes ( Figure 5 ) for Figure 5C of manuscript.

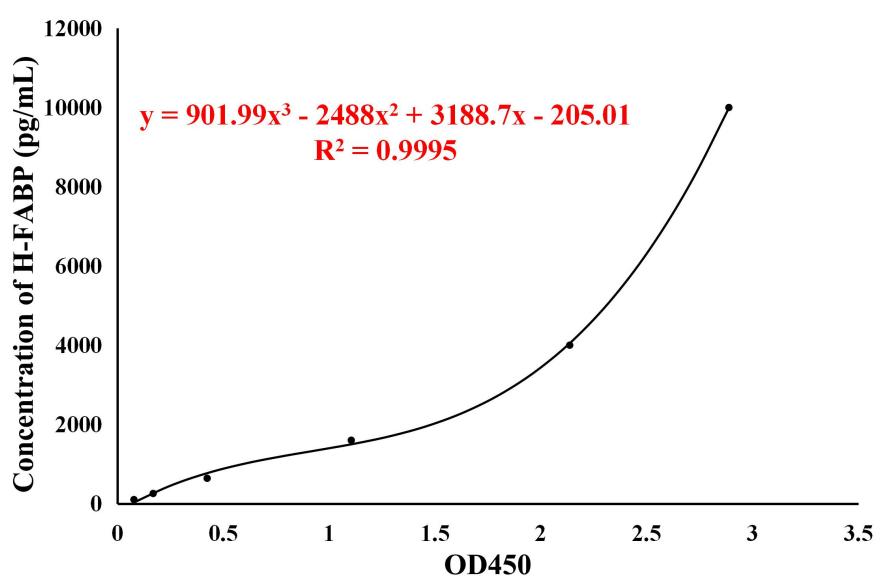


**Supplemental Figure 5 The stability of the H-FABP kit.**

Detection results for H-FABP observed by the naked eyes C: Control line; T: Test line; H-FABP: Heart-type fatty acid binding protein.

## 6. Basic information of fitting curve and OD<sub>450</sub> for table 1

Different concentrations (0, 102, 256, 640, 1600, 4000 and 10000 pg/mL) standard were detected with H-FABP ELISA kit (Hycult Biotech, Catalog number: HK401; Lot number:19750K0517-Z). The fitting curve was shown in Figure 6. The OD values of H-FABP detection with 0.5 ng/mL and 1.0 ng/mL of 3 tests are shown in supplemental Table 1, the OD<sub>450</sub> of different concentration standard was shown in supplemental table 1 for fitting curve.



**Supplemental Figure 6 Fitting curve for H-FABP**

H-FABP: Heart-type fatty acid binding protein.

**Supplemental Table 1 OD<sub>450</sub> of recovery experiment for H-FABP**

Standard (ng/mL)		OD <sub>450</sub>		
		1	2	3
H-FABP	0.5	0.2415	0.2516	0.2384
	1.0	0.5391	0.5461	0.5078



---

**Supplemental Table 2 OD450 of different concentration of H-FABP for fitting curve**

<b>Concentration of standard H-FABP (pg/mL)</b>	<b>OD450</b>
10000	2.8886
4000	2.1368
1600	1.1049
640	0.4243
256	0.1692
102	0.0783
0	0.0437

---

---

## 7. Basic information of samples and H-FABP content of sample for Figure 6

Basic informations of 46 clinical samples were shown in Table 3 and Table 4. 16 plasma and 30 blood samples were detected by ELISA kit, and the OD<sub>450</sub>, content of H-FABP were shown in Table 2; H-FABP concentration is calculated according to fitting curve of supplemental figure 6.

**Supplemental Table 3 Basic information of plasma sample and detected result by ELISA and this kit**

No.	Sex	Age	Sample	OD <sub>450</sub>	Adjusted concentration by ELISA (ng/mL)	Result by this Kit (P/N)	Notes
1	Male	50	Plasma	0.9351	2.0689	P	
2	Female	45	Plasma	1.9983	5.4252	P	
3	Male	54	Plasma	2.8051	14.3298	P	
4	Female	58	Plasma	0.049	0.1094	N	
5	Male	53	Plasma	0.1006	0.3132	N	
6	Male	55	Plasma	0.5574	1.5149	P	
7	Female	49	Plasma	0.08	0.2339	N	
8	Male	71	Plasma	1.5232	3.2326	P	
9	Female	64	Plasma	0.085	0.2534	N	
10	Male	67	Plasma	0.2937	0.9364	N	
11	Female	54	Plasma	0.1051	0.3302	N	
12	Female	63	Plasma	0.3555	1.0959	P	
13	Male	54	Plasma	0.1629	0.5371	N	
14	Male	52	Plasma	0.0787	0.2288	N	
15	Female	37	Plasma	0.3303	1.0329	P	

16	Female	55	Plasma	0.1652	0.5449	N	
----	--------	----	--------	--------	--------	---	--

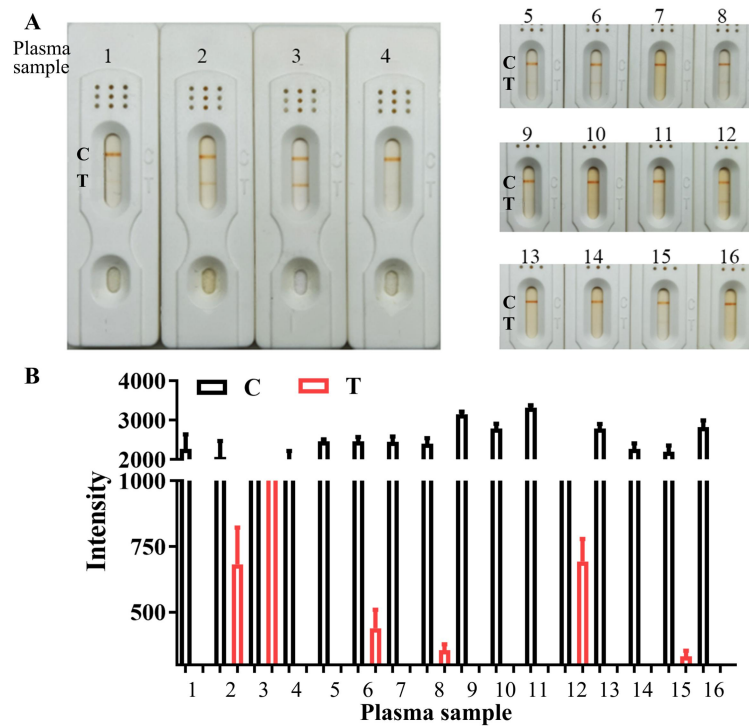
**Supplemental Table 4 Basic information of blood sample and detected result by ELISA and this kit**

No.	Sex	Age	Sample	OD <sub>450</sub>	Adjusted concentration by ELISA (ng/mL)	Result by this Kit (P/N)	Notes
1	Male	55	Blood	0.4293	1.2655	P	
2	Female	57	Blood	0.2841	0.9101	N	
3	Male	65	Blood	0.1961	0.6471	N	
4	Female	68	Blood	0.0624	0.1640	N	
5	Female	62	Blood	0.0902	0.2735	N	
6	Male	55	Blood	0.0969	0.2992	N	
7	Female	47	Blood	0.8944	2.0136	P	
8	Male	45	Blood	0.0692	0.1913	N	
9	Female	27	Blood	0.5655	1.5291	P	
10	Male	84	Blood	0.4658	1.3418	P	
11	Female	63	Blood	0.2632	0.8512	N	
12	Female	53	Blood	0.1426	0.4667	N	
13	Female	68	Blood	0.658	1.6803	P	
14	Female	58	Blood	0.6056	1.5969	P	
15	Female	58	Blood	3.1295	20.5460	P	
16	Male	61	Blood	0.3736	1.1395	P	
17	Female	68	Blood	0.1854	0.6123	N	
18	Female	57	Blood	0.0934	0.2858	N	
19	Male	45	Blood	0.1985	0.6548	N	

20	Female	44	Blood	0.2779	0.8928	N	
21	Male	30	Blood	0.3833	1.1623	P	
22	Female	68	Blood	0.1598	0.5265	N	
23	Male	49	Blood	0.3035	0.9628	N	
24	Male	68	Blood	0.2234	0.7330	N	
25	Male	70	Blood	0.1735	0.5729	N	
26	Female	50	Blood	0.1666	0.5496	P	False positive
27	Female	44	Blood	0.2791	0.8962	N	
28	Male	58	Blood	0.0322	0.0393	N	
29	Male	83	Blood	0.3298	0.9125	N	
30	Male	83	Blood	0.1613	0.5316	N	

## 8. Detection results of 16 plasma samples by this kit for Figure 6A and Figure 6B

The detection results of plasma samples are shown in supplemental Figure 7. A total of 16 specimens, of which 6 were positive for H-FABP and 10 were negative by ELISA, according to 1ng/mL as the reference value. There were 6 positive results, 10 negative results and no false result (Figure 7A, 7B). Figure 7B is a summary produced by GraphPad Prism of the interpretation results of 3 different persons by ImageJ. The number 1-5 samples of manuscript in Figure 6A are 1-4, 12 in supplemental Figure 7.

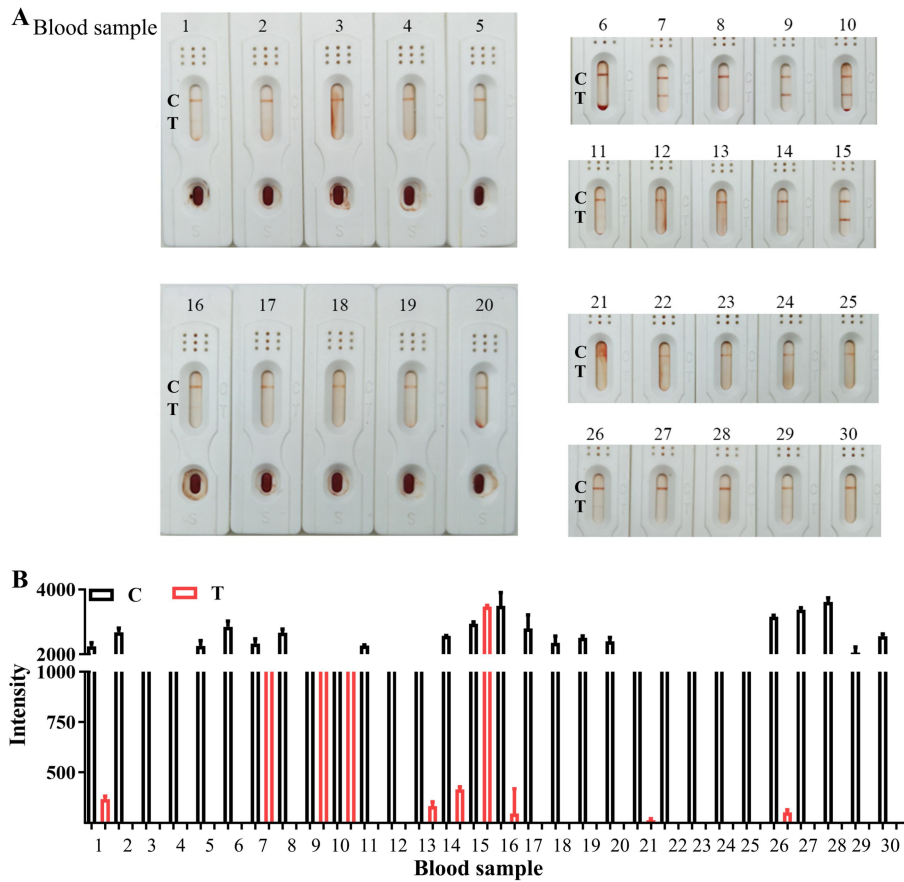


**Supplemental Figure 7 Detection results of 16 plasma samples using this kit for H-FABP.**

C: Control line; T: Test line; H-FABP: Heart-type fatty acid binding protein.

## 9. Detection results of 30 blood samples by this kit for Figure 6C and Figure 6D

The detection results of blood samples are shown in supplemental Figure 8. A total of 30 specimens, of which 9 were positive for H-FABP and 21 were negative by ELISA, according to 1ng/mL as the reference value. There were 10 positive results, 1 false positives (number 21) and no false negative. Figure 8B is a summary produced by GraphPad Prism of the interpretation results of 3 different persons by ImageJ. The number 1-5 samples of manuscript are 6-10 in supplemental Figure 8.



**Supplemental Figure 8 Detection results of 30 blood samples using this kit for H-FABP.**

C: Control line; T: Test line; H-FABP: Heart-type fatty acid binding protein.