#### **Supplemental materials**

Development of an Effective Neutralizing Antibody Assay for SARS-CoV-2 Diagnosis

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#### 1. Full image of colloidal gold for Figure 2B

Colloidal gold is rose-red, and the supernatant of colloidal gold is clear and translucent after 10000 rpm×10 min; and the colloidal gold after centrifugation can be fully resuspended. In addition, both before and after centrifugation, a red laser can pass through the colloidal gold solution, indicating that the colloidal gold has a good Tyndall effect. The full image is shown in Figure 1 for Figure 2B in manuscript.



Figure 1 Appearance of colloidal gold before and after centrifugation. Au-NPs: colloidal gold nanoparticles.

#### 2. Selection of colloidal gold concentration

Initially, 0.25 mM colloidal gold was used for kit preparation, and although it successfully labeled ACE2 protein, the color development of even the control band C line was relatively weak (Figure 2). Finally1.00 mM colloidal gold was selected for the preparation of the kit according to color rendering of the T line and C line.



Figure 2 Detection result of this assay kit by 0.25 mM colloidal gold. C: control line; T: test line.

#### 3. Full image of conjugating pH selection for Figure 3B

pH is an important condition affecting the conjugating effect of protein and colloidal gold. Colloidal gold is conjugated to recombinant protein ACE2 by non-covalent bonds, and four different pH (6.0, 6.5, 7.0 and 8.0) are experimentally compared on the basis of the results of the color reaction, we found that color rendering works best when the pH is 6.5 by detecting negative sample (Figure 3), and the full image is shown in Figure 2 for Figure 3B in manuscript.



Figure 3 Full image of pH selection. Au-NPs: colloidal gold nanoparticles; ACE2: angiotensin converting enzyme 2; C: control line; T: test lin.

# 4. Full image of ACE2 conjugating concentration of colloidal gold for Figure 3D and Figure 3E

The labeling concentration of ACE2 affects the stability of the conjugated complex and limit of detection of lateral flow kit. Two different concentration (6  $\mu$ g/mL, Figure 4A and 8 $\mu$ g/mL, Figure 4B) are experimentally compared based on the results of the color reaction, we found that the immune response elicited by the 6  $\mu$ g/mL was more effective. The full image is shown in figure 4 for Figure 3 D and Figure 3E in manuscript.



Figure 4 Full image of ACE2 conjugating concentration of colloidal gold. Con.: concentration; NAb: neutralizing antibody; ACE2: angiotensin converting enzyme 2; N: negative; C: control line; T: test line; Red arrow: Indicates a colour result.

#### 5. Selection of BSA concentration for blocking reagents

The effectiveness of BSA with a final concentration of 1% and 2% as a blocking reagent was compared, the results show that when the BSA concentration is 2%, the LOD of this kit and the effect is better than 1% BS A(Figure 5A, 5B). Therefore, a 2% BSA concentration was selected in the following study.



Figure 5 Detection results of this kit with different BSA concentration. Con.: concentration; C: control line; T: test line.

## 6. Full image of coating concentration of RBD in test line selection for Figure 3G, Figure 3H, Figure 3I and Figure 3J

The coating concentration of test line has a significant regulatory effect on the limit of detection of the lateral flow kit. Four different concentrations of RBD (0.4 mg/mL Figure 6A, 0.2 mg/mL Figure 6B, 0.08 mg/mL Figure 6C and 0.04 mg/mL Figure 6D) are experimentally compared based on the above pH and concentration of ACE2-AuNPs, the condition of 0.08 mg/mL is optimal by comparing the detection effect of different concentrations of neutralizing antibody. The full image is shown in Figure 4 for Figure 3G, Figure 3H, Figure 3I and Figure 3J in manuscript.



Figure 6 Full image of coating concentration of RBD in test line selection. Con.: concentration; NAb: neutralizing antibody; RBD: receptor binding domain; N: negative; C: control line; T: test line.

#### 7. Limit of detection of lateral flow kit for NAb of SARS-CoV-2

Anti-SARS-Cov-2 NAb (1 mg/mL) were diluted to different concentrations with negative serum and added to the kit for the LOD. Finally, the LOD of NAb in serum was determined to be  $2 \mu g/mL$  by the naked eye and the full image was shown in Figure 7A for Figure 4A in manuscript.

Another NAb is extracted and purified from a patient, diluted to different concentrations for LOD detection. Finally, the LOD is still 2  $\mu$ g/mL by the naked eye (Figure 7B) and GraphPad Prism (Figure 7C), and the R<sup>2</sup> of fitted curve for NAb detection was 0.9698 (Figure 7C). These results indicate good stability in the LOD detection.



Figure 7 Limit of detection of the lateral flow kit used for different NAb detection in human serum. C.: concentration; NAb: neutralizing antibody; N: negative; C: control line; T: test line.

#### 8. Full image of specificity detection of lateral flow kit

The experiment of kit-specificity was performed to avoid the cross-reaction of the kit with other proteins or antibody, generating in false-positive results. Full image was shown in Figure 8 for Figure 4B in manuscript.



Figure 8 The kit of specificity detection results. C: control line; T: test line; Anti-N IgG: anti-nucleoprotein of SARS-CoV-2 immunoglobulin G; Anti-N IgM: anti-nucleoprotein of SARS-CoV-2 immunoglobulin M; H1N1: hemagglutinin 1 neuraminidase 1; ANA: antinuclear antibody; RF: rheumatoid factor; +: positive.

#### 9. Stability detection of lateral flow kit for NAb of SARS-CoV-2

After lateral flow kits were stored in a dry and dark environment at 37°C for 30 days, they were used for stability assays. Full image was shown in Figure 9 for Figure 4C in manuscript. In addition, LOD detection were also performed for kits stored at different time points (1 week, 2 week, 3 week and 4week), All analyses were completed within 15 minutes. The LODs of the lateral flow kits for NAb were 2  $\mu$ g/mL (Figure 10), the same as the previous test results. No false positive results were found in any of the tests.



Figure 9 Full image of stability detection of lateral flow kit. Con.: concentration; NAb: neutralizing antibody; N: negative; C: control line; T: test line; Red arrow: Indicates a colour result.



Figure 10 Stability detection of the lateral flow kit at different points in time. NAb: neutralizing antibody; N: negative; C: control line; T: test line.

### 10 Basic information and detection results of contributors for Figure 5 and Table 1

The clinical samples, which from the first affiliated hospital of Henan university and school of medicine of Henan university, were detected by ELISA or this research kit. A total of 684 specimens, including 311 pre-immunization samples, 170 samples 21 days after the first vaccination, 182 samples 21 days after the second vaccination for verification and 21 samples 6 months after the second vaccination. The basic information of all the contributors are shown in Table 2-Table 5.

#### **10.1 Basic information and detection results of pre-immunization the contributors**

The 311 samples included 5 cases under 20 years old, 173 cases between 20 and 39 years old and 133 cases between 40 and 59 years old. The basic information and detection results by ELISA or this kit of pre-immunization the contributors are shown in Table 1.

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
1	male	23	11.53%	-	
2	female	21	12.45%	-	
3	male	39		-	
4	female	32		-	
5	male	44		-	
6	male	42		-	
7	female	31		-	
8	female	25	8.79%	-	
9	female	44		-	
10	female	32		-	
11	male	51		-	
12	male	27	8.72%	-	
13	male	40		-	
14	female	48		-	
15	male	42		-	
16	male	50		-	

 Table 1 The basic information and detection results by

 ELISA or this kit of pre-immunization the contributors.

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
17	male	40		-	
18	female	44		-	
19	female	34		-	
20	female	44		-	
21	female	33		-	
22	male	30		-	
23	female	23	12.49%	-	
24	male	47		-	
25	male	26	11.23%	-	
26	female	54		-	
27	female	40		-	
28	male	55		-	
29	female	36		-	
30	male	30		-	
31	female	43		-	
32	male	52		-	
33	male	42		-	
34	male	31		-	
35	male	25	13.16%	-	
36	female	44		-	
37	male	53		-	
38	female	36		-	
39	female	49		-	
40	female	33		-	
41	male	50		-	
42	male	35		-	
43	male	33		-	
44	female	30		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
45	female	45		-	
46	female	44		-	
47	female	32		-	
48	male	37		-	
49	male	47		-	
50	male	27	6.05%	-	
51	male	54		-	
52	male	42		-	
53	male	31		-	
54	female	25	9.97%	-	
55	male	44		-	
56	male	53		-	
57	male	36		-	
58	female	49		-	
59	female	41		-	
60	female	50		-	
61	female	35		-	
62	male	42		-	
63	male	47		-	
64	male	32		-	
65	male	53		-	
66	female	26	10.33%	-	
67	male	48		-	
68	female	38		-	
69	female	31		-	
70	female	33		-	
71	male	37		-	
72	male	31		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
73	male	18		-	
74	male	26	9.84%	-	
75	male	36		-	
76	female	44		-	
77	female	29		-	
78	male	56		-	
79	female	31		-	
80	female	50		-	
81	female	42		-	
82	male	33		-	
83	male	39		-	
84	female	48		-	
85	male	28	9.38%	-	
86	female	33		-	
87	male	22	15.27%	-	
88	female	30		-	
89	female	32		-	
90	female	42		-	
91	female	31		-	
92	female	25	6.47%	-	
93	female	44		-	
94	female	32		-	
95	male	51		-	
96	male	27	9.84%	-	
97	male	40		-	
98	male	48		-	
99	female	42		-	
100	female	50		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
101	male	40		-	
102	female	44		-	
103	female	34		-	
104	male	44		-	
105	female	33		-	
106	male	30		-	
107	male	23	11.76%	-	
108	male	33		-	
109	male	37		-	
110	female	37		-	
111	male	35		-	
112	female	29		-	
113	female	28	9.23%	-	
114	female	34		-	
115	male	44		-	
116	male	21	16.17%	-	
117	male	29		-	
118	female	46		-	
119	female	32		-	
120	female	49		-	
121	female	46		-	
122	male	35		-	
123	male	27	8.20%	-	
124	male	27	8.09%	-	
125	male	40		-	
126	male	48		-	
127	male	42		-	
128	male	50		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
129	male	40	~,	-	
130	male	44		-	
131	male	34		-	
132	male	44		-	
133	male	33		-	
134	male	30		-	
135	female	23	13.70%	-	
136	female	47		-	
137	male	21	16.79%	-	
138	female	31		-	
139	female	39		-	
140	female	36		-	
141	male	28	6.20%	-	
142	male	23	12.83%	-	
143	female	34		-	
144	female	48		-	
145	female	36		-	
146	male	46		-	
147	female	22	14.20%	-	
148	male	41		-	
149	male	43		-	
150	male	29		-	
151	female	34		-	
152	female	21	17.10%	-	
153	male	23	10.96%	-	
154	female	36		-	
155	male	48		-	
156	female	47		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
157	female	46		-	
158	female	22	13.21%	-	
159	female	21	14.00%	-	
160	female	39		-	
161	female	32		-	
162	female	44		-	
163	male	42		-	
164	male	31		-	
165	male	25	8.71%	-	
166	male	44		-	
167	female	32		-	
168	female	51		-	
169	male	27	10.24%	-	
170	female	40		-	
171	female	48		-	
172	male	42		-	
173	male	50		-	
174	male	40		-	
175	female	44		-	
176	male	34		-	
177	male	44		-	
178	female	33		-	
179	female	50		-	
180	male	40		-	
181	female	44		-	
182	male	34		-	
183	male	44		-	
184	female	33		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
185	female	30		-	
186	male	23	10.76%	-	
187	male	47		-	
188	female	22	12.58%	-	
189	male	31		-	
190	male	39		-	
191	female	36		-	
192	female	28	8.57%	-	
193	female	23	10.45%	-	
194	male	37		-	
195	female	48		-	
196	male	45		-	
197	male	46		-	
198	male	30		-	
199	female	23	11.41%	-	
200	female	47		-	
201	female	21	15.07%	-	
202	male	31		-	
203	female	39		-	
204	male	36		-	
205	male	28		-	
206	female	23	12.88%	-	
207	male	33		-	
208	female	47		-	
209	female	47		-	
210	male	37		-	
211	female	54		-	
212	male	28		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
213	female	40	~ ,	-	
214	male	33		-	
215	male	42		-	
216	female	35		-	
217	female	54		-	
218	female	38		-	
219	female	48		-	
220	male	28		-	
221	female	44		-	
222	female	33		-	
223	male	38		-	
224	male	36		_	
225	female	19		-	
226	male	32		-	
227	male	46		-	
228	female	29		-	
229	male	36		-	
230	male	38		-	
231	male	49		-	
232	male	51		-	
233	female	30		-	
234	female	55		-	
235	male	45		-	
236	male	24	10.09%	-	
237	male	22	13.62%	-	
238	female	35		-	
239	female	36		-	
240	male	46		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
241	female	36	· ·	-	
242	male	34		-	
243	male	41		-	
244	male	23	11.67%	-	
245	male	23	10.66%	-	
246	female	54		-	
247	female	20	14.75%	-	
248	female	31		-	
249	male	39		-	
250	female	36		-	
251	female	28		-	
252	male	23	10.18%	-	
253	female	19		-	
254	female	48		-	
255	male	33		-	
256	female	46		-	
257	male	30		-	
258	female	23	10.87%	-	
259	female	47		-	
260	male	19	15.81%	-	
261	female	31		-	
262	female	39		-	
263	female	36		-	
264	male	28		-	
265	male	23	9.48%	-	
266	male	42		-	
267	female	47		-	
268	female	33		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
269	male	37		-	
270	female	54		-	
271	female	28		_	
272	male	44		-	
273	male	39		-	
274	female	36		_	
275	female	28		_	
276	female	23	8.63%	-	
277	female	17		_	
278	female	48		_	
279	female	41		_	
280	male	46		_	
281	female	22	10.59%	_	
282	female	42		_	
283	male	47		_	
284	female	36		_	
285	female	36		_	
286	male	21	13.92%	_	
287	male	23	11.63%	_	
288	female	46		-	
289	female	48		_	
290	female	42		-	
291	female	46		-	
292	female	22	11.76%	-	
293	female	21	13.62%	-	
294	male	39		-	
295	female	32		-	
296	male	44		-	

NO.	Sex	Age	Inhibition rate by ELISA (%)	Detection results by this kit (+/-)	Notes
297	male	42		-	
298	male	31		-	
299	female	25	11.96%	-	
300	female	44		-	
301	female	32		-	
302	male	51		-	
303	male	27	9.26%	-	
304	male	40		-	
305	female	48		-	
306	male	42		-	
307	female	50		-	
308	male	40		-	
309	female	44		-	
310	male	34		-	
311	male	44		-	

#### 10.2 Basic information and detection results of first vaccination the contributors

The 170 samples included 6 cases under 20 years old, 108 cases between 20 and 39 years old, 56 cases between 40 and 59 years old. The basic information and detection results by ELISA or this kit of first vaccination contributors are shown in Table 2.

NO	Say	Ago	<b>ELISA</b> inhibition	Test results of	Notos
NO.	бех	Age	rate (%)	reagent kit (+/-)	notes
1	female	22	16.37%	-	
3	male	39		-	
4	female	36		-	
5	male	28		-	
6	female	23	13.30%	-	
7	male	37		-	
8	male	48		-	
9	male	45		-	
10	male	46		-	
11	female	30		-	
12	male	23	13.07%	-	
13	female	47		-	
14	female	21	13.61%	-	
15	female	31		-	
16	male	39		-	
17	male	36		-	
18	male	28		-	
19	female	23	11.24%	-	
20	female	33		-	
21	female	47		-	
22	female	47		-	
23	male	37		-	

Table 2 The basic information and detection results byELISA or this kit of first vaccination the contributors.

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
24			rate (%)	reagent kit (+/-)	
24	male	54		-	
25	male	28		-	
26	male	40		-	
27	male	33		-	
28	male	42		-	
29	female	35		-	
30	male	54		-	
31	male	38		-	
32	male	48		-	
33	female	28		+	
34	female	44		-	
35	female	33		-	
36	female	38		-	
37	male	36		-	
38	male	19	11.04%	-	
39	male	32		-	
40	male	46		-	
41	female	29		-	
42	male	36		-	
43	female	38		-	
44	female	49		-	
45	female	51		-	
46	male	30		-	
47	male	55		-	
48	male	45		-	
49	male	24		-	
50	male	22	15.26%	-	
51	female	35		-	

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
52	female	26		-	
53	mala	16		_	
54	female	40			
55	female	36			
55	formala	34		-	
50	lemale	41		-	
57	male	23	14.42%	-	
58	male	23	15.83%	-	
59	female	54		-	
60	male	20	9.74%	-	
61	female	31		-	
62	male	39		-	
63	female	36		-	
64	female	28		-	
65	female	23		-	
66	female	19	13.52%	-	
67	female	48		-	
68	female	36		-	
69	female	38		-	
70	male	49		-	
71	male	51		-	
72	male	30		-	
73	male	55		-	
74	female	45		-	
75	female	24		-	
76	male	22	16.90%	-	
77	female	35		-	
78	female	36		-	
79	male	46		-	

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
80	female	26		-	
81	male	30			
82	mala	34			
02	inale	41		-	
83	male	23		-	
84	male	23		-	
85	female	54		-	
86	male	20	10.52%	-	
87	female	31		-	
88	female	39		-	
89	female	36		-	
90	male	28		-	
91	male	23		-	
92	male	19	7.70%	-	
93	female	48		-	
94	female	33		-	
95	female	46		-	
96	female	30		-	
97	male	23		-	
98	male	47		-	
99	male	19	9.47%	-	
100	male	31		-	
101	male	39		-	
102	male	36		-	
103	male	28		-	
104	male	23		-	
105	male	42		-	
106	male	47		-	
107	male	33		-	

NO.	Sex	Age	ELISA inhibition	Test results of reagent kit $(+/-)$	Notes
108	1	27		-	
100	male	37			
110	male	54		-	
110	temale	28		-	
111	female	44		-	
112	male	39		-	
113	female	36		-	
114	female	28		-	
115	female	23		-	
116	male	17	17.69%	-	
117	male	48		-	
118	female	41		-	
119	female	46		-	
120	female	22	20.37%	-	
121	male	33		-	
122	female	30		-	
123	male	23		-	
124	male	47		-	
125	male	22	13.49%	-	
126	female	31		-	
127	female	39		-	
128	male	36		-	
129	female	28		-	
130	male	23		-	
131	female	37		-	
132	female	48		-	
133	female	45		-	
134	female	46		-	
135	female	30		-	

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
136	formala	23		-	
137	iemale	47			
137	female	21			
130	male	21	13.52%	-	
139	male	31		-	
140	male	39		-	
141	male	36		-	
142	female	28		-	
143	female	23		-	
144	male	33		-	
145	female	47		-	
146	female	47		-	
147	male	37		-	
148	male	54		-	
149	male	28		-	
150	female	40		-	
151	male	33		-	
152	male	42		-	
153	female	35		-	
154	female	54		-	
155	male	54		-	
156	female	28		-	
157	male	40		-	
158	male	33		-	
159	female	42		-	
160	female	35		-	
161	male	54		-	
162	male	38		-	
163	female	48		-	

NO.	Sex	Age	ELISA inhibition rate (%)	Test results of reagent kit (+/-)	Notes
164	male	28		-	
165	male	44		-	
166	female	33		-	
167	female	38		-	
168	female	36		+	
169	male	19	6.67%	-	
170	female	32		-	

## 10.3 Basic information and detection results of second vaccination the contributors

The 182 samples included 5 cases under 20 years old, 125 cases between 20 and 39 years old and 52 cases between 40 and 59 years old. The basic information and detection results by ELISA or this kit of second vaccination contributors are shown in Table 3.

NO	Sav	A go	ELISA inhibition	Test results of reagent	Notos
NO.	Sex	Age	rate (%)	kit (+/-)	notes
1	male	47		+	
2	female	21		+	
3	female	31	57.58%	+	
4	female	39		+	
5	female	36	51.92%	+	
6	male	28		+	
7	male	23	83.77%	+	
8	male	33	42.32%	+	
9	female	47		+	
10	male	47		+	
11	male	37		+	
12	male	54		+	
13	female	28		+	
14	female	40		+	
15	female	33	43.73%	+	
16	female	42		+	
17	male	35	89.67%	+	
18	female	54		+	
19	male	38		+	
20	male	48		+	
21	female	28		+	
22	female	44		+	

 Table 3 The basic information and detection results by

 ELISA or this kit of pre-immunization the contributors.

NO.	Sex	Age	ELISA inhibition rate (%)	Test results of reagent kit(+/-)	Notes
23	male	33	80.48%	+	
24	female	38		+	
25	male	36		+	
26	female	19	85.52%	+	
27	male	32	70.56%	+	
28	male	46		+	
29	male	29	76.51%	-	
30	male	36		+	
31	female	38		+	
32	male	49		+	
33	female	51		+	
34	female	30	63.52%	+	
35	female	55		+	
36	male	45		+	
37	male	24	70.94%	+	
38	male	22	78.29%	-	
39	female	35	47.38%	+	
40	female	36		+	
41	female	46		+	
42	female	36		+	
43	male	34	47.49%	+	
44	male	41		+	
45	male	23	94.14%	+	
46	male	23	75.95%	+	
47	male	54		+	
48	male	20	78.34%	+	
49	female	31	83.58%	+	
50	male	39		+	

NO.	Sex	Age	ELISA inhibition rate (%)	Test results of reagent kit (+/-)	Notes
51	male	36		+	
52	male	28		+	
53	female	23	85.66%	+	
54	female	23	82.11%	+	
55	female	36		+	
56	female	48		+	
57	male	47		+	
58	male	46		+	
59	male	22	72.06%	+	
60	male	21		+	
61	female	39		+	
62	male	32	58.67%	+	
63	female	44		+	
64	female	42		+	
65	female	31	70.82%	+	
66	male	25	74.57%	+	
67	male	44		+	
68	male	32	64.53%	+	
69	male	51		+	
70	male	27	67.60%	+	
71	female	40		+	
72	female	48		+	
73	male	42		+	
74	female	50		+	
75	female	40		+	
76	female	44		+	
77	male	34	57.99%	+	
78	male	44		+	

NO.	Sex	Age	ELISA inhibition rate (%)	Test results of reagent kit(+/-)	Notes
79	female	33	84.42%	+	
80	male	50		+	
81	female	40		+	
82	male	44		+	
83	female	34	85.16%	+	
84	female	44		+	
85	male	33	96.17%	+	
86	female	30	89.74%	+	
87	female	23	73.14%	+	
88	female	47		+	
89	male	22	87.65%	+	
90	male	44		+	
91	male	33	89.46%	+	
92	male	30	73.32%	+	
93	male	23	62.27%	+	
94	female	47		+	
95	female	22	64.90%	+	
96	male	31	71.95%	+	
97	female	39		+	
98	female	36	73.97%	-	
99	female	28		+	
100	male	23	51.08%	-	
101	male	37		+	
102	female	48		+	
103	male	45		+	
104	female	46		+	
105	male	30	78.48%	+	
106	female	23	81.75%	+	

NO.	Sex	Age	ELISA inhibition rate (%)	Test results of reagent kit (+/-)	Notes
107	female	47		+	
108	female	21		+	
109	female	31	47.03%	+	
110	female	39		+	
111	female	36		+	
112	female	28		+	
113	male	23	79.54%	+	
114	male	33	62.24%	+	
115	male	47		+	
116	male	47		+	
117	female	37		+	
118	female	54		-	
119	male	46		+	
120	female	36		-	
121	female	34	62.79%	+	
122	male	41		+	
123	female	23	93.15%	-	
124	male	23	87.59%	+	
125	male	54		+	
126	male	20		+	
127	male	31	72.79%	+	
128	female	39		+	
129	male	36		+	
130	female	28		+	
131	female	23	77.82%	+	
132	female	19	83.95%	+	
133	male	48		+	
134	male	36		+	
NO.	Sex	Age	ELISA inhibition rate (%)	Test results of reagent kit (+/-)	Notes
-----	--------	-----	------------------------------	--------------------------------------	-------
135	male	38		+	
136	female	49		+	
137	female	51		+	
138	female	30	81.62%	+	
139	female	55		+	
140	male	45		+	
141	male	24	48.76%	-	
142	male	22	86.54%	+	
143	male	35	62.27%	+	
144	male	36		+	
145	male	46		+	
146	male	36		+	
147	male	34	64.22%	+	
148	male	41		+	
149	male	23	72.39%	+	
150	male	23	58.18%	+	
151	male	54		+	
152	male	20		+	
153	female	31	70.86%	+	
154	female	39		+	
155	male	36		+	
156	female	28		+	
157	female	23	68.89%	+	
158	male	19	80.42%	+	
159	male	48		+	
160	female	33	64.50%	+	
161	female	46		+	
162	male	23	94.51%	+	

NO	Sar	1 70	<b>ELISA</b> inhibition	Test results of reagent	Natas
NU.	Sex	Age	rate (%)	kit (+/-)	INOTES
163	female	23	83.25%	+	
164	female	54		+	
165	female	20		+	
166	male	31	46.78%	+	
167	male	39		+	
168	female	36		+	
169	female	28		+	
170	female	23	77.38%	+	
171	male	19	81.03%	+	
172	female	48		+	
173	male	33	58.64%	+	
174	male	46		+	
175	male	30	83.72%	+	
176	female	23	61.34%	+	
177	female	47		+	
178	male	19	81.38%	+	
179	female	31	67.11%	+	
180	male	39		+	
181	female	36		+	
182	female	28		+	

Abbreviations: red mark: Clinical Samples with inconsistent result by ELISA and this assay kit.

#### 10.4 Detection results of pre-immunization sample by naked eye

The detection results of samples from the first affiliated hospital of Henan university and school of medicine of Henan university are shown in Figure 11-22. A total of 684 specimens, including 311 pre-immunization samples, 170 samples 21 days after the first vaccination, 182 samples 21 days after the second vaccination for verification and 21 samples 6 months after the second vaccination.

Detection results of pre-immunization samples (from 1 to 100) by naked eye are shown in Figure 9. All of the detection results are negative.

Negative sample number			2 11 12 E	3 11 13 13	4 • •	5	6 7 6 17	8 11 18	9 19	10 0 20 10 10 0	21 111 0 31 111 6	22 111 <b>E</b> 32 111 <b>E</b>	23 111 0 33 111 6	24 111 0 34	25 111 0 35 111	26 111 0 36	27	28  	29 6 39	30 11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
						0 1														
		42	43	44	45	46	47	48	49 :::: 	50 	61 :::: 0	62 :::: []	63 :::: 0	64 :::: •	65 :::: 0	66 	67	68 :::: 0	69 !!!!	70
							0	0	0		0				0			0		
	51 C T C	52	53	54	55	56	57	58	59	60	71 ::: 0	72 ::: 0	73 ::: •	74	75	76 :::: 0	77	78 :::: 0	79 :::: 0	80
	81 :::: C T	82 :::: E	83	84	85 ::: 0	86 ::: 0	87	88 ::: E	89 :::: 0	90 :::: 0										
	91 ::: C T	92 :::: [	93 	94 ::: [	95 ::: [	96 :::: [	97 ::: E	98 :::: [	99 :::: ]	100										
	0			0	0	0	0			0										



Detection results of pre-immunization s	samples (from	101 to	221) by	naked	eye ar	e show	n in
Figure 12. All of the detection results are neg	gative.						



Figure 12 Detection result by naked eye of some pre-immunization sample from 101-221. N: negative control; P: positive control; C: control line; T: test line.

Detection results of pre-immunization samples (from 222 to 311) by naked eye are shown in Figure 13. All of the detection results are negative.



Figure 13 Detection result by naked eye of some pre-immunization sample from 222-311. N: negative control; P: positive control; C: control line; T: test line.

# 10.5 Detection results of pre-immunization sample by Image J and Graphpad Prism

Detection results of pre-immunization samples (from 1 to 200) by Image J and Graphpad Prism are shown in Figure 14. All of the detection results are negative.





Detection results of pre-immunization samples (from 201 to 311) by Image J and Graphpad Prism are shown in Figure 15. All of the detection results are negative.



Figure 15 Detection result by Image J and Graphpad Prism of pre-immunization samples from 201-311.

### 10.6 Detection Results of first vaccination sample by naked eye

Detection results of samples after the first vaccination (from 1 to 170) by naked eye are shown in Figure 16. All of the detection results are negative.



Figure 16 Detection result by naked eye of 170 samples after 1 dose. N: negative control; P: positive control; C: control line; T: test line.

# 10.7 Detection results of pre-immunization sample by Image J and Graphpad Prism

Detection results of samples after the first vaccination (from 1 to 170) by Image J and Graphpad Prism are shown in Figure 17. All of the detection results are negative.



Figure 17 Detection result by Image J and Graphpad Prism of 170 samples after 1 dose. N: negative control; P: positive control; C: control line; T: test line.

# 10.8 Detection Results of second vaccination sample by naked eye

Detection results of samples after second vaccination (from 1 to 39) by naked eye are shown in Figure 18. All of the detection results are positive except sample 29 and 38, and sample 29 and 38 were negative and passed further verification by ELISA (Figure 5F in manuscript).



Figure 18 Detection result by naked eye of samples 1-39 after 2 dose. N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.

Detection results of samples after second vaccination (from 40 to 81) by naked eye are shown in Figure 19. All of the detection results are positive.



Figure 19 Detection result by naked eye of samples 40-81 after 2 dose. N: negative control; P: positive control; C: control line; T: test line. Detection results of samples after second vaccination (from 82 to 182) by naked eye are shown in Figure 20. All of the detection results are positive except sample 97, 99, 101, 119, 124 and 141, and sample 97 and 124 were negative and passed further verification by ELISA (Figure 5F in manuscript).



Figure 20 Detection result by naked eye of samples 82-182 after 2 dose. N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.

# 10.9 Detection results of pre-immunization sample by Image J and Graphpad Prism

Detection results of samples after the second vaccination (from 1 to 182) by Image J and Graphpad Prism are shown in Figure 21.



Figure 21 Detection result by Image J and Graphpad Prism of 182 samples after 2 dose. N: negative control; P: positive control; C: control line; T: test line.

# 10.10 Detection results of 6 months sample after second vaccination by naked eye, Image J and Graphpad Prism

Detection results of samples 6 months after the second vaccination by naked eye (Figure 22a) and Image J, Graphpad Prism (Figure 22b) are shown in Figure 22.



Figure 22 Detection result by naked eye (Figure 20a) and Image J and Graphpad Prism (Figure 20b) of 182 samples after 2 dose. N: negative control; P: positive control; C: control line; T: test line.

# 11 Basic information and detection results of contributors for Table 2

356 clinical samples after infection, which from the first affiliated hospital of Henan university, were detected by ELISA and this research kit. The 356 samples included 15 cases under 20 years old, 77 cases between 20 and 39 years old, 132 cases between 40 and 59 years old, 110 cases between 60 and 79 years old and 22 cases over 80 years old. The basic information and detection results by ELISA and this kit of contributors are shown in Table 4.

## 11.1 Basic information and detection results of infected contributors

NO	Sov	Ago	<b>ELISA</b> inhibition	Test results of	Notos
NO.	Sex	Age	rate (%)	reagent kit (+/-)	INUICS
1	male	54	95.64%	+	
2	female	93	97.35%	+	
3	male	51	96.67%	+	
4	female	27	97.55%	+	
5	male	40	97.21%	+	
6	male	48	1.65%	-	Infected
7	female	72	97.17%	+	
8	female	50	97.49%	+	
9	female	40	97.27%	+	
10	female	44	6.67%	-	Infected
11	male	34	97.78%	+	
12	male	44	87.50%	+	
13	male	33	97.39%	+	
14	female	30	97.63%	+	
15	male	62	97.67%	+	
16	male	44	97.48%	+	
17	male	32	97.71%	+	
18	female	74	97.64%	+	
19	female	47	97.67%	+	

Table 4 The basic information and detection results byELISA and this kit of infected contributors.

NO	Say	A go	ELISA inhibition	Test results of	Notes
NO.	Sex	Age	rate (%)	reagent kit (+/-)	INOLES
20	female	17	97.41%	+	
21	female	54	97.48%	+	
22	male	40	97.80%	+	
23	female	55	97.72%	+	
24	male	36	97.43%	+	
25	male	30	97.53%	+	
26	female	43	97.50%	+	
27	female	52	97.43%	+	
28	male	42	97.41%	+	
29	male	31	97.81%	+	
30	male	25	95.49%	+	
31	female	44	97.52%	+	
32	male	53	97.62%	+	
33	female	36	97.63%	+	
34	male	49	79.66%	+	
35	female	63	97.48%	+	
36	male	50	97.18%	+	
37	male	35	97.66%	+	
38	male	42	97.24%	+	
39	male	47	89.10%	+	
40	female	32	89.35%	+	
41	male	35	97.63%	+	
42	female	38	95.15%	+	
43	female	54	97.08%	+	
44	female	53	97.18%	+	
45	male	16	97.63%	+	
46	male	58	97.11%	+	
47	male	68	97.72%	+	

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
	female	80	rate (%)	reagent kit (+/-)	Uninfected
40	fomala	12	07.62%		
49		70	97.0270		
50	temale	72	47.86%	+	
51	female	73	8.31%	-	Infected
52	male	18	10.19%	-	Infected
53	male	6	97.42%	+	
54	male	82	3.20%	-	Infected
55	female	23	97.63%	+	
56	female	65	97.41%	+	
57	male	48	97.32%	+	
58	male	77	96.90%	+	
59	male	66	51.68%	-	Infected
60	male	69	97.10%	+	
61	male	56	96.27%	+	
62	male	68	96.12%	+	
63	male	50	97.40%	+	
64	male	63	63.12%	+	
65	male	64	97.32%	+	
66	male	39	97.50%	+	
67	male	48	95.72%	+	
68	male	81	96.61%	+	
69	male	70	6.35%	-	Infected
70	female	59	2.18%	-	Infected
71	female	30	97.49%	+	
72	male	43	97.45%	+	
73	female	70	97.52%	+	
74	female	18	11.72%	-	Infected
75	female	72	12.26%	-	Infected

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
			rate (%)	reagent kit (+/-)	
76	male	69	10.90%	-	Infected
77	male	66	78.03%	+	
78	female	56	2.64%	-	Infected
79	female	73	97.64%	+	
80	female	20	92.27%	+	
81	male	64	97.53%	+	
82	female	65	13.65%	+	Infected
83	male	43	93.88%	-	Infected
84	female	73	88.03%	+	
85	female	67	9.00%	+	Infected
86	male	64	95.76%	-	Infected
87	female	60	97.53%	+	
88	male	58	97.61%	+	
89	male	43	97.42%	+	
90	male	58	97.60%	+	
91	female	11	95.87%	+	
92	female	18	97.44%	+	
93	male	54	12.57%	-	Infected
94	female	58	97.51%	+	
95	male	69	97.31%	+	
96	female	79	94.09%	+	
97	female	54	96.85%	+	
98	female	42	97.48%	+	
99	female	52	97.32%	+	
100	female	47	70.38%	+	
101	female	59	97.33%	+	
102	female	50	95.06%	+	
103	male	85	2.09%	-	Infected

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
104	male	78	2.30%	reagent kit (+/-)	Infected
105	male	53	96.85%	+	
106	male	13	97.55%	+	
107	female	54	97.48%	+	
108	female	67	12.08%	_	Infected
109	male	60	97.11%	+	
110	female	32	94.59%	+	
111	female	73	17.30%	-	Infected
112	male	83	97.11%	+	
113	male	68	9.57%	_	Infected
114	male	68	97.51%	+	
115	female	59	96.92%	+	
116	male	23	97.57%	+	
117	male	47	76.05%	+	
118	female	46	97.41%	+	
119	female	87	9.18%	-	Infected
120	male	69	97.32%	+	
121	female	69	97.30%	+	
122	male	24	97.55%	+	
123	male	70	12.34%	-	Infected
124	female	30	97.54%	+	
125	female	70	2.19%	-	Infected
126	male	51	97.79%	+	
127	male	29	58.81%	+	
128	female	59	97.44%	+	
129	male	61	10.44%	-	Infected
130	male	85	10.90%	+	Infected
131	male	41	97.43%	+	

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
122	C 1	()	rate (%)	reagent kit (+/-)	
132	Temale	64	97.16%	+	
133	female	27	97.35%	+	
134	female	70	97.35%	+	
135	male	71	5.60%	-	Infected
137	female	90	9.17%	-	Infected
138	male	62	97.36%	+	
139	male	28	97.31%	+	
140	female	71	10.56%	-	Infected
141	male	56	97.06%	+	
142	female	69	89.68%	+	
144	female	59	97.42%	+	
145	male	61	14.57%	-	Infected
146	female	62	96.25%	+	
147	male	87	9.21%	-	Infected
148	female	37	97.13%	+	
149	male	67	93.50%	+	
150	male	72	97.08%	+	
151	female	80	97.48%	+	
152	female	69	12.37%	-	Infected
153	female	70	11.07%	-	Infected
154	female	56	18.38%	-	Uninfected
155	male	21	96.74%	+	
156	female	29	97.44%	+	
157	female	46	97.13%	+	
158	male	32	97.39%	+	
159	male	49	97.46%	+	
160	female	46	97.43%	+	
161	male	67	97.46%	+	

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
1.0		17	rate (%)	reagent kit (+/-)	
162	male	17	97.37%	+	
163	male	32	62.19%	-	Infected
164	male	53	97.11%	+	
165	male	52	97.35%	+	
166	male	51	97.35%	+	
167	female	69	97.38%	+	
168	female	70	97.25%	+	
169	male	77	96.80%	+	
170	male	54	96.70%	+	
171	male	60	13.14%	-	Infected
172	male	57	95.86%	+	
173	female	69	95.23%	+	
174	female	61	96.84%	+	
175	male	47	97.13%	+	
176	male	61	15.07%	-	Infected
177	male	45	97.04%	+	
178	male	50	12.94%	-	Infected
179	male	59	95.21%	+	
180	female	60	96.62%	+	
181	male	71	96.09%	+	
182	female	88	17.10%	-	Infected
183	male	69	96.95%	+	
184	male	88	4.17%	-	Infected
185	female	40	97.17%	+	
186	female	85	97.38%	+	
187	female	78	96.70%	+	
188	female	86	97.31%	+	
190	male	44	96.28%	+	

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
101	C 1	(0	rate (%)	reagent kit (+/-)	
191	Iemale	68	/4.9/%	+	
192	male	60	97.26%	+	
193	male	63	97.30%	+	
194	male	85	9.75%	-	Infected
195	male	78	97.29%	+	
196	female	32	97.37%	+	
197	female	72	96.93%	+	
198	female	28	97.31%	+	
199	male	93	2.44%	-	Infected
200	female	73	96.92%	+	
201	female	59	97.17%	+	
202	male	75	52.12%	-	Infected
203	male	61	89.49%	+	
204	male	82	66.72%	+	
205	female	64	33.67%	+	
206	female	39	85.48%	-	Infected
207	female	31	97.34%	+	
208	male	35	97.41%	+	
209	female	51	97.02%	+	
210	male	29	97.09%	-	Infected
211	male	57	97.48%	+	
212	female	58	96.99%	+	
213	female	34	97.18%	+	
214	male	65	97.28%	+	
215	female	44	97.43%	+	
216	male	24	94.27%	+	
217	female	68	96.73%	+	
218	female	16	97.25%	+	

NO.	Sex	Аде	ELISA inhibition	Test results of	Notes
		rate (%)	reagent kit (+/-)		
219	male	58	97.12%	+	
220	female	52	97.49%	+	
221	female	59	95.26%	+	
222	female	45	97.35%	+	
223	male	59	96.99%	+	
224	male	39	96.80%	+	
225	male	54	97.46%	+	
226	female	55	97.13%	+	
227	female	31	97.43%	+	
228	male	40	97.21%	+	
229	female	50	96.59%	+	
230	female	45	4.20%	-	Infected
231	male	62	96.37%	+	
232	male	31	96.78%	+	
233	female	39	5.06%	-	Uninfected
234	female	36	97.23%	+	
235	female	70	97.25%	+	
236	female	74	10.92%	-	Infected
237	female	27	97.31%	+	
238	female	68	97.23%	+	
239	female	56	53.30%	-	Infected
240	male	22	97.25%	+	
241	female	50	97.37%	+	
242	female	72	6.13%	-	Infected
243	female	59	97.03%	+	
244	female	32	97.43%	+	
245	male	64	97.41%	+	
246	female	33	73.14%	-	Infected

NO.	Sex	Age	ELISA inhibition rate (%)	Test results of reagent kit (+/-)	Notes
247	male	61	96.31%	+	
248	female	50	97.12%	+	
249	male	47	66.08%	+	
250	female	45	95.93%	+	
251	female	55	96.22%	+	
252	male	70	94.92%	+	
253	female	43	83.49%	-	Infected
254	male	42	97.39%	+	
255	female	28	96.96%	+	
256	male	23	96.68%	+	
257	male	90	6.76%	-	Infected
260	male	48	96.79%	+	
261	female	69	97.25%	+	
262	female	46	97.17%	+	
263	female	22	96.89%	+	
264	male	85	4.71%	-	Infected
265	male	61	3.42%	-	Infected
266	male	73	10.85%	-	Infected
267	female	66	96.86%	+	
268	male	21	96.15%	+	
269	female	37	51.24%	-	Infected
270	male	55	96.18%	+	
271	male	75	97.31%	+	
272	male	9	96.31%	+	
273	female	59	96.88%	+	
274	female	86	16.48%	-	Infected
276	female	62	96.87%	+	
277	female	25	83.67%	-	Infected

NO.	Sex	Age	ELISA inhibition	Test results of	Notes
278	male	77	94 88%		
270	mala	67	96 20%	-	
2/9		57	90.20%		
280	female	57	94.75%	+	
282	female	49	84.31%	-	Infected
283	male	38	2.58%	-	Infected
284	female	49	6.88%	-	Infected
286	male	50	97.08%	+	
287	male	53	96.91%	-	Infected
288	male	78	14.02%	-	Infected
289	male	43	3.87%	-	Infected
290	male	78	11.98%	-	Infected
291	female	6	96.32%	+	
292	male	77	95.75%	+	
293	female	48	96.45%	+	
294	male	52	92.60%	+	
295	female	33	3.42%	-	Infected
297	male	69	15.73%	-	Infected
298	female	75	5.98%	-	Infected
299	female	80	5.49%	-	Infected
300	female	52	96.89%	+	
301	female	44	96.21%	+	
302	female	67	6.82%	-	Infected
303	male	56	95.41%	+	
304	female	73	96.41%	+	
305	male	26	96.35%	+	
306	male	36	95.04%	+	
307	female	64	95.72%	+	
308	female	67	96.16%	+	

NO.	Sex	Age	ELISA inhibition rate (%)	Test results of reagent kit (+/-)	Notes
309	male	54	59.37%	-	Infected
310	male	33	97.10%	+	
311	female	60	96.55%	+	
312	male	61	82.79%	+	
313	male	51	97.05%	+	
314	male	71	44.26%	-	Infected
315	female	52	96.30%	+	
316	male	55	94.27%	+	
317	male	67	92.38%	+	
319	male	72	96.52%	+	
320	female	57	95.70%	+	
321	male	70	96.85%	+	
322	male	58	96.72%	+	
323	male	47	96.69%	+	
324	male	83	19.03%	-	Infected
325	male	37	97.02%	+	
326	male	54	95.65%	+	
327	female	28	96.65%	+	
328	male	40	96.45%	+	
329	male	33	96.19%	+	
330	male	42	96.61%	+	
331	female	92	0.81%	-	Infected
332	male	54	79.86%	+	
333	female	54	96.38%	+	
334	female	46	96.04%	+	
335	female	78	96.82%	+	
336	male	56	96.40%	+	
337	male	77	57.57%	-	Infected
338	female	38	96.66%	+	
339	female	58	94.77%	+	

NO.	Sex	Age	ELISA inhibition Test results of		
			rate (%)	reagent kit (+/-)	Notes
340	female	73	25.39%	+	
341	male	44	77.86%	+	
342	male	33	96.65%	+	
343	female	72	96.06%	+	
344	male	36	96.87%	+	
345	male	19	96.65%	+	
346	female	56	96.78%	+	
347	male	46	96.94%	+	
348	female	29	96.27%	+	
349	male	36	96.96%	+	
350	female	77	5.72%	-	Infected
351	female	49	96.34%	+	
352	male	51	96.28%	+	
353	male	30	81.05%	+	
354	male	55	95.75%	+	
355	male	83	7.49%	-	Uninfected
356	male	24	96.91%	+	
357	female	22	96.70%	+	
358	female	74	95.34%	+	
359	male	73	96.45%	+	
360	male	46	96.35%	+	
361	male	71	95.33%	+	
362	male	34	96.67%	+	
363	female	41	93.67%	+	
364	male	23	40.70%	+	
365	female	23	95.50%	+	
366	female	54	96.49%	+	

# 11.2 Detection results of infected samples by naked eye

The detection results of infected samples from the first affiliated hospital of Henan university are shown in Figure 9-20 and Table 6. The 356 samples included 15 cases under 20 years old, 77 cases between 20 and 39 years old, 132 cases between 40 and 59 years old, 110 cases between 60 and 79 years old and 22 cases over 80 years old. The detection results of contributors by this kit are shown in Figure 23-29.

Detection results of infected samples (from 1 to 56) by naked eye are shown in Figure 23.



Figure 23 Detection result by naked eye of infected samples (from 1 to 56). N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.

Detection results of infected samples (from 57 to 112) by naked eye are shown in Figure 24.



Figure 24 Detection result by naked eye of infected samples (from 57 to 112). N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.

Detection results of infected samples (from 113 to 158) by naked eye are shown in Figure 25.



Figure 25 Detection result by naked eye of infected samples (from113 to 158). N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.





Figure 26 Detection result by naked eye of infected samples (from 159 to 214). N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.

Detection results of infected samples (from 215 to 270) by naked eye are shown in Figure 27.



Figure 27 Detection result by naked eye of infected samples (from 215 to 270). N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.

Detection results of infected samples (from 271 to 326) by naked eye are shown in Figure 28.



Figure 28 Detection result by naked eye of infected samples (from 271 to 326). N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.

Detection results of infected samples (from 327 to 382) by naked eye are shown in Figure 29.



Figure 29 Detection result by naked eye of infected samples (from 327 to 382). N: negative control; P: positive control; C: control line; T: test line; Red arrow: Indicates a negative result.

# 11.3 Detection results of infected samples by Image J and Graphpad Prism

Detection results of infected samples (from 1 to 112) by Image J and Graphpad Prism are shown in Figure 30.



Figure 30 Detection result by Image J and Graphpad Prism of pre-immunization samples from 1-112.

Detection results of infected samples (from113-214) by Image J and Graphpad Prism are shown in Figure 31.



Figure 31 Detection result by Image J and Graphpad Prism of pre-immunization samples from 113-214.
Detection results of infected samples (from 215-326) by Image J and Graphpad Prism are shown in Figure 32.



Figure 32 Detection result by Image J and Graphpad Prism of pre-immunization samples from 215-326.

N: negative control; P: positive control; C: control line; T: test line.

Detection results of infected samples (from 327-382) by Image J and Graphpad Prism are shown in Figure 33.





N: negative control; P: positive control; C: control line; T: test line.

## 11.4 Distribution of detection results by ELISA and this research kit

All of the clinical samples after infection were detected by ELISA and this research kit, the distribution of two methods was shown in Figure 34. The detect results of 339 samples are consistent by ELISA (Vazyme Biotech Co. Ltd., DD3101) and this research kit, the coincidence rate was 97.83% (339/356).



Figure 34 Distribution of 356 samples detection results by ELISA and this research kit.