Supporting Information

A paper-based chemiluminescence immunoassay device for rapid and high-throughput detection of allergen-specific IgE

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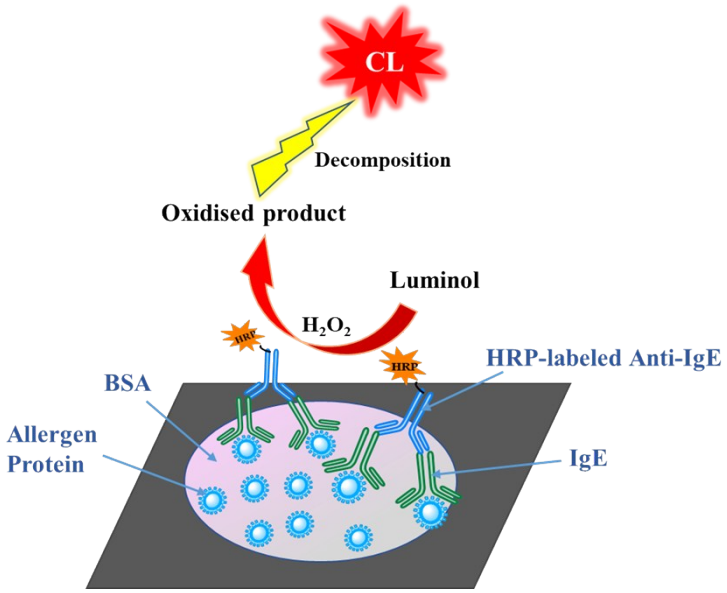

Fig. S1. The illustration of chemiluminescence mechanism in this assay.

(a)

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          1                                                          60
Before    ATGAAGACCCTGCTCCTCACCATCGGCTTCAGCCTCATTGCGATCCTGCAGGCCCAGGAT
After                                                        CAGGAC
                                                               Q  D

          61                                                         120
Before    ACCCCAGCCTTGGGAAAGGACACTGTGGCTGTGTCAGGGAAATGGTATCTGAAGGCCATG
After     ACCCCGGCTCTGGGTAAAGACACCGTTGCTGTTTCTGGTAAATGGTACCTGAAAGCTATG
            T  P  A  L  G  K  D  T  V  A  V  S  G  K  W  Y  L  K  A  M

          121                                                        180
Before    ACAGCAGACCAGGAGGTGCCTGAGAAGCCTGACTCAGTGACTCCCATGATCCTCAAAGCC
After     ACCGCTGACCAGGAAGTTCCGGAAAAACCGGACTCTGTTACCCCGATGATCCTGAAAGCT
            T  A  D  Q  E  V  P  E  K  P  D  S  V  T  P  M  I  L  K  A

          181                                                        240
Before    CAGAAGGGGGGCAACCTGGAAGCCAAGATCACCATGCTGACAAATGGTCAGTGCCAGAAC
After     CAGAAAGGTGGTAACCTGGAAGCTAAAATCACCATGCTGACCAACGGTCAGTGCCAGAAC
            Q  K  G  G  N  L  E  A  K  I  T  M  L  T  N  G  Q  C  Q  N

          241                                                        300
Before    ATCACGGTGGTCCTGCACAAAACCTCTGAGCCTGGCAAATACACGGCATACGAGGGCCAG
After     ATCACCGTTGTTCTGCACAAAACCTCTGAACCGGGTAAATACACCGCTTACGAAGGTCAG
            I  T  V  V  L  H  K  T  S  E  P  G  K  Y  T  A  Y  E  G  Q

          301                                                        360
Before    CGTGTCGTGTTCATCCAGCCGTCCCCGGTGAGGGACCACTACATTCTCTACTGCGAGGGC
After     CGTGTTGTTTTCATCCAGCCGTCTCCGGTTCGTGACCACTACATCCTGTACTGCGAAGGT
            R  V  V  F  I  Q  P  S  P  V  R  D  H  Y  I  L  Y  C  E  G

          361                                                        420
Before    GAGCTCCATGGGAGGCAGATCCGAATGGCCAAGCTTCTGGGAAGGGATCCTGAGCAGAGC
After     GAACTGCACGGTCGTCAGATCCGTATGGCTAAACTGCTGGGTCGTGACCCGGAACAGTCT
            E  L  H  G  R  Q  I  R  M  A  K  L  L  G  R  D  P  E  Q  S

          421                                                        480
Before    CAAGAGGCCTTGGAGGATTTTCGGGAATTCTCAAGAGCCAAAGGATTGAACCAGGAGATT
After     CAGGAAGCTCTGGAAGACTTCCGTGAATTCTCTCGTGCTAAAGGTCTGAACCAGGAAATC
            Q  E  A  L  E  D  F  R  E  F  S  R  A  K  G  L  N  Q  E  I

          481                                                525
Before    TTGGAACTCGCGCAGAGCGAAACCTGCTCTCCAGGAGGACAGTAG
After     CTGGAACTGGCTCAGTCTGAAACCTGCTCTCCGGGTGGTCAGTAG
            L  E  L  A  Q  S  E  T  C  S  P  G  G  Q
```

(b)

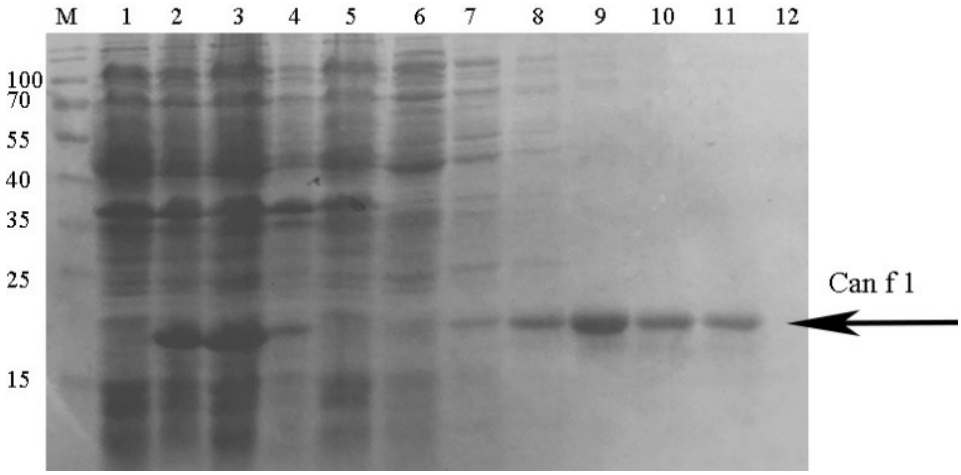

Fig. S2. Expression and purification of Can f 1 in *E. coli.* (a) The nucleotide sequences for codon-optimized Can f 1 gene before and after codon optimization. The underlined part is the codon of signal peptide, which is removed from the sequences when optimized. (b) Lane M, protein molecular weight standard; Lane 1, non-induced recombinant Can f 1 whole cell lysate; Lane 2, IPTG-induced recombinant Can f 1 whole cell lysate; Lane 3, supernatant fraction after ultrasonication; Lane 4, precipitation fraction (inclusion bodies) after ultrasonication. The Can f 1 was denoted with an arrow; Lane 5, unbinding fractions of the supernatant of Can f 1; Lane 6, washing with 10 mM imidazole; Lane 7, washing with 30 mM imidazole; Lane 8, washing with 50 mM imidazole; Lane 9, washing with 100 mM imidazole; Lane 10, washing with 150 mM imidazole; Lane 11, washing with 200 mM imidazole; Lane 12, washing with 250 mM imidazole.

Table S1 Information of 10 patients

No.	Gender	Age	Associated disease	ELISA
1	Female	55	Rhinitis, Eczema	+
2	Male	50	Rhinitis, Eczema	+
3	Female	53	Rhinitis	-
4	Male	15	Rhinitis, Asthma	+
5	Female	35	Bronchial asthma	+
6	Female	21	Rhinitis, Asthma	-
7	Female	29	Rhinitis, Food allergy	+
8	Female	26	Asthma	-
9	Female	27	Bronchial asthma, Rhinitis	+
10	Male	23	Rhinitis, Bronchial Asthma	-

Noted information: +, positive; -, negative

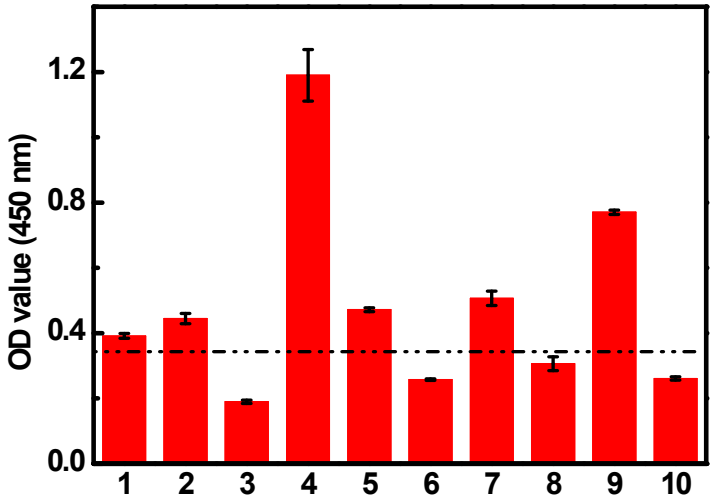

Fig. S3. Detection of Can f 1 sIgEs in 10 patients' sera by commercial ELISA kit. Dotted line indicated the 2.1 times of mean OD value of 5 NHS. All experiments were conducted in quadruplicate.

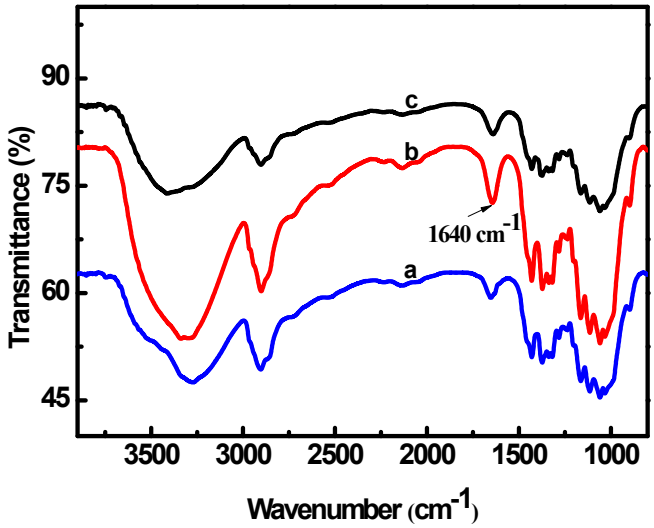

Fig. S4. FT-IR spectra of (a) the original paper; (b) plasma treated paper; (c) the antigen immobilized paper.

H_2O_2

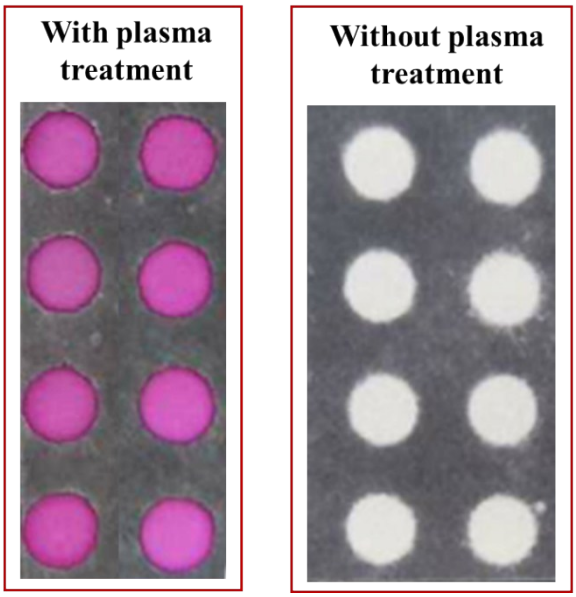

Fig. S5. Verification of aldehyde groups on paper with the Schiff reagent before and after the plasma treatment.

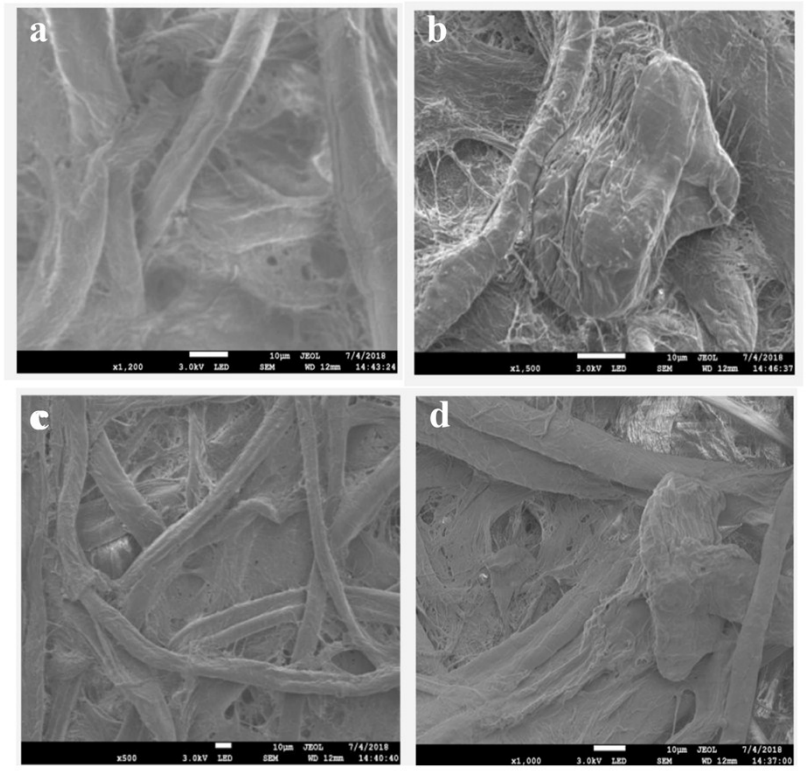

Fig. S6. SEM images showed the characteristics of the paper device before/after oxygen plasma treatment and antibody immobilization. (a) Original paper without treatment; (b) oxygen plasma treated paper; (c) the antigen immobilized paper; and (d) the BSA immobilized paper. Scale bar = 10 μm.

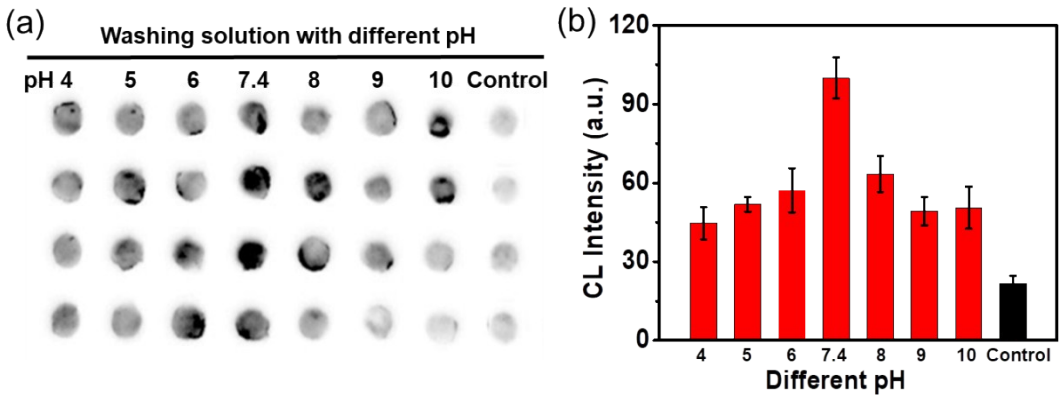

Fig. S7. (a) The CL images of the serum sample washed with different pH buffer solution. (b) The corresponding CL intensity of (a). All experiments were conducted in quadruplicate.

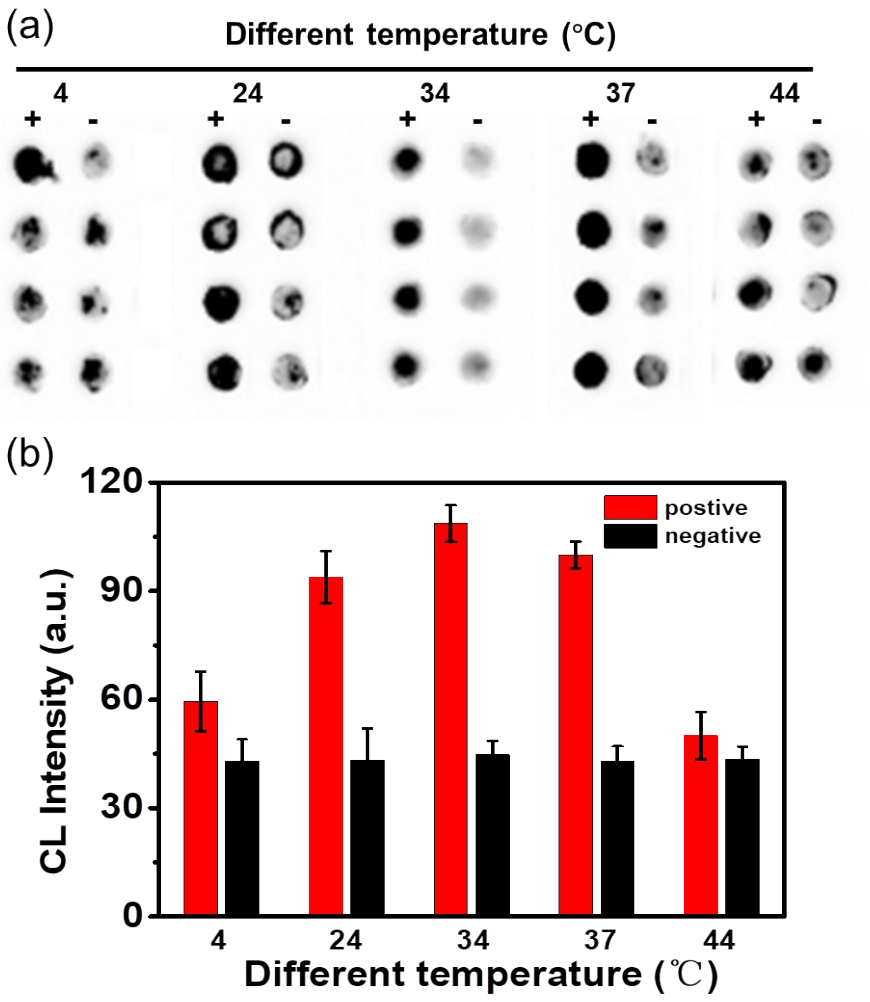

Fig. S8. (a) The CL images of the positive and negative serum sample incubated with different temperature. (b) The corresponding CL intensity of (a). All experiments were conducted in quadruplicate.

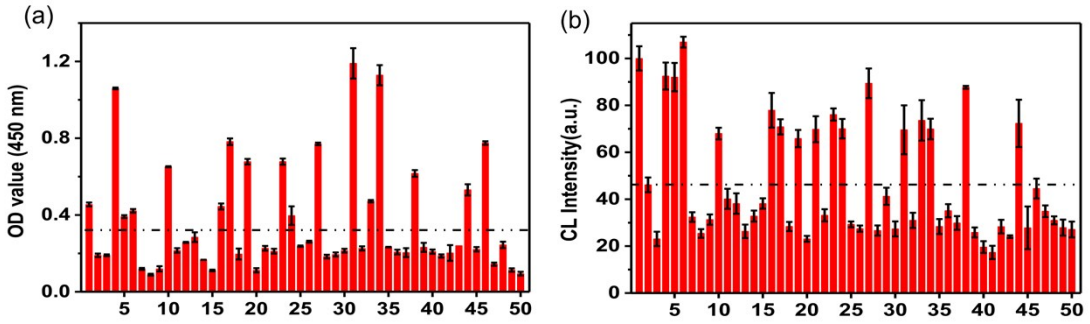

Fig. S9. The OD values at 450 nm of 50 serum samples determined by ELISA kit. Dotted line indicated the 2.1 times of mean OD value of 5 NHS; The CL intensity of 50 serum samples determined by the paper-based device. Dotted line indicated the 1.6 times of mean CL intensity of 5 NHS. All experiments were conducted in quadruplicate.

Table S2. The detailed information of human serum samples and their detection results with commercial ELISA kit and our paper-based device.

No.	Gender	Age	Associated disease	Results	
				ELISA	paper-based device
1	Male	57	Atopic dermatitis	+	+
2	Male	60	Eczema, Cough	-	-
3	Female	53	Rhinitis	-	-
4	Male	56	Rhinitis	+	+
5	Female	55	Rhinitis, Eczema	+	+
6	Male	52	Rhinitis, Cough	+	+
10	Female	19	Rhinitis, Asthma	+	+
11	Male	48	Asthma, Atopic dermatitis	-	-
12	Female	21	Rhinitis, Asthma	-	-
13	Female	35	Rhinitis	-	-
14	Male	14	Rhinitis, Cough	-	-
16	Male	50	Rhinitis, Eczema	+	+
17	Male	19	Rhinitis, Dyspnea	+	+
18	Female	33	Urticaria	-	-
19	Female	17	Rhinitis, Cough	+	+
21	Female	33	Chronic urticaria	-	+
23	Male	43	Conjunctivitis, Cough	+	+
24	Female	27	Bronchial asthma, Rhinitis	+	+
25	Male	27	Hay fever.	-	-
26	Female	26	Asthma	-	-
27	Female	15	Rhinitis, Bronchial asthma	+	+
31	Male	15	Rhinitis, Asthma	+	+
32	Female	32	Rhinitis	-	-
33	Female	35	Bronchial asthma	+	+
34	Female	13	Rhinitis, Asthma	+	+

35	Male	23	Rhinitis, Bronchial Asthma	-	-
36	Female	50	Atopic dermatitis	-	-
38	Male	30	Atopic dermatitis	+	+
44	Female	26	Rhinitis, Bronchial asthma	+	+
45	Male	31	Rhinitis	-	-
46	Female	13	Rhinitis, Asthma	+	-
48	Male	29	Bronchial asthma, Rhinitis	-	-
The rest	Healthy people without a history of allergic symptoms			-	-

Noted information: +, positive; -, negative.

Table S3. The accuracy for each microzone in one sample with the paper-based device. All experiments were conducted in quadruplicate.

Samples	1 (+)	2 (+)	3 (-)	4 (+)	5 (+)	6 (+)	7 (-)	8 (-)	9 (-)	10 (+)
+	70%	67%		72%	100%	87%				64%
-			100%				100%	83%	100%	
Samples	11 (-)	12 (-)	13 (-)	14 (-)	15 (-)	16 (+)	17 (+)	18 (-)	19 (+)	20 (-)
+						67%	75%		88%	
-	83%	71%	89%	88%	63%			89%		100%
Samples	21 (+)	22 (-)	23 (+)	24 (+)	25 (-)	26 (-)	27 (+)	28 (-)	29 (-)	30 (-)
+	75%		83%	61%			75%			
-		100%			91%	91%		71%	78%	67%
Samples	31 (+)	32 (-)	33 (+)	34 (+)	35 (-)	36 (-)	37 (-)	38 (+)	39 (-)	40 (-)
+	67%		73%	56%				81%		
-		80%			75%	70%	60%		100%	67%
Samples	41 (-)	42 (-)	43 (-)	44 (+)	45 (-)	46 (+)	47 (-)	48 (-)	49 (-)	50 (-)
+				71%		64%				
-	67%	100%	100%		67%		71%	70%	92%	67%

Noted information: +, positive; -, negative.