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Development of an up-converting phosphor technology-based 10-channel lateral flow assay for profiling antibodies against *Yersinia pestis*

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ABSTRACT

In this study, a 10-channel up-converting phosphor technology-based lateral flow (TC-UPT-LF) assay was developed to profile antibodies against *Yersinia pestis*. Ten expressed *Y. pestis* proteins were covalently conjugated with an up-converting phosphor particle to develop double-antigen sandwich immunochromatographic strips to detect corresponding antibodies. After optimization one by one, each strip was integrated into a TC-UPT-LF disc for simultaneously detection of different antibodies. A scanning biosensor was also developed to acquire the results. The performance of the TC-UPT-LF assay was evaluated by using standard samples and plague monkey serum samples. Fifty-one patient serum samples were detected by the TC-UPT-LF assay. The TC-UPT-LF disc could be stable for 10 days at 37 °C with an average CV of 10.3%. Its sensitivity and qualitative results are comparable to those of ELISA. Its linearity fitting coefficient of determination (R^2) for different antibody detection is between 0.93 and 0.99. Besides F1 antibody, the LcrV and YopD antibodies also showed higher positive ratio than the other seven antibodies, as 100% (13/13) and 92% (12/13) in monkey sera and 86.3% (44/51) and 66.7% (34/51) in patient sera, respectively. It is suggested that the TC-UPT-LF assay has been successfully developed for multi-detection and LcrV and YopD can be the potential diagnostic markers of the plague.

1. Introduction

Techniques that are used in anti-bioterrorism efforts, clinical investigation, food safety control, and even forensic identification should allow for high-throughput on-site bio-detection and should be simple to operate, highly accurate, and objective and quantitative. Over the past few years, many methods have been developed to achieve this goal, including gene and protein chip techniques (Bacarese-Hamilton et al., 2004; Ehricht et al., 2009; Servoss et al., 2009; Yoo et al., 2009). However, the high-cost, time-consuming and tedious operation made the chip-based assays only suitable for research laboratory, not for on-site detection. The immunochromatographic assay (also called lateral flow, LF) as the most widely used on-site detecting method has the potential to be developed as a multi-assay platform for simultaneous mid-throughput detection of multiple analytes. Two aspects of improvement to the LF assay are required in order to meet this requirement.

On the one hand, it should be developed to a quantitative assay. The traditional LF assays usually use colored particles, such as colloid gold (Zhou et al., 2009) or dye (Oh et al., 2009) as labels to enable easy observation of results via color change, however, their lower quantitative capability and low sensitivity also limit their application in field detection. To overcome these drawbacks, many new materials have been employed as labels, including up-converting phosphor particles (UCPs) (Niedbala et al., 2000), semiconductor quantum dots (QDs) (Jokerst et al., 2009), liposome beads (Edwards et al., 2006), carbon nanotubes (Jensen et al., 2009; Onac et al., 2006) and other organic or inorganic nanoparticles (Richardson et al., 2001), etc. These materials can all improve the quantitative capability or decrease the limit of detection (LOD) of LF assays, and among them, QDs and UCPs may be the most promising markers and have attracted more attention. Owing to the advantage of narrow emission bands, QDs have ambitious potential in parallel multi-detection, however, commercially available QDs are hardly photostable enough to be used for quantitative measurements with low LODs (Liu and Lin, 2007). While UCPs in general still permit the lowest LOD. Using an up-converting phosphor technology-based LF (UPT-LF) assay, 10^3 cfu/ml *Escherichia coli* could be detected against a background of 10^9 other organisms per milliliter. For smaller analytes, e.g. drugs of abuse, LOD

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in the order of 5 ng/ml was obtained in a competitive LF assay (Niedbala et al., 2001).

UCPs are kinds of rare-earth-containing crystal particles with the unique property of up-converting phenomenon, that is, they can be excited by low-energy infrared light (absorbing two or more low-energy photons) and emit high-energy visible light (emitting a high-energy photon) (van De Rijke et al., 2001). UCPs generate large enough anti-Stokes shifts that result in well-separated emission and excitation bands and they do not bleach or fade, and the different colors' of UCPs can be excited simultaneously with the same emission spectrum. Those unique optical properties render UCPs to be ideal labels and separate UPT from other LF assay in the biological detection field. UPT-LF assay has potential for quantitative detection, high sensitivity, and high stability and thus allow repetitive re-analysis. Up until now, several kinds of UPT-LF strips have been developed for quantitative detection of pathogens, including *Yersinia pestis* (Yan et al., 2006), *Brucella* (Qu et al., 2009), hepatitis B virus (Li et al., 2009), *Streptococcus suis* serotype 2 (Zheng et al., 2008), *Schistosoma* circulating anodic antigen (Corstjens et al., 2008), *E. coli* O157:H7 (Niedbala et al., 2001), etc.

On the other hand, LF assays should be developed to a one-step multi-target detection method (Corstjens et al., 2007; Niedbala et al., 2001). In previous reports, researchers usually coated two or more test lanes on the LF strip in parallel with each lane corresponding to one target. Corstjens et al. (2007) developed a consecutive flow multiplex method for TB/HCV or HIV/HCV detection using same

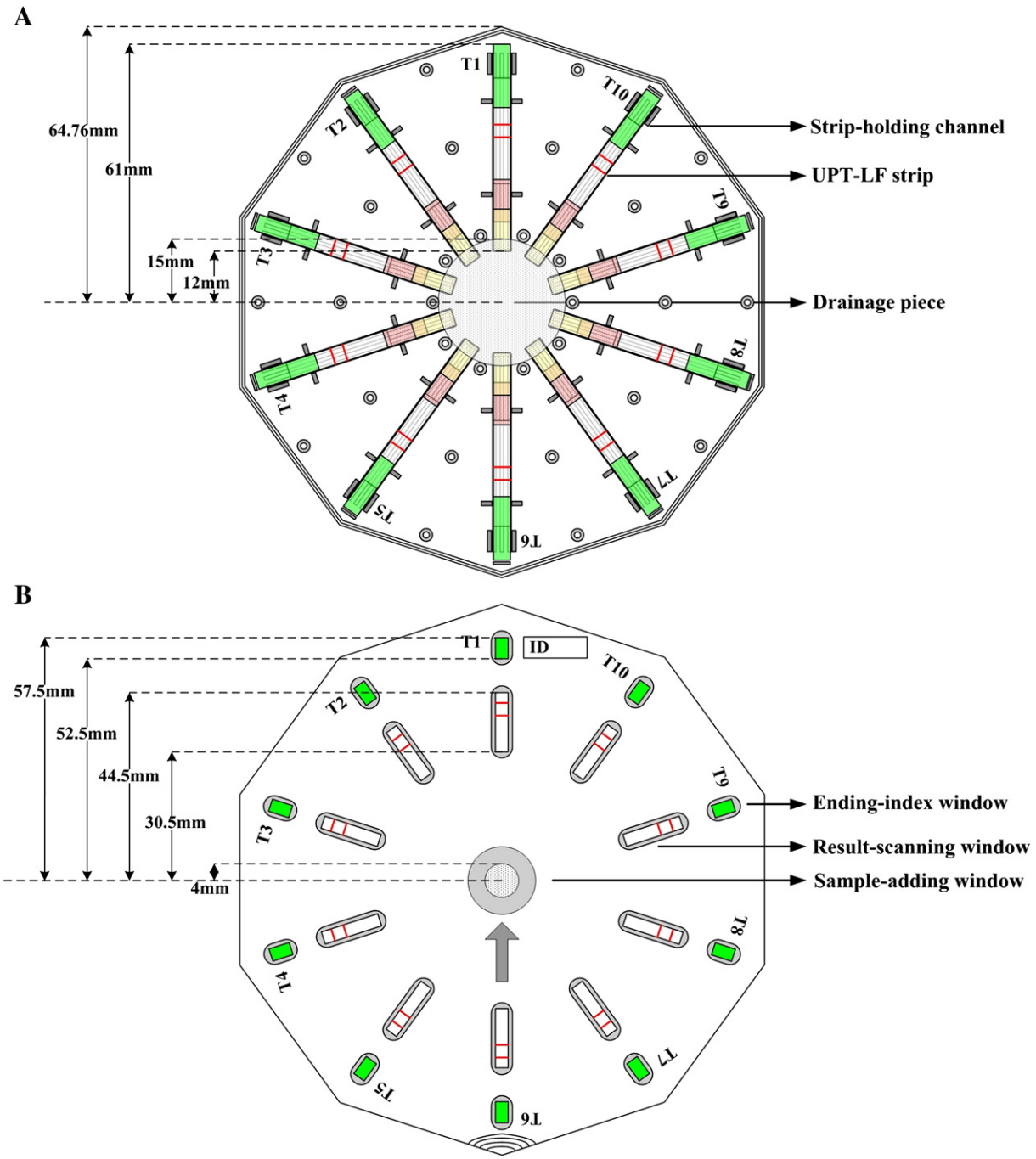

Fig. 1. The sketch map of TC-UPT-LF disc. The cartridge of disc consists of the base (A) and the upper cover (B). There are 10 strip-holding channels arranged on the base in the central symmetry. Ten strips were set in the strip-holding channels with the drainage piece overlapping with the sample pad of every strip. The upper cover engages with the base, and the sample-adding window, the result-scanning window and the ending-index window correspond to the drainage piece, the analysis membrane and the absorbent pad respectively.

Table 1
Primers of genes encoding proteins of *Y. pestis*.

Gene ID	Sensitive primer (5′–3′)	Antisensitive primer (5′–3′)	Amplicon (bp)	Restriction enzymes
YopD	gggatccatagcgaa agaggtgaaaatag	ggtcgacttagac aacaccaaaagcgg	469	BamH I/Sal I
YPO1089	acgtggatccaaaa caccactcattctgc	acgtgtcgacttag tttcttgctgtattgc	1084	BamH I/Sal I
YPO1303	gggatccaactcc aaggatgtttctgg	ggtcgacttaaaata catactcttcaacac	385	BamH I/Sal I
YPO1435	acgggatccccaa ctcataaagatcagc	acgaagcttgtgttgc ctgtaactgggtttgg	802	BamH I/Hind III
YPO2118	acgtatccgtgat tatcgagatatggc	acgtgtcgacttagc tccgcttgtgaataac	217	BamH I/Sal I
YPO2131	acgtggatccacag ccaatgccaactctg	acgtgtcgacttacc attcagcctcgtatg	1398	BamH I/Sal I
YPO1613	acgggatccatga aaaggtatttatctc	acgaagctttcaa tattggacaggcgc	577	BamH I/Hind III
YPO3827	acgggatcctatct ggatcgtgtgaccg	acgaagcttggtgg cgtatttaggtagcc	739	BamH I/Hind III
LcrV	ccggaattcatgat tagagcctacgaac	cacgtcgacttaggc aaagtgagataattc	811	Ecol I/Sal I

optical spectra UCP particle as a marker. Niedbala et al. (2001) developed a rapid immunoassay for detecting four analytes using two different particles. They relied on different (antibody or antigen) biomolecules-UCP conjugates on one strip for multi-detection. It is susceptible for immunochromatography to false-binding of conjugates to non-paired test lanes, and UCP particles with different optical spectra require a complex instrument for signal acquisition. Both limited the immunoassay's multi-detection capability and performance. Therefore, it is necessary to develop a new multiplex detecting method that can simultaneously detect 10–20 targets with a relatively simple instrument. In this research, a 10-channel UPT-based LF (TC-UPT-LF) disc assay was developed using a double-antigen sandwich immunoassay to detect antibodies against *Y. pestis*. The TC-UPT-LF disc holds 10 kinds of LF strips with each in one channel for detecting the corresponding analytes simultaneously and quantitatively using a biosensor with a scanning function.

The F1 capsule antigen or its antibody is a commonly used target for *Y. pestis* diagnosis. However, there exists the risk of false-negative detection because a naturally-occurring F1 antigen-lacking *Y. pestis* mutant was found to be as highly virulent as the wild strain (Quenee et al., 2008). Therefore, discovery of complementary diagnostic targets is urgently needed. Previous studies in our laboratory (Li et al., 2005) showed that among about 200 known or predicted proteins of *Y. pestis*, more than 10 of them had strong immunogenicity to induce antibody production, which could be detected in the sera of a variety of plague infected animals by the protein chip method. Ten of these proteins were chosen for developing a double-antigen sandwich TC-UPT-LF assay to detect their corresponding antibodies in order to lay a foundation to evaluate more serum samples from different animals or humans with plague infections for screening new plague diagnostic markers.

2. Materials and methods

2.1. TC-UPT-LF stuffs

UCP ($NaYF_4$:Yb^{3+},Er^{3+}) with a diameter of about 200–300 nm was prepared and kindly provided by Dr. Yan Zheng from Shanghai Kerune Phosphor Technology Co. Ltd. (Shanghai, China). The peak of its exciting spectrum is 980 nm and that of its emitting spectrum is 541.5 nm.

The nitrocellulose membrane (SHF 1350225) and the glass fiber (GFCP20300) were obtained from Millipore Corp. (Bedford, MA). Papers (No. 470 and No. 903) were purchased from Schleicher & Schuell, Inc. (Keene, NH). The plastic cartridge of the TC-UPT-LF disc, showed in Fig. 1, and the laminating card were designed by our group and manufactured by Shenzhen Jincanhua Industry Company (Shenzhen, China) and Shanghai Liangxin Biotechnology Company (Shanghai, China), respectively.

The TC-UPT biosensor was designed and produced via collaboration between our laboratory and Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences (Shanghai, China).

2.2. Monkey and patient serum samples

Eighteen monkey sera (five healthy controls and 13 positive samples) and 59 human sera (51 from plague patients and eight from healthy people) were collected by the Qinghai Institute for Endemic Disease Control of China. The positive sera, verified by an indirect haemoagglutination assay for F1 antibody, were obtained respectively from monkeys challenged by *Y. pestis* 144 strain, which is one of China's reference strain isolated from marmot with high virulence to both animals and humans, and plague patients.

2.3. Expression of Y. pestis proteins

An *E. coli* expression system was employed to express nine proteins of *Y. pestis*, including YPO3827, YPO2131, LcrV, YPO1089, YPO1303, YopD, YPO1435, YPO1613 and YPO2118. The entire coding regions of these genes were amplified using PCR with the primers listed in Table 1 and cloned into plasmid pET28a or pET32a (Invitrogen Life Technologies, Carlsbad, California, USA). The recombinant plasmids were then transformed into *E. coli* BL21 cells (Invitrogen Life Technologies, Carlsbad, California, USA). Expression of the target proteins was induced by 1 mM IPTG and purified under

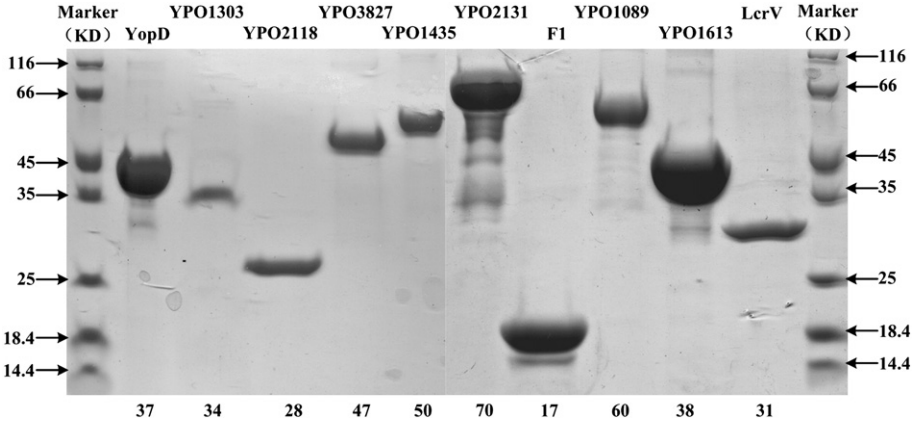

Fig. 2. SDS-PAGE result of the 10 purified proteins.

Table 2
Comparison of TC-UPT-LF disc and single-target LF strip precision.

Method	CV% of repeated tests									
	YPO3827	YPO2131	LcrV	YPO1089	YPO1303	YopD	YPO1435	YPO1613	F1	YPO2118
Disc	8.5	8.4	8.2	10.9	14.8	9.9	4.9	16.7	8.4	12.2
Strip	15.0	14.0	13.2	8.2	7.1	8.2	14.4	13.6	12.0	13.0

native or denatured conditions with a QIA expressionist™ Ni-NTA affinity chromatography (Qiagen, Germantown, Md.). Protein purity was verified by SDS-PAGE and the purified proteins were used for the following assays. F1 antigen was purified from *Y. pestis* live attenuated strain EV as reported previously (Wang et al., 2008).

2.4. Polyclonal antibody preparation

The polyclonal antibody corresponding to each of *Y. pestis* proteins was prepared by immunizing 3–4 kg clean-level New Zealand white rabbit using the conventional method reported elsewhere (Sutherland et al., 2004). All animal experiments were conducted in accordance with the Guidelines for the Welfare and Ethics of Laboratory Animals of China and Beijing Institute of Microbiology and Epidemiology. Rabbits were immunized three times with successive injections of 200, 400, and 800 μg protein hypodermically for the first and second times and intravenously for the last immunization. Specific antibodies in the sera were evaluated by indirect ELISA. Once its titer was higher than 1:25,600, blood was obtained from rabbits for collection of serum and purification of specific immunoglobulin G (IgG) with a caprylic acid-saturated ammonium sulfate precipitation method. Potency and quantity of the antibodies were measured by indirect ELISA and UV spectrometry, respectively.

2.5. TC-UPT-LF assay

The UCP-protein conjugates and UPT-LF strips were prepared according to the method described previously (Li et al., 2009). For developing double-antigen sandwich LF strips to detect antibodies, antigens (0.8 mg/ml, 1 μl/cm) and their corresponding antibodies (2 mg/ml, 1 μl/cm) were dispensed on the nitrocellulose membrane as the test lane (T) and control lane (C), respectively. UCP-antigen conjugate (1 mg/ml, 30 μl/cm) was fixed in the glass fiber as the conjugate pad. The plastic cartridge of TC-UPT-LF disc (Fig. 1) was designed to allow 10 UPT-LF strips to react simultaneously. The cartridge was comprised of a base and an upper cover that engaged with the base. Ten UPT-LF strips were set in the strip-holding channels of the base with the drainage piece overlapping with the sample pad of every strip. After the cartridge was assembled, the sample-adding window, the result-scanning window and the ending-index window of the upper cover just corresponded to the drainage piece, membrane and absorbent pad, respectively. By means of the symmetrical structure and the drainage piece, the samples applied through the sample-adding window could be distributed synchronously and uniformly into the 10 single-target strips, and thus this one-step assay could simultaneously detect 10 targets in one sample.

The principle and operating procedure of the TC-UPT biosensor were similar to those of the single-channel biosensor described previously (Yan et al., 2006). A high-precision two-dimensional scanning system was introduced in the newly developed biosensor to implement linear and rotary motions, so as to realize the scanning detection of 10 strips on the disc. The biosensor will capture the signals on 10 strips consecutively and respectively. The scanning and data report for 10 strips could be completed within 2 min.

The uniform sample-treating procedure was obtained through the systematic optimization of experimental conditions corresponding to each single-target strip. The serum sample was first diluted 10 times with the sample-treating buffer (0.03 M phosphate buffer containing 2% BSA, 0.1% SDS, 0.1% Triton X-100, pH7.2) and then 100 μl were applied per strip for single-target LF strip or 1000 μl per disc for TC-UPT-LF disc. After 15 min, the strip or disc could be scanned with a UPT biosensor for the results. The areas of the peaks corresponding to the test and control lanes were referred to as T and C respectively, and the ratio of T/C is the result of measurement. Samples with a T/C ratio higher than the cutoff threshold (mean + 2SD) were regarded as positive and vice versa.

2.6. Performance evaluation of TC-UPT-LF assay by standard samples

Standard samples with serial concentrations (0, 5, 7, 10, 30, 50, 70, 100, 300, 500 and 700 μg/ml), prepared by diluting the purified rabbit IgG corresponding to each of proteins with normal rabbit serum, were used to evaluate the performance of TC-UPT-LF assay.

For the TC-UPT-LF disc, the chromatographic consistency of each channel is critical for the synchronous and uniform multi-detection. Therefore, the coefficients of variation (CV) of the measurements between 10 same single-target LF strips and TC-UPT-LF disc detection were compared. A standard sample with a concentration of 1 mg/ml was used during the experiment.

Standard samples of each concentration were tested three times by the disc. Then, the quantitative curve was drawn and the sensitivity, linearity, and reproducibility corresponding to each kind of strip integrated in the disc were also evaluated. Because the mass concentration of antibodies between different animal species is not comparable, we employed an ELISA titer instead of the mass concentration of antibody during the quantitative test.

The TC-UPT-LF disc was stored at 37 °C for the accelerated aging test. The discs stored at 37 °C were taken out at different intervals during 21-day's evaluation to test the standard samples with three repeats for each concentration. The precision of detection was used as an index to evaluate the stability of the disc.

2.7. Performance evaluation of TC-UPT-LF assay by monkey sera

Eighteen monkey serum samples (five healthy controls and 13 positive samples confirmed by F1-based indirect haemoagglutination

Table 3
Comparison of ELISA and TC-UPT-LF assay sensitivity.

Method	Detection sensitivity for 10 targets[a] (μg/ml)									
	YPO3827	YPO2131	LcrV	YPO1089	YPO1303	YopD	YPO1435	YPO1613	F1	YPO2118
ELISA	3.13	1.56	1.56	0.20	0.39	6.25	1.56	0.39	0.39	0.39
UPT	18.20	7.67	0.97	0.41	2.75	97.11	3.90	30.36	0.37	3.39

[a] Each target is an antibody against a certain *Y. pestis* protein.

assay) were employed for parallel detection by both TC-UPT-LF and ELISA. The discrepant results were confirmed by a Western blotting (WB) assay.

The indirect ELISA method, with a coating of 30 to 50 ng expressed protein per well, was used to detect the antibody against the corresponding protein in the standard samples and monkey sera for evaluating the sensitivity and specificity of the TC-UPT-LF assay. The mean OD value of negative controls multiplied by 2.1 was adopted as the cutoff threshold.

WB assay was used as the confirmatory test for discrepant results between the TC-UPT-LF assay and ELISA. Prior to separation by electrophoresis on a sodium dodecyl sulfate (SDS)-12% polyacrylamide gel, the target protein solution and loading buffer were mixed with the same proportion and the mixture was loaded with 15 μg of the target protein in the lane. After electrophoresis, the protein was transferred to a polyvinylidene difluoride membrane (Osmonics Inc., Minnetonka, Minn.) by using a semi-dry transfer procedure (40 mA for

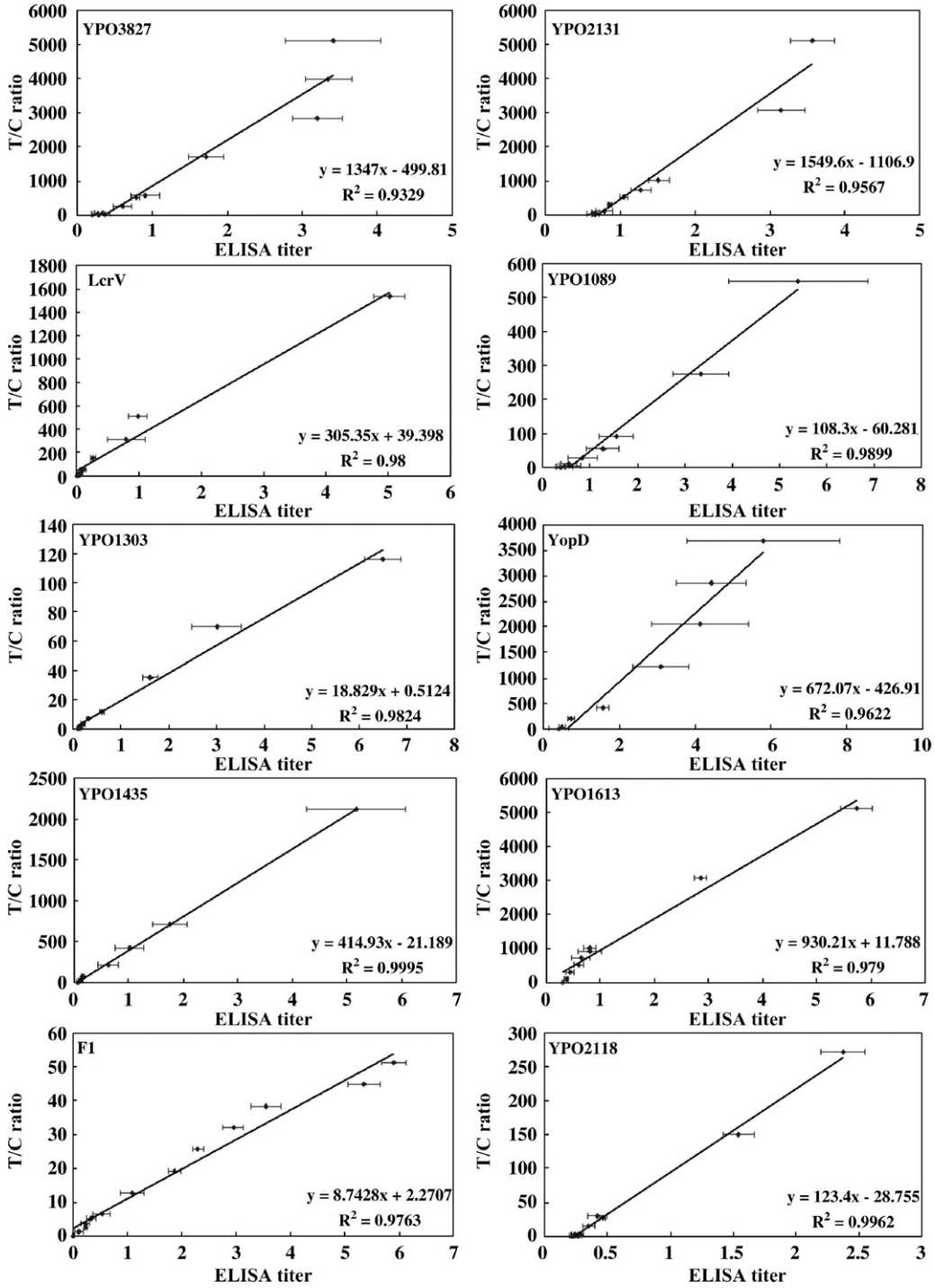

Fig. 3. Standard quantitative curves corresponding to the tests of the 10 targets. The dose–response relationship of the strips had a positive linearly correlation. With the measurements of UPT assay (T/C ratio) as *x*-axis and the ELISA titer (reciprocal type) of standard samples as *y*-axis, the quantitative equation and the linear fitting coefficient of determination (R^2) were deduced. By putting the T/C ratio of a given sample into the equation, the quantitative result demonstrated as an ELISA titer will be feedback.

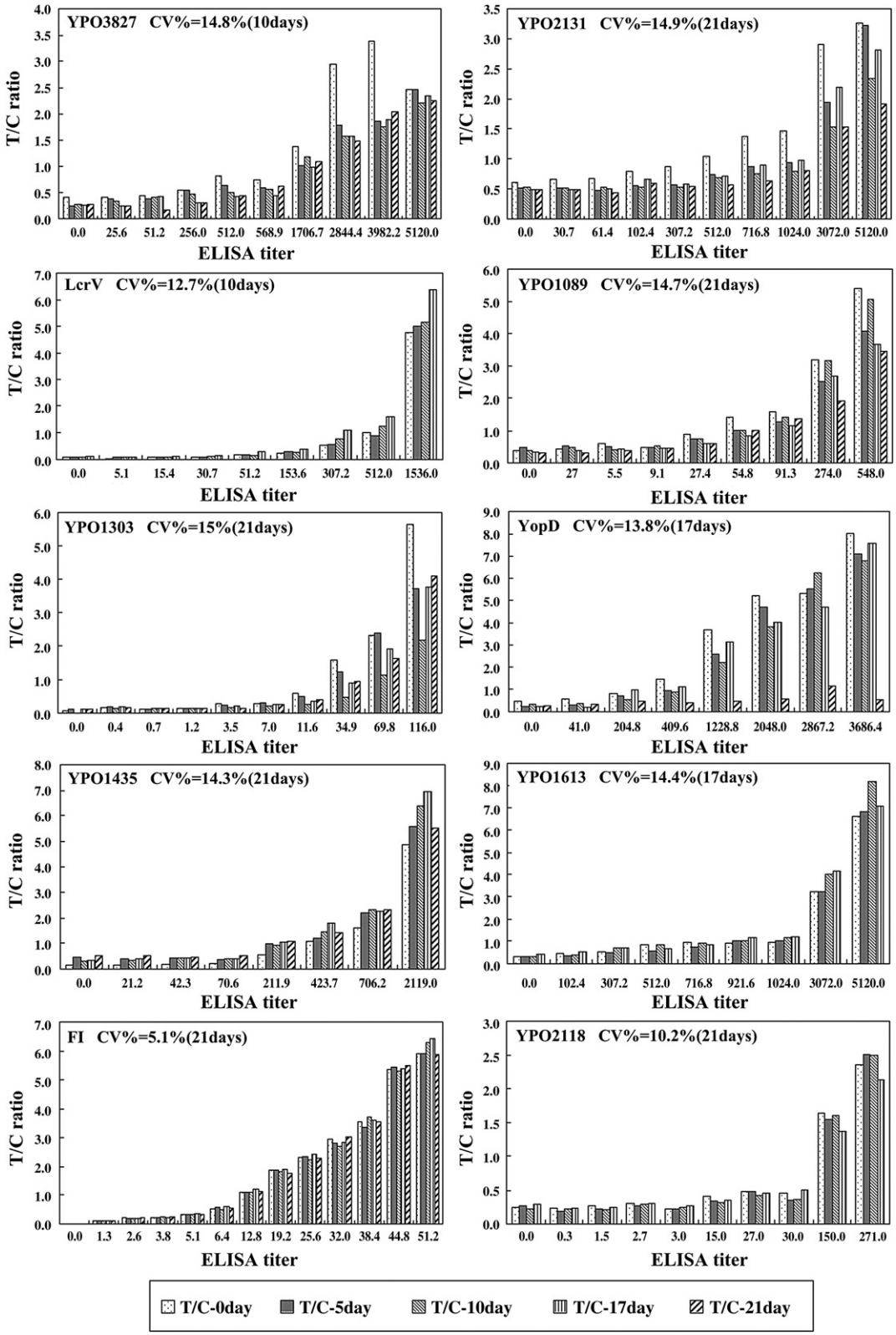

Fig. 4. The stability estimation of the TC-UPT-LF disc. Each histogram corresponds to one strip detecting a certain target, with the ELISA titer (reciprocal type) of the standard sample as *x*-axis and the T/C ratio of UPT assay as *y*-axis. The different legends represent different detection time points. The standard was defined that the mean value of CV% within the stability phase should not exceed 15%. The CV% value showed in each histogram is the mean value of a series CV% within the labeled time. The T/C ratios of samples with the same concentration had no significant difference statistically among time points within stability phase.

2 h) with a Trans-Blot SD kit (Bio-Rad Laboratories, Hercules, Calif.). Monkey sera were diluted 1:1 with 2× TBS buffer (40 mM Tris–HCl, 1 M NaCl, pH7.5), and incubated with the above immunoblotted membrane for one hour at 37 °C, and then, probed by a secondary HRP-conjugated goat anti-monkey antibody for 1 h at 37 °C. Finally, the results were visualized by a mixture of H_2O_2 and DAB chromogenic agent.

Table 4
The results of UPT assay and ELISA for 10 targets in 13 plague monkey sera.

Result of UPT and ELISA	Number of samples										
	YPO3827	YPO2131	LcrV	YPO1089	YPO1303	YopD	YPO1435	YPO1613	F1	YPO2118	Total
Both positive	3	2	12	3	7	12	0	1	13	6	59
Both negative	3	8	1	10	4	0	12	10	0	5	53
Discrepant result	7	3	0	0	2	1	1	2	0	2	18

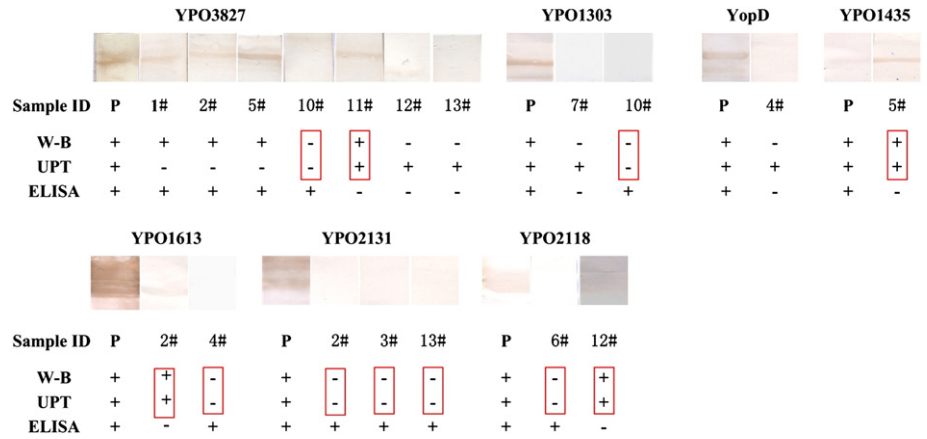

Fig. 5. The confirmatory experiment of WB assay. Samples showing the conflicting results between UPT assay and ELISA were further tested by WB assay. Eleven out of 18 UPT tests had the same results as with WB assay, while the others showed the identical results in both ELISA and WB assay.

2.8. Detection of patient sera by TC-UPT-LF disc

Fifty-nine human sera samples were detected by the TC-UPT-LF assay as the method described above.

3. Results and discussion

3.1. Expression of 10 Y. pestis proteins and preparation of their antibodies

All 10 proteins were successfully expressed in *E. coli* as soluble (YPO1613, LcrV and YopD) or inclusion body (YPO2131, YPO3827, YPO1435, YPO1303, YPO2118 and YPO1089) form. The proteins expressed as inclusion body were refolded with a urea gradient dialysis method. The purity of the 10 *Y. pestis* proteins was verified by SDS-PAGE, as shown in Fig. 2. These proteins were used for UCP-labeling and as the test lane in the strips.

These proteins were also used to immunize rabbits for preparing the corresponding specific antibodies. The ELISA titers and quantities (mg/ml) of antibodies were as follows: 1:51,200 and 9 for YPO3827, 1:204,800 and 20 for YPO2131, 1:204,800 and 40 for LcrV, 1:109,600 and 12 for YPO1089, 1:25,600 and 22 for YPO1303, 1:51,200 and 25 for YopD, 1:204,800 and 29 for YPO1435, 1:204,800 and 17 for YPO1613, 1:204,800 and 12 for FI antigen, and 1:51,200 and 17 for YPO2118, respectively. These antibodies were used as control lanes in the strips for each corresponding protein employed as a test lane, and also for diluting as standard samples.

3.2. Chromatographic consistency of TC-UPT-LF assay

The standard samples with the concentration of 1 mg/ml were tested 10 times by the single-target LF strips and the TC-UPT-LF disc, and their coefficients of variations (CV) with the corresponding protein are shown in Table 2. The mean CV of the former and latter was 11.9% and 10.3%, respectively, which indicated that the reproducibility of the disc was a little bit better than that of the strip. This is probably due to the fact that the traditional plastic cartridge of the single-target strip does not consider the overflow loss of sample during the flow reaction. This problem was solved by the drainage design in the TC-UPT-LF disc, so that the sample added into the disc can be distributed evenly and accurately.

3.3. Sensitivity, linearity, and reproducibility of TC-UPT-LF assay

The standard samples with the serial concentrations were tested simultaneously by TC-UPT-LF disc and ELISA method. Their sensitivities to the corresponding proteins are listed in Table 3. The data demonstrated that the sensitivities of UPT assay were similar to those of ELISA for detection of antibodies against LcrV, YPO1089, YPO1435 and F1 antigen, while for the others the sensitivity of ELISA was higher than that of UPT assay. This might be due to the expressed protein used in the experiments, which did not fit the immunochromatographic pattern well and needed to be optimized.

The quantitative curves (Fig. 3) of the 10 targets were drawn with the T/C ratio as the *x*-axis and the ELISA titer of standard samples as the *y*-axis. The molecular weight and affinity of antibodies vary dramatically among different animal species, hence there is no concordance between the mass concentration and the affinity of antibodies. The ELISA titer is the most common representative for antibody activity and is comparable among different animal species. Therefore, in our research the final quantitative result of UPT assay was demonstrated as an ELISA titer. As shown in the Fig. 3, UPT assay demonstrated a good linear response with the linear fitting coefficient of determination (R^2) (0.93–0.99) for each corresponding target protein, with a mean CV of 7.1% to 15.0%, indicating the good reproducibility of the strips.

3.4. Stability of TC-UPT-LF assay

The estimation of stability is shown in Fig. 4. With criterion of the mean CV before the expiration date should not be higher than 15%, the stability of different strips stored at 37 °C varied with 10 days for the LcrV- strip, 17 days for the YopD- and YPO1613-strips, and 21 days for the other seven strips. Therefore, the stability of the disc was considered to be 10 days at 37 °C.

Table 5
The results of UPT assay for 10 targets in 51 plague patient sera.

Target antibody	YPO3827	YPO2131	LcrV	YPO1089	YPO1303	YopD	YPO1435	YPO1613	F1	YPO2118
Positive number	18	24	34	24	11	44	28	11	51	2
Positive ratio	35.3%	47.1%	66.7%	47.1%	21.6%	86.3%	54.9%	21.6%	100%	3.9%

3.5. Antibody profiling in plague monkey sera

The antibody profiling results for the 10 targets in the 13 plague monkey sera are shown in Table 4. Most of the results (112/130) were in accordance between TC-UPT-LF and ELISA, while 18 of them were different. WB assay was used as the confirmatory method to validate any discrepant results (Fig. 5). Using the result of WB assay as the gold standard, 11 results of UPT assay and seven results of ELISA were correct, respectively. To 130 tests, the accurate ratio of TC-UPT-LF and ELISA were 94.6% (123/130) and 91.5% (119/130), respectively, without significant difference statistically (p 0.05) between the two methods.

In all positive plague monkey sera, except for the 100% positive reaction with the antibody against F1 antigen, the antibody against the LcrV and YopD showed a relatively higher positive ratio (12/13) than the other antibodies.

3.6. Antibody profiling in plague patient sera

For the quantitative result of monkey sera has been verified no statistical difference between the UPT and the ELISA, we then detected human sera using TC-UPT-LF assay to evaluate its feasibility in clinical practice.

The antibody profiling for the 10 targets in the 51 plague patient sera (Table 5) demonstrated that in all positive plague sera, antibody against F1 antigen was 100% positive, and those against YopD and LcrV were 86.3% (44/51) and 66.7% (34/51), respectively, indicating the potential application of LcrV and YopD as diagnostic markers. About half of the patients showed antibodies against YPO2131, YPO1089 and YPO1435.

4. Conclusion

In this study, we developed a TC-UPT-LF assay to make one-step quantitative detection of 10 targets possible. This assay does not require a complex optical instrument because the UCP used in ten strips is the same. Its independent immunochromatographic detection avoids the risk of steric hindrance and non-specific binding of multiple detections in one strip. This platform has been successfully used for profiling antibodies against *Y. pestis* with satisfactory repeatability, sensitivity, specificity and stability. By confirming the diagnostic significance of F1 antigen, we also found that LcrV and YopD are potential diagnostic markers of plague. This technique is a versatile platform that could be not only used for multi-antibody detection, but also adapted for multi-pathogen examination and for potential vaccine target evaluation in basic research.

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