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RESEARCH ARTICLE

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Assessment of recombinant antigens Tp0100 and Tp1016 of *Treponema pallidum* for serological diagnosis of syphilis

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Abstract

Objective: To discover novel serodiagnostic candidates for the serological diagnosis of syphilis.

Methods: Two recombinant *Treponema pallidum* proteins Tp0100 and Tp1016 were expressed, purified, and identified by Western Blotting. A total of 600 clinical serum samples were tested with the Tp0100-based ELISA, the Tp1016-based ELISA, and the commercial LICA Syphilis TP kit (ChIVD, Beijing, China). The sensitivities were determined by testing 340 samples from individuals with clinically diagnosed primary, secondary, latent, and tertiary syphilis. The specificities were determined by screening 260 samples from healthy controls and individuals with potentially cross-reactive infections, including leptospirosis, Lyme disease, hepatitis B, tuberculosis, rheumatoid arthritis, systemic lupus erythematosus. Kappa (κ) values were applied to compare the agreement between clinical syphilis diagnosis and the Tp0100-based ELISA, the Tp1016-based ELISA, or the LICA Syphilis TP test.

Results: Using clinical syphilis diagnosis as the gold standard, Tp0100 exhibited an overall sensitivity of 95.6% and specificity of 98.1% for testing IgG antibody while Tp1016 demonstrated only an overall sensitivity of 75.0% and specificity of 79.6%. In contrast, the LICA Syphilis TP test revealed an overall sensitivity of 97.6% and specificity of 96.2%. In addition, the overall percent agreement and corresponding κ values were 96.7% (95% CI 95.6%–97.8%) and 0.93 for the Tp0100-based ELISA, 77.0% (95%

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CI 74.3%–79.7%) and 0.54 for the Tp1016-based ELISA, and 97.0% (95% CI 96.0%–98.0%) and 0.94 for the LICA Syphilis TP test, respectively.

Conclusion: The recombinant *T. pallidum* protein Tp0100 shows promise as a novel diagnostic antigen in the serological tests for syphilis.

KEYWORDS
ELISA, recombinant protein, serodiagnosis, syphilis, Tp0100, Tp1016, *Treponema pallidum*

1 | INTRODUCTION

Syphilis is a chronic multisystemic disease caused by infection with the spirochete *Treponema pallidum* (*T. pallidum*) and acquired usually via sexual exposure or via vertical mother–fetus transmission or by blood transfusions.[1] Despite efforts being made to eliminate the disease, syphilis remains a global severe health concern because the syphilis vaccine is not available,[2] with an estimated 18 million cases worldwide and 5.6 million new cases annually.[3] Furthermore, occurrence of congenital syphilis is increasing, which results in more than 0.5 million stillbirths or neonatal deaths each year.[4] Additionally, active syphilis infection greatly increases risk of HIV infection and transmission.[5]

Owing to its diverse manifestations which often make clinicians confused, the clinical diagnosis of syphilis usually requires a combination of a clinician's diagnosis (including the patient's clinical manifestations, and/or history of sexual contact) and laboratory tests. Currently, diagnosis remains primarily dependent on serologic tests[6,7] due to the inability to culture *T. pallidum* in vitro.[8] The serological tests for the diagnosis of syphilis are divided into non-treponemal tests and treponemal tests. The non-treponemal tests, such as the venereal disease research laboratory (VDRL) test and, more commonly, the rapid plasma reagin (RPR) test are used to measure nonspecific anti-cardiolipin antibodies (reagin) and to monitor the treatment effect. These tests are inexpensive, simple to perform, and widely available, but the positive results require further confirmatory serologic tests to detect specific treponemal antibodies. The traditional treponemal tests, including the fluorescent treponemal antibody absorption (FTA-ABS) test, the *T. pallidum* hemagglutination (TPHA) assay, and the *T. pallidum* particle agglutination (TPPA) assay, are supposed to possess high sensitivity and specificity. However, these tests are expensive, labor-intensive, time-consuming, highly operator-dependent, and not suitable for high-volume screening.[9]

The traditional algorithm starts with a nontreponemal test,[10] followed by a confirmatory treponemal test, which is not suitable for large-scale syphilis screening. In recent years, to improve the efficiency of high-throughput screening, many laboratories in Europe[11,12] and the USA[13] recommended a reverse algorithm, which uses an automated recombinant antigen-based treponemal assay, such as enzyme immunoassay (EIA) and chemiluminescence immunoassay (CIA), to screen suspected samples. Reactive tests are followed by a quantitative non-treponemal test and the discordant samples are retested with another different treponemal test. The recombinant *T. pallidum* proteins-based EIAs and CIAs have been of great interest to researchers due to their objectivity, reproducibility, and automation.[14,15] However, current commercially available EIA/CIA kits have exhibited 17%–32% of discordant results in three studies.[16,17] There is no consensus on which recombinant *T. pallidum* protein is best for sensitivity and specificity in serological diagnosis of syphilis.[18] Therefore, assessment of additional sensitive and specific antigens is essential for the accurate serological diagnosis of syphilis.

Tp0100, a putative thioredoxin,[19] and Tp1016 (TpN39b), a basic membrane protein[20] have been previously reported to be reactive with sera from *T. pallidum*-infected rabbits as well as patients with different stages of syphilis.[21,22] In the current study, we evaluated the potential of these two recombinant proteins as diagnostic antigens. The sensitivities and specificities of two recombinant proteins were determined by screening sera collected from patients with clinically confirmed syphilis and sera from healthy controls or individuals with potentially cross-reactive infections, respectively. For direct comparison, the same serum samples were also measured by the LICA Syphilis TP test, a commercial CIA. Our data showed that the Tp0100-based ELISA was highly sensitive and highly specific in detecting IgG antibodies in individuals infected with different stages of *T. pallidum*. Therefore, Tp0100 may be a promising candidate for serological diagnosis of syphilis.

2 | MATERIALS AND METHODS

2.1 | Propagation of T. pallidum Nichols strain and isolation of genomic DNA

T. pallidum Nichols strain, a generous gift from Dr. Weiming Gu (Skin Diseases and Sexual Transmitted Diseases Hospital), was inoculated intratesticularly and passaged in mature male New Zealand White rabbits as described previously.[23] This study was approved by the Institutional Review Committee of the University of South China. The QIAamp DNA minikit (Qiagen) was used to extract the genomic DNA of *T. pallidum* according to the manufacturer's protocols.

2.2 | Human and rabbit serum samples

The current study was approved by the Human Ethics Committee of the University of South China, and all participants gave informed

consent. Human serum samples were collected from patients with clinically diagnosed primary (n = 21), secondary (n = 78), latent (n = 228), and tertiary (n = 13) syphilis, healthy controls (n = 60), and hepatitis B (n = 56), tuberculosis (n = 20), rheumatoid arthritis (RA) (n = 40), systemic lupus erythematosus (SLE) (n = 40) at the First People's Hospital of Changde between March 2019 and August 2020. According to the literature,[9] the diagnosis and staging criteria of syphilis were determined by combining patient's clinical signs, symptoms, sexual contact history, and laboratory serological tests as followings: primary syphilis, typical chancre present, usually coupled with regional lymphadenopathy, sexual contact with a syphilis patient and positive dark-field examination and/or TPPA and RPR; secondary syphilis, generalized cutaneous rash, mucocutaneous lesions or lymphadenopathy and positive TPPA and RPR; latent syphilis, no clinical manifestations and with a possible history of infection and positive TPPA and RPR; tertiary syphilis, typical clinical symptoms of more than 2 years duration, such as nodular syphilid, syphilitic gumma, neuropsychiatric illness, and cardiovascular involvement, and positive RPR and TPPA. Serum samples from individuals with confirmed leptospirosis (n = 24) and Lyme disease (n = 20) were obtained from the Hunan Provincial Center for Disease Control and Prevention and the Chinese Center for Disease Control and Prevention, respectively. The RPR (KHB) test and TPPA (Fujirebio) test were performed to test all the serum samples according to the manufacturers' protocols. Normal rabbit sera and *T.pallidum*-infected rabbit sera used for the Western blotting analysis were from healthy New Zealand White rabbits and New Zealand White rabbits infected with *T. pallidum* Nichols strain at 28 days postinfection, respectively.

2.3 | Expression, purification, and identification of recombinant proteins

Polymerase chain reaction (PCR) was performed to amplify the genes of *Tp0100* and *Tp1016* from genomic DNA of *T. pallidum* Nichols strain by using the following primers: forward primer 5′-GACACGGATCCATGAGCTGCAGCCG-3′ (*BamH*I) and the reverse primer 5′-GTGTCAAGCTTTTAATCACGCGCCAGTTCTTTG-3′ (*Hind*III) for *Tp0100*. forward primer 5′-GACACGGATCCATGGGCAGATACATAGTTC-3′(*BamH*I) and 5′-GTGTCCTCGAGTCACCAGTCGAGCACCTTG-3′ (*Xho*I) for *Tp1016*. The amplified genes were cloned into the expression plasmid pET28a (Merck Millipore, Darmstadt, Germany). The expression strain *E.coli* BL21(DE3) containing constructs were induced to express recombinant proteins overnight at 37°C with 0.7 mM (Tp0100) or 0.1 mM (Tp1016) isopropyl beta-D-thiogalactopyranoside (IPTG). Bacteria were collected and lysed in a buffer containing 300 mM NaCl, 1% Triton X-100, 20% glycerol, 50 mM Tris–HCl (pH 7.8), and 10 mM imidazole. The target proteins were purified by using Ni-NTA agarose beads (Qiagen), and the concentration of recombinant proteins was determined by BCA protein detection kit (Cwbio).

2.4 | Western blotting analysis

The purified recombinant proteins were separated by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred electrophoretically to polyvinylidene fluoride (Merck Millipore). After blocking with 5% bovine serum albumin (BSA) for 2 h at room temperature, the membrane was incubated overnight at 4°C with anti-His-tagged monoclonal antibody (Millipore) (1:1000), rabbit sera (1:500), or human sera (1:500). Finally, before being incubated with corresponding horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Merck Millipore) (1:10,000), goat anti-rabbit IgG (Abcam) (1:12,000) or goat anti-human IgG (Abcam) (1:8000) at 37°C for 1 h, membranes were washed five times for 10 min. The membrane was developed with the ECL Western Blot Kit (Pierce). The immunoreactive bands were quantified using software Alphaimager 2200.

2.5 | Indirect IgG enzyme-linked immunosorbent assays (ELISAs)

The recombinant protein antigens Tp0100 and Tp1016 were diluted with carbonate buffer (pH 9.6) to a concentration of 10 μg/ml and then added to 96-well microplates (Corning Costar) at 100 μl per well and incubated overnight at 4°C. The microplates were washed five times with PBS containing Tween-20 (PBST) and blocked with 5% BSA at 37°C for 2 h. Hundred μl of the serum samples was loaded at a dilution of 1:500 in PBST with 5% BSA. Following 1-h incubation at 37°C, the microplates were washed with PBST 5 times to remove unbound antibodies. The diluted HRP-conjugated goat anti-human IgG (Merck Millipore) at a dilution of 1:20,000 was added to each well and incubated for 45 min at 37°C. For detection of the immunocomplexes, plates were developed for 15 min at 37°C with 100 μl of tetramethylbenzidine-H_2O_2 substrate each well. The reaction was stopped with 100 μl of 0.2 M sulfuric acid following 15-min incubation at room temperature in the dark, and absorbance at 450 nm was measured using a Synergy HT Multi-Mode microplate reader (BioTek Instruments). for obtaining sample-to-cutoff ratio (S/CO) values. Each sample was tested in triplicate.

2.6 | LICA Syphilis TP test

The light-initiated chemiluminescent assay (LICA) is a new method derived from the luminescent oxygen channeling immunoassay. The LICA Syphilis TP (ChIVD) is a commercial available CIA kit (China Medical Device Registration Approval No. 20173401251), which uses double-antigen sandwich immunoassay method based on recombinant *T. pallidum* antigens TpN15, TpN17, and TpN47 for qualitative detection of the coresponding both IgG and IgM antibodies.[24] In this study, the LICA Syphilis TP test was performed to screen all the serum samples described above on a fully automated system

Chemclin® 1500 analyzer (ChIVD, Beijing, China) according to the manufacturer's instructions. The chemiluminescent reaction was measured in relative light units (RLUs), with the RLU/cutoff (S/CO) radio ≥1.0 indicating a positive result, and the S/CO radio <1 indicating a negative result.

2.7 | Statistical analysis

SPSS 18.0 prism software (SPSS) was used for statistical analysis of the data in this study. With the clinical diagnosis of syphilis (the combination of the serological tests and diagnosis from clinicians) as the gold standard, the agreement and kappa (κ) coefficients were calculated to evaluate the agreement between the ELISAs or LICA Syphilis TP test and clinical diagnosis. The agreement by κ values was considered almost perfect (0.81–1.0), substantial (0.61–0.8), moderate (0.41–0.6), fair (0.21–0.4), slight (0–0.2), or poor (<0).

3 | RESULTS

3.1 | Expression, purification, and identification of recombinant proteins Tp0100 and Tp1016

The *Tp0100* and *Tp1016* genes were successfully amplified by PCR. The His-tagged recombinant proteins Tp0100 and Tp1016 were expressed as an inclusion body and in the supernatant, respectively. The observed molecular masses were 22 kDa for Tp0100 and 39 kDa for Tp1016 (Figure 1A,C). SDS-PAGE analysis showed that the purity of the two recombinant proteins was more than 96% (Figure 1B,D). The immunoreactivities of Tp0100 and Tp1016 were confirmed by Western blotting. The anti-His monoclonal antibody, rabbit sera infected with *T. pallidum* ($n = 3$) or sera from patients with secondary syphilis ($n = 12$) showed to be reactive with these two recombinant proteins. However, no reactivities between recombinant proteins and sera from normal rabbits ($n = 2$) and sera from uninfected human ($n = 6$) were observed (Figure 2). These results preliminarily indicated that the two recombinant proteins had good immunoreactivity and some degree of antigenic specificity.

3.2 | Evaluation of recombinant proteins Tp0100 and Tp1016 by indirect ELISAs

To assess the sensitivities of these two recombinant proteins for the clinical diagnosis of syphilis, Tp0100 and Tp1016 were tested against 340 serum samples from clinically confirmed syphilis patients by Tp0100-based and Tp1016-based ELISAs. The overall sensitivity of the Tp0100-based ELISA and Tp1016-based ELISA was 95.6% and 75.0%, respectively. The sensitivities of the Tp0100-based ELISA and Tp1016-based ELISA were 90.5% and 61.9%, 98.7% and 69.2%, 94.7% and 78.1%, 100% and 76.9% for primary, secondary, latent, and tertiary syphilis, respectively (Table 1).

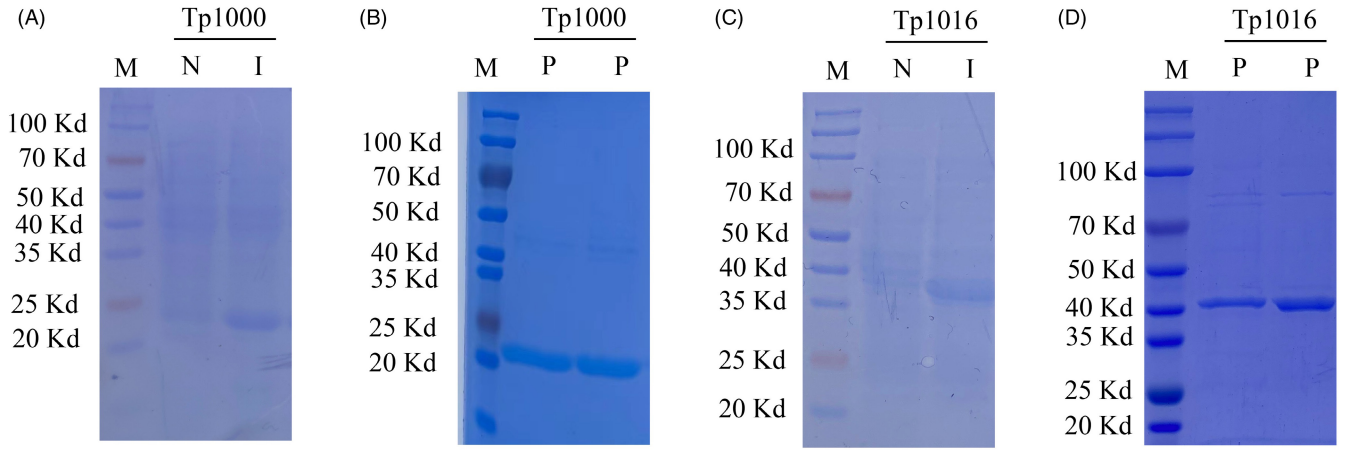

FIGURE 1 SDS-PAGE analysis of the expression and purification of recombinant proteins. (A, B) Tp0100 (22 kDa); (C, D) Tp1016 (39 kDa). I, induced with IPTG; M, molecular mass markers; N, noninduced; P, purification.

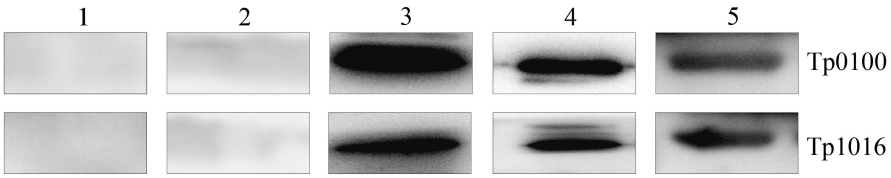

FIGURE 2 Western blotting identification of recombinant proteins Tp0100 and Tp1016 with sera from normal rabbits (1), sera from uninfected human (2), anti-His monoclonal antibody (3), sera from *T. pallidum*-infected rabbits (4), and sera from patients with secondary syphilis (5).

To determine the specificities of these two recombinant proteins, 260 serum samples from healthy controls (n = 60) and potentially cross-reactive infections (n = 200), including leptospirosis (n = 24), Lyme disease (n = 20), hepatitis B (n = 56), tuberculosis (n = 20), RA (n = 40), SLE (n = 40), were tested. The overall specificity of the Tp0100-based ELISA and Tp1016-based ELISA was 98.1% and 79.6%, respectively (Table 2).

3.3 | LICA Syphilis TP test

It was found that the LICA Syphilis TP showed an overall sensitivity of 97.6%, with sensitivities of 95.2%, 100%, 96.9%, and 100% for primary, secondary, latent, and tertiary syphilis, respectively (Table 1). The overall specificity is 96.2%, indicating positive reactions to sera from healthy controls (96.7%), patients with leptospirosis (91.7%), Lyme disease (90.0%), hepatitis B (96.4%), tuberculosis (95.0%), RA (100%), and SLE (97.5%) (Table 2).

3.4 | Direct comparison of the agreement between clinical syphilis diagnosis and the recombinant antigen-based IgG ELISAs or the LICA Syphilis TP

For 600 clinical serum samples, the agreement and κ values of the two recombinant antigen-based IgG ELISAs and the LICA Syphilis TP test were compared with clinical diagnosis, which is the gold standard. The overall percent agreements and corresponding κ values were as follows: Tp0100, 96.7% (95% CI 95.6%–97.8%), κ = 0.93; Tp1016, 77.0% (95% CI 74.3%–79.7%), κ = 0.54; LICA Syphilis TP, 97.0% (95% CI 96.0%–98.0%), κ = 0.94 (Table 3). The results indicated that the Tp0100-based ELISA and the LICA Syphilis TP had high agreement with clinical syphilis diagnosis.

TABLE 1 Comparison of sensitivity between recombinant protein-based ELISAs and LICA Syphilis TP at diverse phases of syphilis

	IgG (ELISAs) or IgG + IgM (LICA test)		
Protein and sera	Positive	Negative	Sensitivity (%)
Tp0100			
Primary syphilis	19	2	90.5
Secondary syphilis	77	1	98.7
Latent syphilis	216	12	94.7
Tertiary syphilis	13	0	100
All	325	15	95.6
Tp1016			
Primary syphilis	13	8	61.9
Secondary syphilis	54	24	69.2
Latent syphilis	178	50	78.1
Tertiary syphilis	10	3	76.9
All	255	85	75.0
LICA Syphilis TP			
Primary syphilis	20	1	95.2
Secondary syphilis	78	0	100
Latent syphilis	221	7	96.9
Tertiary syphilis	13	0	100
All	332	8	97.6

4 | DISCUSSION

Serological diagnosis of syphilis remains challenging despite the availability of commercially available diagnostic kits. Recombinant antigens have several advantages over the crude *T. pallidum* antigens used for TPPA or TPHA test. Firstly, since *T. pallidum* cannot be cultured in vitro,[8] crude *T. pallidum* antigens used for syphilis

TABLE 2 Comparison of specificity between recombinant protein-based ELISAs and LICA Syphilis TP in healthy controls and individuals with potentially cross-reactive infections

	IgG (ELISAs) or IgG + IgM (LICA test)		
Protein and sera	Positive	Negative	Specificity (%)
Tp0100			
Healthy controls	1	59	98.3
Leptospirosis	1	23	95.8
Lyme disease	1	19	95.0
Hepatitis B	1	55	98.2
Tuberculosis	1	19	95.0
RA	0	40	100
SLE	0	40	100
All	5	255	98.1
Tp1016			
Healthy controls	7	53	88.3
Leptospirosis	4	20	83.3
Lyme disease	6	14	70.0
Hepatitis B	18	38	67.9
Tuberculosis	2	18	90.0
RA	10	30	75.0
SLE	6	34	85.0
All	53	207	79.6
LICA Syphilis TP			
Healthy controls	2	58	96.7
Leptospirosis	2	22	91.7
Lyme disease	2	18	90.0
Hepatitis B	2	54	96.4
Tuberculosis	1	19	95.0
RA	0	40	100
SLE	1	39	97.5
All	10	250	96.2

TABLE 3 Direct comparison of recombinant protein-based IgG ELISAs and LICA Syphilis TP to clinical diagnosis

Assay	Clinical diagnosis (gold standard)		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Agreement (%) (95% CI)	κ value
	Positive	Negative				
Tp0100						
Positive	325	5	95.6 (92.8–97.3)	98.1 (95.6–99.2)	96.7 (95.6–97.8)	0.93
Negative	15	255				
Tp1016						
Positive	255	53	75.0 (70.1–79.3)	79.6 (74.3–84.1)	77.0 (74.3–79.7)	0.54
Negative	85	207				
LICA Syphilis TP						
Positive	332	10	97.6 (95.4–98.8)	96.2 (93.1–97.9)	97.0 (96.0–98.0)	0.94
Negative	8	250				

serodiagnosis need to be extracted from Treponemes passaged in rabbit testicular tissue and often contain contamination of other proteins, resulting in false positive tests. The recombinant antigen is easy to be produced on a large scale and economically in vitro and has high purity, which may reduce the occurrence of false positives caused by cross-reaction. Moreover, the use of recombinant antigens in automated EIAs or CIAs will facilitate more standardization of the immunological tests.[13] Moreover, new tests such as CIAs based on the recombinant antigens can distinguish between recent and previously treated syphilis infections.[25]

In the past decades, with the elucidation of *T. pallidum* genome and application of bioinformatics and immunoproteomics,[21,22,26,27] many recombinant *T. pallidum* proteins, such as TpN15 (Tp0171), TpN17 (Tp0435), TpN44.5 (TmpA, Tp0768), TpN47 (Tp0574), Tp0453, Tp92 (Tp0326),TpF1 (Tp1038), Tp463, Tp0663, and Tp0821, have been evaluated for their diagnostic value,[18,28–34] and some of them have been used as diagnostic antigens in commercial tests.[35,36] However, there is still no universally accepted serological test, and diagnosis of syphilis often relies on a combination of several assays.[37] Therefore, it is imperative to screen other more sensitive and specific antigens for serological diagnosis of syphilis.

Theoretically, surface-exposed outer membrane proteins may have superior sensitivity because of their direct exposure to the immune system and continual stimulation of effective antibody responses.[18] However, in fact, only an extremely low density of proteins could be found in the outer membrane of *T. pallidum*.[38] Moreover, despite the fact that lipoproteins used in current commercial tests for syphilis screening as diagnostic antigens, such as TpN44.5, TpN15, TpN17, and TpN47, are proved to be preliminarily located in inner membrane, they are able to stimulate high levels of antibody responses.[39] All these indicate that screening diagnostic antigens can not be limited to surface-exposed outer membrane proteins.

In the present study, Tp0100, a putative periplasmic thioredoxin, which reduces c-type cytochromes,[19] and Tp1016, a nonlipidated basic membrane protein,[20] were expressed in inclusion body form and soluble form in *E. coli*, respectively. Previous works from McKevitt and Brinkman showed that both recombinant Tp0100 and Tp1016 were reactive with sera from *T. pallidum*-infected rabbits at 14, 28, 56, and 84 days postinfection or with sera from patients with primary, secondary, and early latent syphilis.[21,22] Consistent with these results, in this study, the Western blotting analysis showed that these two recombinant proteins were reactive with sera from *T. pallidum*-infected rabbits or individuals with secondary syphilis. However, no reactivities were found between recombinant proteins Tp0100 and Tp1016 and sera from healthy controls. These results preliminarily indicated that the two recombinant proteins had good immunoreactivity and some degree of antigenic specificity.

To further assess the serodiagnostic potential of two recombinant proteins, we applied the Tp0100-based ELISA and the Tp1016-based ELISA as well as the LICA Syphilis TP test to detect 600 clinical serum samples, including 340 samples from confirmed syphilis patients and 260 samples from healthy controls and individuals with potentially cross-reactive diseases. The LICA Syphilis TP, a commercial available CIA kit, is highly sensitive and specific and could be used for *T. pallidum* antibody detection and syphilis screening.[24] Using the clinical diagnosis as the gold standard, the LICA Syphilis TP revealed an overall higher sensitivity (97.6%), especially in early syphilis stage, than the Tp0100-based ELISA (95.6%) but an overall lower specificity (96.2%) than the Tp0100-based ELISA (98.1%). The possible explanation for these results is that a combination of multiple treponemal antigens and the total antibody (IgG and IgM) detection in the LICA Syphilis TP may enhance the sensitivity than a single treponemal antigen and IgG antibody detection in the Tp0100-based ELISA. However, at the same time, the use of multiple treponemal antigens in the LICA Syphilis TP also may increase incidence of cross-reactivity, reducing the specificity of the test. In addition, the high agreement and corresponding κ values in this study indicated that the Tp0100-based ELISA and the LICA Syphilis TP were in perfect agreement with clinical diagnosis. Therefore, the recombinant Tp0100 protein may be an effective candidate for serological diagnosis of syphilis. However, Tp1016-based ELISA was found to exhibit only an overall sensitivity of 75.0%, specificity of 79.6%, 77.0% of percent agreement, and 0.54 κ value, suggesting that Tp1016 is not suitable for a diagnostic candidate.

There are several limitations in this study. One limitation is that it is difficult to obtain adequate serum samples from patients with primary and tertiary syphilis due to the widespread use of antibiotics in China. Another limitation is that inadequate or incorrect clinical data, including some patients using false names, denying sexual contact history to protect their privacy, or unclear course of illness, leads to potential misclassification of patients and difficulty of distinguishing between different stages of syphilis (e.g. early latent syphilis and late latent syphilis). Additionally, to increase the sensitivity of the detection of syphilis, especially primary syphilis, an IgM Tp0100-based ELISA or an IgM/IgG Tp0100-based ELISA needs to be further developed and evaluated.

Taken together, these results indicate that Tp0100 could be as a new diagnostic marker for screening syphilis. Nevertheless, before the Tp0100 antigen can be definitively demonstrated to be helpful in the accurate serodiagnosis of syphilis, more extensive testing with a larger number of clinical serum samples, especially serum samples from primary and tertiary syphilis patients and other control sera from individuals with infertility, cancer, HIV or HCV positive patients, will need to be performed.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

TThe datasets used and/or analyzed during this study are available from the corresponding author Tiebing Zeng on reasonable request.

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