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The optimum condition of the toluidine red unheated serum test for the replacement of the venereal disease research laboratory test in cerebrospinal fluid for neurosyphilis diagnosis

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ABSTRACT

Background: The cerebrospinal fluid (CSF) venereal disease research laboratory (VDRL) test remains the standard for the laboratory diagnosis of neurosyphilis. The toluidine red unheated serum test (TRUST) is an alternative to the VDRL test as a serological test for syphilis, but it lacks guidelines for its use in CSF for neurosyphilis diagnosis.

Methods: A total of 210 suspected neurosyphilis patients were included, consisting of 124 neurosyphilis patients and 86 syphilis/non-neurosyphilis patients. The TRUST was modified into the CSF-TRUST-10 test with 10 μL of antigen by referring to the CSF-VDRL test, and the CSF-TRUST-17 test with 17 μL of antigen by referring to its procedures in serum. The diagnostic performance of the CSF-TRUST-10 and CSF-TRUST-17 tests and the concordance between them and the CSF-VDRL test were evaluated.

Results: The diagnostic performance of the CSF-TRUST-10 and CSF-TRUST-17 tests for diagnosing neurosyphilis were comparable to the CSF-VDRL test, as well as the positive rate. The agreement rate was 98.7% between the qualitative CSF-TRUST-10 and CSF-VDRL tests. A total of 91.4% of the quantitative CSF-TRUST-10 results were consistent with the CSF-VDRL test, and the discordant results were no more than two titres. The agreement rate was 98.1% between the qualitative CSF-TRUST-17 and CSF-VDRL tests and 87.6% between the quantitative CSF-TRUST-17 and CSF-VDRL tests.

Conclusions: The CSF-TRUST with 10 μL of antigen could be an alternative for the CSF-VDRL test for neurosyphilis diagnosis. Our results provide a basis for using the TRUST to guide the diagnosis of neurosyphilis.

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1. Introduction

Neurosyphilis refers to an infection of the central nervous system by *Treponema pallidum*, and neurosyphilis can develop at any time during the course of syphilis. Globally, there are about 18 million people infected with *T. pallidum* [1]. The organism invades the CNS relatively early in the infection in approximately one-quarter of syphilis patients and leaves an estimated 4–9% of patients eventually developing late manifestations of neurosyphilis [2]. The clinical manifestation spectrum of neurosyphilis varies from an asymptomatic state to various neurological symptoms and even death. At present, the cerebrospinal fluid (CSF) venereal disease research laboratory (VDRL) test remains the standard for the laboratory diagnosis of neurosyphilis [3]. The VDRL test is based on an antigen composed of an alcoholic solution containing measured amounts of cardiolipin, cholesterol, and sufficient purified lecithin to measure antibodies to lipoidal material released from damaged host cells as well as to lipoprotein-like material and possibly by cardiolipin released from the treponemes [4]. However, the CSF-VDRL is technically cumbersome, the antigen suspension needs to be freshly prepared, and the results should be read with a light microscope. Thus, the reproducibility of the VDRL method depends on the reagent and the operator. In resource-limited settings, the CSF-VDRL test may not be widely available [5].

The VDRL test has been modified into the rapid plasma reagin test (RPR) and toluidine red unheated serum test (TRUST), in which sized charcoal particles or toluidine red particles are added to the VDRL antigen as markers to achieve direct visual observation of the results of an antigen-antibody reaction [4]. For the diagnosis of syphilis, the RPR test and TRUST are equivalent serologic methods to the VDRL method for serum samples [6,7]. For the diagnosis of neurosyphilis, the CSF RPR has been recommended as an alternative for the CSF-VDRL for neurosyphilis diagnosis [7]. In China, most laboratories perform the TRUST [8]. Lin et al. analyzed data involving 210 HIV-negative neurosyphilis patients and found that the CSF-TRUST had comparable sensitivity (76.2% versus 81.4%) and specificity (93.1% versus 90.3%) to the CSF-VDRL test [9]. Our previous study that included 824 syphilis patients also demonstrated that the overall agreement between the CSF-TRUST and the CSF-VDRL tests was 97.3% (802/824), indicating that the CSF-TRUST could be used as an option for CSF examination in settings without the CSF-VDRL test in place [10].

According to the manufacturer's instructions, the original design of the TRUST was for serum examination using 17 μL of antigen, so there were no guidelines for the operation and interpretation of the CSF-TRUST [11]. An antigen amount of 10 μL used for CSF-VDRL could be available for CSF-TRUST reference [12]. Here, this study aimed to further determine the optimum amount of antigen of the CSF TRUST for the replacement of the CSF VDRL test as a guideline for the diagnosis of neurosyphilis.

2. Methods

2.1. Study population and ethics statement

Between August 2017 and January 2021, a total of 210 patients who attended Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, were suspected of having neurosyphilis and underwent lumbar punctures. All of these patients underwent nontreponemal tests and treponemal tests using both serum and CSF. This study was approved by the Ethics Committee of the School of Medicine, Xiamen University (No. 2022-012), and it was conducted in compliance with the national legislation of China and the Declaration of Helsinki guidelines. Written informed consent was obtained from all of the study participants.

2.2. Diagnostic criteria

The diagnostic criteria for neurosyphilis were based on those as described in the guidelines of the Centers for Disease Control in the United States and Europe [6,7]. Briefly, neurosyphilis was defined as syphilis at any stage with a positive *T. pallidum* particle agglutination (TPPA) assay result based on a CSF sample and a combination of the following findings: (1) reactive VDRL in a CSF sample, (2) clinical symptoms or signs consistent with neurosyphilis without other known causes for these clinical abnormalities, and (3) elevated CSF protein concentration (>500 mg/L) or leukocyte count (>5 cells/μL) in the absence of other known causes of these abnormalities. Verified neurosyphilis was defined as meeting conditions (1) and (2); likely neurosyphilis was defined as meeting conditions (2) and (3); and possible neurosyphilis was defined as meeting condition (2). The above three classifications were all treated as neurosyphilis.

Table 1
The parameters of the operating procedures of the CSF-VDRL, CSF-TRUST-10, and CSF-TRUST-17 tests.

	CSF-VDRL	CSF-TRUST-10	CSF-TRUST-17
Specimen (μL)	50	50	50
Antigen (μL)	10	10	17
Reaction circle diameter (mm)	16	18	18
Rotation rate (rpm)	180	100	100
Rotation duration (min)	8	8	8

Abbreviations: CSF, cerebrospinal fluid; VDRL, venereal disease research laboratory; TRUST, toluidine red unheated serum test.

2.3. Laboratory tests

The TRUST (Shanghai Rongsheng Biotech Co., Ltd. Shanghai, China) and TPPA (Fujirebio, Tokyo, Japan) tests in both serum and CSF samples were performed for all participants, as well as the VDRL (Becton, Dickinson Co., Maryland, USA) test in CSF. The serological tests and CSF-VDRL test were performed according to the manufacturer's instructions and as previously reported [13].

There was no standard procedure of the TRUST on CSF. The CSF-TRUST was performed according to that of the serum tests using an 18 mm circle card test. First, 50 μL of the specimen was placed on a plastic-coated card; next, the specimen was spread within the area of the circle; and antigen was added with one drop of antigen. A mechanical rotator was used to mix the antigen and antibody. The mixture was rotated for 8 min at 100 rpm. The quantitative test was performed in a manner comparable to the qualitative test, except that serial twofold dilutions of the samples were prepared in 0.9% saline. The quantitative results were reported as the highest dilution giving a reactive result. In this study, the CSF-TRUST referred to the CSF-TRUST-10 test with 10 μL of antigen or the CSF-TRUST-17 test with 17 μL of antigen. The parameters of the operating procedures of the CSF-VDRL, CSF-TRUST-10 and CSF-TRUST-17 tests are shown in Table 1.

In TPPA test, the gelatin particles sensitized with *T. pallidum* antigen would be combined with the syphilis antibodies and produce agglutination reaction. The results of TPPA test could be visually evaluated because of the magenta color of gelatin particles [14]. The CSF-TPPA test was performed in a manner similar to that of the serum tests. The CSF protein levels were quantified using a Roche Cobas 8000 automatic biochemistry analyzer (Roche Diagnostics, F. Hoffmann La Roche Ltd., Switzerland). The CSF leukocyte counts were measured using an Automatic Blood Cell XE5000 Analyzer (Sysmex International Reagents, Co., Ltd, Japan).

2.4. Statistical analysis

The variables are presented as medians (interquartile range [IQR]) for the continuous variables or frequencies (%) for the categorical variables. The diagnostic performance of the nontreponemal tests was determined and was compared to the results of the diagnostic criteria for neurosyphilis using sensitivity and specificity according to the Yerushalmy model. McNemar's test and McNemar-Bowker test were used to test the difference between correlated proportions on a 2 × 2 classification table and $m \times m$ contingency table, respectively [15]. The strength of agreement between the paired groups of binary and ordinal categorical variables was defined according to the kappa and weighted kappa values, respectively, and was classified as almost perfect (κ, 0.81–1.00), substantial (κ, 0.61–0.80), moderate (κ, 0.41–0.60), fair (κ, 0.21–0.40), slight (κ, 0.00–0.20), and poor (κ < 0.00) [16]. All statistical analyses were performed using SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, USA). A two-sided *P* value of <0.05 was considered statistically significant.

3. Results

3.1. Demographic characteristics of the study population

A total of 210 participants were included in the study, consisting of 124 neurosyphilis patients and 86 syphilis/non-neurosyphilis patients. The neurosyphilis group included 91 (73.4%) males and 33 (26.6%) females, and their median age was 51 (IQR, 38–58) years. The non-neurosyphilis group included 38 (44.2%) males and 48 (55.8%) females, and their median age was 42 (IQR, 30–50) years.

3.2. The diagnostic performance of the CSF-TRUST-10 and CSF-TRUST-17 tests for neurosyphilis

In our study, with the diagnosis of neurosyphilis as the reference standard, the sensitivities of the CSF-VDRL, CSF-TRUST-10, and

Table 2
Comparison of the sensitivities and specificities of the CSF-VDRL, CSF-TRUST-10, and CSF-TRUST-17 tests for neurosyphilis diagnosis.

	Sensitivity comparison in neurosyphilis patients (n = 124)		P_1	Specificity comparison in syphilis/non-neurosyphilis patients (n = 86)		P_2
CSF-TRUST-10[a]	CSF-VDRL			CSF-VDRL		
	Positive	Negative		Positive	Negative	
Positive	57	0	0.500	0	0	n.a.
Negative	2	27		0	66	
CSF-TRUST-17	CSF-VDRL			CSF-VDRL		
	Positive	Negative		Positive	Negative	
Positive	85	0	0.125	0	0	n.a.
Negative	4	35		0	86	
CSF-TRUST-17	CSF-TRUST-10[a]			CSF-TRUST-10[a]		
	Positive	Negative		Positive	Negative	
Positive	57	0	>0.999	0	0	n.a.
Negative	0	29		0	66	

Abbreviation: CSF, cerebrospinal fluid; VDRL, venereal disease research laboratory; TRUST, toluidine red unheated serum test; n.a., not available.
[a] The results of the CSF-TRUST-10 test were missing for 38 neurosyphilis patients and 20 syphilis/non-neurosyphilis patients.

CSF-TRUST-17 tests were 71.8% (89/124), 66.3% (57/86), and 68.5% (85/124), respectively. There was no significant difference in the pairwise comparison of sensitivity (CSF-TRUST-10 versus CSF-VDRL, $P = 0.500$; CSF-TRUST-17 versus CSF-VDRL, $P = 0.125$; CSF-TRUST-10 versus CSF-TRUST-17, $P > 0.999$) (Table 2). The specificity was 100.0% for the CSF-VDRL, CSF-TRUST-10, and CSF-TRUST-17 tests. In consideration of the differences in sex and age between the neurosyphilis and syphilis/non-neurosyphilis patients, the sensitivity and specificity were further evaluated by a stratification analysis using sex and age, and there was no significant difference (data not shown).

3.3. The concordance between the qualitative CSF-TRUST-10 and CSF-TRUST-17 tests and the CSF-VDRL

As the CSF-VDRL test is in clinical practice, the positive rates of the CSF-TRUST-10 and CSF-TRUST-17 tests were compared. There was no significant difference in the positive rate between the CSF-TRUST-10 test and the CSF-VDRL test (37.5% [57/152] versus 38.8% [59/152], $P = 0.500$). The positive and negative predictive values of the CSF-TRUST-10 test were 100.0% and 97.9%, respectively. The agreement rate between the two tests was 98.7% (150/152), and the Kappa value was 0.972 (P for $\kappa < 0.001$), indicating that the strength of agreement was almost perfect.

In consideration of that the results of the CSF-TRUST-10 test were missed in 27.6% (58/210) of the participants, the distribution of basic characteristics of the participants with missing values was compared to that of the participants who were included in the analysis. The results showed that there was no significant difference in the age distribution (median age [IQR], 46 [37–56] versus 48 [32–57], $P = 0.697$) or neurosyphilis prevalence (65.5% versus 56.6%, $P = 0.239$) between participants with missing values and those included in the analysis. However, the sex ratio of the participants with missing values was significantly different from that of the included patients (male: female, 43:15 versus 86:66, $P = 0.019$). Therefore, further stratified analysis was carried out according to sex and age. After stratification by sex and age, there was still no significant difference in the positive rate between the two tests in each subgroup ($P > 0.05$), and the kappa value between the two tests varied from 0.926 to 1.0 in each subgroup (P for $\kappa < 0.001$).

There was no significant difference in the positive rate between the CSF-TRUST-17 test and the CSF-VDRL test (40.5% [85/210]

Table 3
Concordance between the qualitative CSF-TRUST-10 and CSF-TRUST-17 tests and the CSF-VDRL.

	CSF-VDRL		P for χ^2	Positive predictive value	Negative predictive value	Agreement	κ	P for κ
	Positive	Negative						
CSF-TRUST-10								
The whole cohort (n = 152)[a]			0.500	100.0%	97.9%	98.7%	0.972	<0.001
Positive	57	0						
Negative	2	93						
Subgroups by sex								
Male (n = 86)			>0.999	100.0%	100.0%	100.0%	1.0	<0.001
Positive	39	0						
Negative	0	47						
Female (n = 66)			0.500	100.0%	95.8%	97.0%	0.926	<0.001
Positive	18	0						
Negative	2	46						
Subgroups by age								
<60 (n = 127)			0.500	100.0%	97.6%	98.4%	0.965	<0.001
Positive	43	0						
Negative	2	82						
≥60 (n = 25)			>0.999	100.0%	100.0%	100.0%	1.0	<0.001
Positive	14	0						
Negative	0	11						
CSF-TRUST-17								
The whole cohort (n = 210)			0.125	100.0%	96.8%	98.1%	0.961	<0.001
Positive	85	0						
Negative	4	121						
Subgroups by sex								
Male (n = 129)			0.500	100.0%	97.0%	98.4%	0.969	<0.001
Positive	63	0						
Negative	2	64						
Female (n = 81)			0.500	100.0%	96.6%	97.5%	0.939	<0.001
Positive	22	0						
Negative	2	57						
Subgroups by age								
<60 (n = 176)			0.250	100.0%	97.3%	98.3%	0.964	<0.001
Positive	65	0						
Negative	3	108						
≥60 (n = 34)			>0.999	100.0%	92.9%	97.1%	0.939	<0.001
Positive	20	0						
Negative	1	13						

Abbreviations: CSF, cerebrospinal fluid; VDRL, venereal disease research laboratory; TRUST, toluidine red unheated serum test.
[a] The results of the CSF-TRUST-10 test were missing for 58 participants.

versus 42.4% [89/210], $P = 0.125$). The positive and negative predictive values of the CSF-TRUST-17 test were 100.0% and 96.8%, respectively. The agreement rate between the two tests was 98.1% (206/210), and the Kappa value was 0.961 (P for $\kappa < 0.001$), indicating that the strength of agreement was almost perfect. After stratifying by sex and age, there was still no significant difference in the positive rate between the two tests in each subgroup ($P > 0.05$), and the kappa value between the two tests varied from 0.939 to 0.969 in each subgroup (P for $\kappa < 0.001$) (Table 3).

3.4. The concordance between the quantitative CSF-VDRL and the CSF-TRUST-10 and CSF-TRUST-17 tests

Compared with the quantitative CSF-VDRL test, 91.4% (139/152) of the quantitative CSF-TRUST-10 results were in complete agreement with the results of that test. For the patients having the same qualitative CSF-TRUST-10 results as the CSF-VDRL test, 6.7% (10/150) of them had one-titre lower quantitative CSF-TRUST-10 results than the CSF-VDRL test, and 0.7% (1/150) of them had one-titre higher quantitative CSF-TRUST-10 results than the CSF-VDRL test. For the remaining two patients with discordant qualitative CSF-TRUST-10 results with the CSF-VDRL test, the quantitative CSF-VDRL results were all 1:1. There was no significant difference in the proportional distribution between the results of the two quantitative tests ($P > 0.05$). The weighted Kappa value between the two quantitative tests was 0.939 (P for $\kappa < 0.001$), indicating that the strength of agreement was almost perfect (Table 4).

Compared with the quantitative CSF-VDRL test, 87.6% (184/210) of the quantitative CSF-TRUST-17 results were in complete agreement. For the patients having the same qualitative CSF-TRUST-17 results as the CSF-VDRL test, 8.3% (17/206) of them had one-titre lower quantitative CSF-TRUST-17 results than the CSF-VDRL test; 2.4% (5/206) of them had one-titre higher quantitative CSF-TRUST-17 results than the CSF-VDRL test. For the remaining four patients with discordant qualitative CSF-TRUST-17 results with the CSF-VDRL test, the quantitative CSF-VDRL results were all 1:1. The proportional distribution of the quantitative CSF-TRUST-17 results was significantly different from that of the quantitative CSF-VDRL test ($P = 0.007$). The weighted Kappa value between the two quantitative tests was 0.919 (P for $\kappa < 0.001$), indicating that the strength of agreement was almost perfect (Table 4).

4. Discussion

During a syphilis infection, an antibody-like substance called reagin, which is formed by the host in response to lipids released from damaged host cells early in infection with *T. pallidum* and lipid-like material from the treponemal cell surface, can be detected in the patient's serum [17]. In syphilis infection of the central nervous system, reagin can be detected in the CSF. The CSF-VDRL test remains the only recommended test for the laboratory diagnosis of neurosyphilis until now [3]. However, the situation is even more challenging in China, as there is no commercial VDRL reagent approved by the National Medical Products Administration for CSF-VDRL examination [8]. Thus, this study determined the optimum amount of antigen of the CSF TRUST in replacement of the CSF VDRL test for neurosyphilis diagnosis. In our results, there was no significant difference in the diagnostic performance for neurosyphilis between the CSF-TRUST-10 tests and CSF-VDRL tests or with either positive rate. The agreement rates between the two tests were as high as 98.7%. The 1.3% discordant results only occurred in the condition of very low CSF-VDRL titres (≤1:1). For quantitation, the consistency between the CSF-VDRL and CSF-TRUST-10 tests was outstanding, of which the agreement rate was 91.4% and the weighted Kappa value was 0.939 (P for $\kappa < 0.001$), and the discordant results were no more than two titres, indicating that the strength of

Table 4
Concordance between the quantitative CSF-TRUST-10 and CSF-TRUST-17 tests and the CSF-VDRL test.

	CSF-VDRL									P for χ^2	Agreement	Weighted κ	P for weighted κ
	Negative	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128				
CSF-TRUST-10[a]										0.066	91.4%	0.939	<0.001
Negative	93	2	0	0	0	0	0	0	0				
1:1	0	16	2	0	0	0	0	0	0				
1:2	0	0	12	2	0	0	0	0	0				
1:4	0	0	1	8	5	0	0	0	0				
1:8	0	0	0	0	6	1	0	0	0				
1:16	0	0	0	0	0	2	0	0	0				
1:32	0	0	0	0	0	0	1	0	0				
1:64	0	0	0	0	0	0	0	0	0				
1:128	0	0	0	0	0	0	0	0	1				
CSF-TRUST-17										0.007	87.6%	0.919	<0.001
Negative	121	4	0	0	0	0	0	0	0				
1:1	0	20	4	0	0	0	0	0	0				
1:2	0	2	17	2	0	0	0	0	0				
1:4	0	0	3	11	9	0	0	0	0				
1:8	0	0	0	0	8	0	0	0	0				
1:16	0	0	0	0	0	5	2	0	0				
1:32	0	0	0	0	0	0	1	0	0				
1:64	0	0	0	0	0	0	0	0	0				
1:128	0	0	0	0	0	0	0	0	1				

Abbreviation: CSF, cerebrospinal fluid; VDRL, venereal disease research laboratory; TRUST, toluidine red unheated serum test.
[a] The results of the CSF-TRUST-10 test were missing for 58 participants.

agreement between the two tests was almost perfect.

Similarly, the diagnostic performance of the CSF-TRUST-17 test for neurosyphilis was comparable to that of the CSF-VDRL test, as was the positive rate. However, a larger amount of TRUST antigen did not bring a better diagnostic performance. The results of the quantitative CSF-TRUST-17 test fluctuated more, of which 2.4% were higher and 10.0% were lower than those of the CSF-VDRL test, resulting in a proportional distribution that was significantly different from that of the quantitative CSF-VDRL test.

As a modification based on the VDRL test, the TRUST used sized toluidine red particles added to the antigen as markers. Thus, the presence of flocculation of the antigen-antibody complex on the slides could be read macroscopically immediately after rotation [4]. Additionally, the TRUST antigen is added with choline chloride and ethylene diamine tetraacetic acid to enhance the reactivity of the test and stabilized the antigen suspension. The TRUST could be performed at room temperature (23–29 °C), and the TRUST reagents are stored at 2–8 °C. Compared to the VDRL test, the TRUST is easy to operate and is accessible in China, and there are multiple commercial TRUST reagents approved by the National Medical Products Administration for use in serum testing in the laboratory diagnosis of syphilis. A nationwide survey that included hospitals located in 116 cities in China demonstrated that of 154 hospitals that were capable of performing serological tests on CSF for syphilis, only 11.0% (17/154) of the hospitals could perform CSF VDRL tests, whereas most laboratories could only perform the CSF TRUST [8]. Our results indicated high consistency of the results between the CSF-TRUST and CSF VDRL tests, which provided strong evidence for the application of the CSF-TRUST in the diagnosis of neurosyphilis.

To the best of our knowledge, this is the first study to determine the optimum amount of antigen of the CSF TRUST in replacement of the CSF VDRL test for neurosyphilis diagnosis. However, this study has several limitations that need to be addressed. First, the included participants were suspected of having a diagnosis of neurosyphilis and had an indication for lumbar puncture and CSF examination for syphilis, but they were not selected randomly, raising the concern of selection bias. Additionally, for this reason, the prevalence of neurosyphilis, as well as the positive rate of the CSF-VDRL test, might be higher in the present study than in similar studies. Second, to be noted, although the sensitivity of the CSF-TRUST-10 test was very close to that of the CSF-VDRL, a rare missed diagnosis would still be unfortunate. Thus, a diagnosis or exclusion of neurosyphilis should be based on not only the result of CSF-TRUST-10 test but also a combination of clinical manifestations and other CSF parameters. Additionally, the sensitivity of TRUST in CSF still needs to be improved.

In conclusion, our study demonstrated that the CSF-TRUST using 10 μL or 17 μL of antigen had comparable diagnostic performance with the CSF-VDRL test for neurosyphilis. The CSF-TRUST with 10 μL of antigen was highly recommended as the optimal alternative for the CSF-VDRL test; the qualitative results of the CSF-TRUST could completely replace those of the CSF-VDRL test in clinical practice. Our results provide a basis for using TRUST to guide neurosyphilis diagnosis.

Author contribution statement

Yao Xiao: Analyzed and interpreted the data; Wrote the paper.
Man-Li Tong: Performed the experiments.
Yang Yang: Contributed reagents, materials, analysis tools or data.
Wei-Ming Gu; Li-Li Liu; Tian-Ci Yang: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] P.L.F. Neto, R.R.S. Fonseca, M.E.S. Avelino, E.M. Vilhena, M. Barbosa, C.A.F. Lopes, S.T.M. Gomes, B.J. Sequeira, R.V. Laurentino, F.B. Freitas, A.B. Oliveira-Filho, L.F.A. Machado, Prevalence and factors associated with syphilis in people living with HIV/AIDS in the state of para, Northern Brazil, Front. Public Health 9 (2021), 646663.
[2] T. Wong, K. Fonseca, M.A. Chernesky, R. Garceau, P.N. Levett, B. Serhir, Canadian public health laboratory Network laboratory guidelines for the diagnosis of neurosyphilis in Canada, can, J. Infect. Dis. Med. Microbiol. 26 (Suppl A) (2015) 18A–22A.
[3] K.A. Workowski, L.H. Bachmann, P.A. Chan, C.M. Johnston, C.A. Muzny, I. Park, H. Reno, J.M. Zenilman, G.A. Bolan, Sexually transmitted infections treatment guidelines, 2021, Mmwr Recommend. Rep. 70 (4) (2021) 1–184.
[4] S.A. Larsen, B.M. Steiner, A.H. Rudolph, Laboratory diagnosis and interpretation of tests for syphilis, Clin. Microbiol. Rev. 8 (1) (1995) 1–21.
[5] H. Gonzalez, I.J. Koralnik, G.D. Huhn, L.C. Tantalo, E.M. Ritz, Z. Orban, C.M. Marra, A dual-platform point-of-care test for neurosyphilis diagnosis, Sex. Transm. Dis. 48 (5) (2021) 353–356.

[6] U.S. CDC, Syphilis (Treponema pallidum) | 2018 case definition. https://ndc.services.cdc.gov/case-definitions/syphilis-2018/, 2021 (Accessed 16 April 2021).
[7] M. Janier, M. Unemo, N. Dupin, G.S. Tiplica, M. Potocnik, R. Patel, 2020 European guideline on the management of syphilis, J. Eur. Acad. Dermatol. Venereol. 35 (3) (2021) 574–588.
[8] F.Z. Du, Q.Q. Wang, Z.J. Zheng, X. Zhang, G.J. Liang, X.S. Chen, R.L. Zhang, The challenge of diagnosis and treatment of neurosyphilis in China: results from a nationwide survey, Sex Health 18 (4) (2021) 333–339.
[9] L. Zhu, X. Gu, R.R. Peng, C. Wang, Z. Gao, P. Zhou, Y. Gao, M. Shi, Z. Guan, A.C. Sena, Comparison of the cerebrospinal fluid (CSF) toluidine red unheated serum test and the CSF rapid plasma reagin test with the CSF venereal disease research laboratory test for diagnosis of neurosyphilis among HIV-negative syphilis patients in China, J. Clin. Microbiol. 52 (3) (2014) 736–740.
[10] W. Gu, Y. Yang, L. Wu, S. Yang, L.K. Ng, Comparing the performance characteristics of CSF-TRUST and CSF-VDRL for syphilis: a cross-sectional study, BMJ Open 3 (2) (2013).
[11] L. Shanghai, Rongsheng Biotech Co., syphilis toluidine red untreated serum test (TRUST). http://www.rsbio.com/, 2021 (Accessed 1 December 2021).
[12] BD, BBL™ VDRL test Control serum set. https://www.bd.com/, 2021 (Accessed 1 December 2021).
[13] Y. Xiao, W. Li, Q.-L. Li, Q.-Y. Xu, Y. Yang, T.-C. Yang, L.-L. Liu, W.-M. Gu, Which is the optimum antigen concentration for the venereal disease research laboratory test of cerebrospinal fluid for neurosyphilis diagnosis: 10 or 17 μL? Front. Med. 9 (2022).
[14] G.L. Coates, L. Guarenti, S.P. Parker, J.F. Willumsen, A.M. Tomkins, Evaluation of the sensitivity and specificity of a Treponema pallidum dried blood spot technique for use in the detection of syphilis, Trans. R. Soc. Trop. Med. Hyg. 92 (1) (1998) 44.
[15] A.H. Bowker, A test for symmetry in contingency tables, J. Am. Stat. Assoc. 43 (244) (1948) 572–574.
[16] J.R. Landis, G.G. Koch, The measurement of observer agreement for categorical data, Biometrics 33 (1) (1977) 159–174.
[17] K. Gao, X. Shen, Y. Lin, X.Z. Zhu, L.R. Lin, M.L. Tong, Y. Xiao, H.L. Zhang, X.M. Liang, J.J. Niu, L.L. Liu, T.C. Yang, Origin of nontreponemal antibodies during Treponema pallidum infection: evidence from a rabbit model, J. Infect. Dis. (2018) 835–843.