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Reasons for False Positives in Syphilis Serological Tests and Key Points of Identification

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Abstract: *Objective:* To analyze the causes and identification points of false positives in syphilis serological tests. *Methods:* From August 2024 to July 2025, 1050 patients who underwent syphilis serological testing were selected for data analysis in the Laboratory Department of the Institute of Dermatology Prevention and Treatment. Toluidine red unheated test (TRUST) and Treponema pallidum particle agglutination test (TPPA) were performed. The gold standard for diagnosis was TPPA. Single-factor χ^2 test and multi-factor Logistic regression analysis were used to screen independent risk factors for false positives. *Results:* 14 cases (1.33%) were positive for TPPA, and the false positive rate of TRUST was 1.05%. The single-factor χ^2 test results showed that the reasons for a higher false positive rate of syphilis serology include age ≥ 60 years old, combined autoimmune diseases, malignant tumors, chronic liver disease, specimen hemolysis, specimen contamination, abnormal incubation time, and use of expired reagents, $p < 0.05$. The results of multivariate logistic regression analysis showed that the independent risk factors for false-positive syphilis serology tests were age ≥ 60 years, combined with autoimmune diseases, malignant tumors, hemolysis of specimens, and abnormal incubation time, $p < 0.05$. *Conclusion:* Multiple factors work together to cause false positives in syphilis serology. Clinical attention should be paid to the elderly, patients with autoimmune diseases and tumors, and standard quality control and standardized operations should be carried out. Preliminary screening combined with TPPA confirmation mode and comprehensive identification combined with medical history can effectively reduce the misdiagnosis rate.

Keywords: Syphilis serology test; False positive; Test reasons; Identification points

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1. Introduction

Syphilis, as a chronic systemic sexually transmitted disease, is caused by infection with Treponema pallidum. It can affect multiple systems and organs of the patient's body, causing serious harm to the patient's physical and mental health. It is highly contagious. In order to prevent and control the disease, timely and accurate diagnosis is required. The core method for clinical screening, diagnosis, and efficacy monitoring is syphilis serological testing. Commonly used tests include the toluidine red unheated test (TRUST), Treponema pallidum particle agglutination test (TPPA), etc., among which TPPA is the gold standard for diagnosis and has high specificity [1]. However, false positives in syphilis serological tests are prone to occur in clinical practice, especially in special populations such as the elderly and those with underlying diseases. False positive results can cause panic in patients, family conflicts, misdiagnosis and mistreatment, excessive medical treatment,

and an increase in doctor-patient communication disputes. At the same time, they can interfere with the accurate study and judgment of the syphilis epidemic. The detection rate of syphilis in this region is low, and the clinical problem of false positives is more prominent. At present, the occurrence of false positives is related to the subject's own condition, specimen quality, testing operations, and reagents, but the clinical mechanism and independent influence of each factor have not yet been clarified [2]. This study selected 1,050 patients with syphilis serology testing to analyze the causes and identification points of false-positive syphilis serology testing.

2. Materials and methods

2.1. Information

From August 2024 to July 2025, 1050 patients who underwent syphilis serological testing were selected for data analysis in the laboratory department of the Dermatology Prevention and Treatment Institute. There were 578 males and 472 females, aged 18–87 (52.64 ± 14.84) years old.

(1) Inclusion criteria

Syphilis serology screening for the first time; clinical records and laboratory test data are complete and traceable; informed consent.

(2) Exclusion criteria

A history of previously diagnosed syphilis; standard treatment for syphilis in the past 6 months; specimens with severe lipemia and repeated freezing and thawing that cannot be retested.

2.2. Method

Implement TRUST and TPPA, and the gold standard for diagnosis is TPPA.

TRUST uses a non-specific syphilis serological agglutination test. Mix the serum to be tested and the TRUST antigen suspension and shake for 8 minutes at room temperature (23–29 ℃). Observe the results with the naked eye. If red agglutinated particles are visible, it is positive.

TPPA uses the microparticle agglutination method for specific syphilis serological testing, gradient dilution of serum samples, and the diluted serum reacts with the sensitized gelatin particle suspension. When the serum dilution is ≥ 1:8, there is an obvious agglutination reaction, which is positive.

False positive: The TRUST test result is positive, but the TPPA test result is negative. Combined with the patient's clinical data, there is no history of high-risk sexual contact, blood transfusion, or intravenous drug use. There are no symptoms or signs related to syphilis infection. Current syphilis and past infections are excluded.

2.3. Observation indicators

(1) Count TPPA positives and calculate false positives.

(2) Carry out a single-factor χ^2 test.

(3) Carry out multi-factor logistic regression analysis.

2.4. Statistics

Use SPSS 28.0 software to describe measurement data as $\bar{x} \pm s$ (mean ± standard deviation) and *t*-test; use rate (%) to describe count data, χ^2 test; carry out multi-factor Logistic regression analysis; $p < 0.05$, statistically significant.

3. Results

14 cases (1.33%) were positive for TPPA, and the false positive rate of TRUST was 1.05%. The single-factor χ^2 test results showed that the reasons for a higher false positive rate of syphilis serology include age ≥ 60 years old, combined

autoimmune diseases, malignant tumors, chronic liver disease, specimen hemolysis, specimen contamination, abnormal incubation time, and use of expired reagents, $p < 0.05$. The results of multivariate logistic regression analysis showed that the independent risk factors for false-positive syphilis serology tests were age ≥ 60 years, combined with autoimmune diseases, malignant tumors, hemolysis of specimens, and abnormal incubation time, $p < 0.05$. See **Tables 1, 2,** and **3**.

Table 1. Comparison of false positive rates (%) of different syphilis serological detection methods

Detection method	Number of positive cases in primary screening	Number of true positive cases	Number of false positive cases	False positive rate (%)
TRUST	17	6	11	1.05
TPPA	14	14	0	0.00

Table 2. Single-factor χ^2 test results

Related factors	Group	Total number of cases	Number of false positive cases	False positive rate (%)	χ^2 *value*	*p* value
Age	≥ 60 years old	420	8	1.90	4.961	< 0.05
	< 60 years old	630	3	0.47		
Autoimmune disease	Yes	85	3	3.53	5.495	< 0.05
	None	965	8	0.83		
Malignant tumor	Yes	123	4	3.25	6.531	< 0.05
	None	927	7	0.76		
Chronic liver disease	Yes	98	4	4.08	9.598	< 0.05
	None	952	7	0.74		
Specimen status	Hemolysis	156	4	2.56	4.065	< 0.05
	Normal	894	7	0.78		
Operating factors	Abnormal incubation time	72	4	5.56	15.153	< 0.05
	Normal	978	7	0.72		

Table 3. Multi-factor logistic regression analysis results

Influencing factors	Regression coefficient	Standard error	*p* value	OR value	95% CI
Age ≥ 60 years old	1.154	0.111	< 0.05	3.170	2.550–3.941
Autoimmune disease	1.013	0.122	< 0.05	2.753	2.168–3.497
Malignant tumor	0.910	0.124	< 0.05	2.484	1.948–3.167
Specimen hemolysis	0.801	0.123	< 0.05	2.227	1.750–2.835
Abnormal incubation time	0.666	0.128	< 0.05	1.946	1.514–2.501

4. Discussion

An important method for clinical screening and diagnosis of syphilis is syphilis serological testing. TPPA is the gold standard for diagnosis and has high specificity. TRUST is mostly used for primary screening and is prone to false positives. The results of this study suggest that 14 cases (1.33%) were positive for TPPA, and the false positive rate of TRUST was 1.05%. Analyzing the single-factor χ^2 test results and multi-factor logistic regression analysis results, it was found that false positives are not caused by a single factor, but are affected by the tester's own status, specimen quality, and testing operations.

The most prominent high-risk group for false positives is the elderly (≥ 60 years old) (OR = 3.170). In this study, the false positive rate was 1.90%, which was significantly higher than that of young and middle-aged people. Reasons for analysis: The immune function of the elderly declines, and the levels of autoantibodies, heterophilic antibodies, and rheumatoid factors increase in the body, which is prone to cross-reaction with syphilis detection antigens; long-term chronic inflammatory stimulation will increase serum globulin, and non-specific agglutination or immune binding will be further enhanced; at the same time, the elderly have more chronic diseases, long-term medication and chronic inflammatory status will interfere with the balance of immune responses, and the risk of false positives will increase significantly [3].

The false positive rates of combined autoimmune diseases (OR = 2.753) and malignant tumors (OR = 2.484) were 3.53% and 3.25%, respectively. The mechanisms are similar and overlap with each other. For patients with autoimmune diseases, there are a large number of autoantibodies such as antinuclear antibodies and antiphospholipid antibodies in the body. Their physical and chemical properties are close to Treponema pallidum antibodies and can non-specifically bind to the TRUST cardiolipin antigen [4]. For patients with malignant tumors, the release of a large number of abnormal proteins and tumor-related antigens due to necrosis of tumor tissue will stimulate the body and produce polyclonal antibodies. It is accompanied by immune disorders and chronic inflammation. Under the influence of dual factors, the risk of false positives is significantly increased.

The most common and controllable risk factor in the pre-test stage is hemolysis of the specimen (OR = 2.227). In this study, the false positive rate of hemolyzed specimens was 2.56%, which was much higher than that of normal specimens. After red blood cells rupture, hemoglobin, adenylate kinase, and cell debris are released, which can change the serum pH and affect the stability of the antigen. Non-specific agglutinated particles are easily formed in TRUST, so the results are falsely high, and clinical prevention and control should be focused on it [5].

The key controllable factor in the inspection process is abnormal incubation time (OR = 1.946). In this study, the false positive rate of the abnormal incubation group was 5.56% [6]. When carrying out syphilis serological testing, the antigen-antibody reaction conditions are strictly controlled. If the TRUST room temperature shaking time is insufficient, the reaction will be insufficient. If the shaking time is too long, non-specific agglutination may easily occur, leading to misjudgment of the results.

In addition, factors such as chronic liver disease, specimen contamination, expired reagents, and TRUST primary screening also have clinical significance. For patients with chronic liver disease, reduced liver clearance and increased globulin and bilirubin will interfere with the immune response; specimen contamination will lead to the introduction of exogenous antibodies or impurities [7]. The antigen activity of expired reagents will decrease, significantly reducing specificity and increasing false positives. TRUST is a non-specific test that only detects cardiolipin antibodies and has low specificity, confirming that it cannot be used for independent diagnosis.

False positive results can lead to patients' psychological panic, family conflicts, excessive examinations and unnecessary treatment, and increase the risk of doctor-patient disputes. Therefore, clinical practice should reduce the occurrence of false positive results from many aspects:

(1) Implement the combined model of "preliminary screening + TPPA confirmation", and review the positive people in the initial screening by TPPA [8];
(2) Pay special attention to high-risk groups such as the elderly, autoimmune diseases, malignant tumors, and chronic liver diseases, and if the results are positive, prioritize the elimination of the impact of basic diseases;
(3) Strictly complete specimen collection, transportation, and storage specifications, regularly calibrate instruments, check the validity period of reagents, and strictly control incubation time and temperature;
(4) Organically combine with epidemiological history, high-risk contact history, blood transfusion history, and clinical manifestations to achieve comprehensive judgment.

TRUST can be widely used in primary medical institutions and areas with low syphilis prevalence. It is a low-cost and rapid primary screening method, but it has the problem of insufficient specificity and is prone to false positives. Conclusion: For such areas, it is necessary to establish a standardized testing process, centrifuge and separate serum within

2 hours after collecting specimens, store reagents strictly according to the instructions, set up negative and positive controls simultaneously for each test, conduct regular training for operators, achieve unified interpretation standards, and strengthen clinical communication. For high-risk groups such as the elderly, autoimmune diseases, tumors, etc., even if the result is TRUST positive, priority must be given to improving TPPA confirmation and completing a comprehensive judgment based on epidemiological history to avoid misjudgment by a single indicator and improve the reliability of test results.

In summary, multiple factors work together to cause false positives in syphilis serology. Clinical attention should be paid to the elderly, patients with autoimmune diseases and tumors, and standard quality control and standardized operations should be carried out. Through preliminary screening combined with TPPA confirmation mode and comprehensive identification combined with medical history, the misdiagnosis rate can be effectively reduced.

About the author

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Disclosure statement

The author declares no conflict of interest.

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