Validation of automated AIX1000 rapid plasma reagin system for the diagnosis of syphilis.

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ABSTRACT

Here we validate usage of automated AIX1000 rapid plasma reagin (RPR) system in syphilis diagnosis.

Despite the existence of numerous diagnostic methods and the efficacy of antibiotic treatment, the prevalence and occurrence of syphilis persist at elevated levels with emerging increasing trend.

Manual nontreponemal serological testing has traditionally been a cornerstone in syphilis diagnosis, despite significant limitations such as time consumption, labor intensiveness, and subjective interpretation of agglutination reactions, requiring expertise for accurate assessment.

The AIX1000 system is an automated platform designed for nontreponemal RPR testing. Our findings indicate that results obtained from the AIX1000 system are comparable to those from manual VDRL nontreponemal assays. Notably, the AIX1000 system offers objective and consistent data interpretation through pattern recognition software, ensuring accuracy and reproducibility. Additionally, the system provides complete traceability from sample to result, along with storage of result images, offering a convenient and robust tool for syphilis diagnosis.

Keywords: Syphilis, *Treponema pallidum*, AIX1000, RPR, VDRL, nontreponemal test

INTRODUCTION

Syphilis, a chronic venereal disease in humans caused by *Treponema pallidum* subsp. *pallidum* (*T. pallidum*), progresses through multiple stages (Plagens-Rotman et al., 2021). This pathogenic gram-negative bacterium, characterized by its motile spirochete form, is typically transmitted through sexual contact, gaining entry into the host through breaches in squamous or columnar epithelium (Xie et al., 2022).

The primary mode of syphilis transmission is through sexual exposure. Less commonly, the disease can be spread through the transfusion of unscreened blood or blood products, artificial insemination, organ or bone marrow transplantation, or indirect transmission via infected materials (in case of an immediate exposure). Additionally, since the later weeks of the first trimester of pregnancy, the organism can pass transplacentally to the fetus, leading to the development of congenital syphilis (Xiong et al., 2023).

According to the World Health Organization's estimates, around 22.3 million individuals worldwide aged 15 to 49 were afflicted with syphilis in 2020, with approximately 5.6 million new cases emerging annually (Mercuri et al., 2022). In the Czech Republic, a nation with roughly 10.5 million inhabitants, recent years have seen an average of 700 to 800 cases of syphilis annually, as reported by the Institute of Health Information and Statistics of the Czech Republic (Vrbová et al., 2019).

The majority of syphilis cases have been identified among men who have sex with men (MSM), individuals engaging in promiscuous behavior, or those involved in sex work. There has been a significant rise in syphilis incidence among MSM, especially among those who are HIV-positive. As the number of syphilis-infected individuals continues to grow, timely diagnosis and treatment are crucial for reducing syphilis prevalence (Xiong et al., 2023).

For effective containment of syphilis transmission, a precise and streamlined diagnostic method is of great importance (Luo et al., 2021). Swift and precise identification of syphilis is critical for prompt treatment and for curtailing further spread of the infection.

Serological assays represent the primary diagnostic methods widely employed in clinical laboratories (Tuddenham et al., 2020). Traditionally, serological screening for syphilis has relied on manual nontreponemal tests like the rapid plasma reagin (RPR) and the Venereal Disease Research Laboratory (VDRL) tests. These tests involve examining serum or plasma for the presence of total antibodies (IgM and IgG) targeting lipoidal antigens, such as cardiolipin, released from compromised host cells. Reactive specimens are subsequently subjected to further pathogen-specific assays to confirm a syphilis diagnosis (Satyaputra et al., 2021).

When conducted manually, RPR and/or VDRL tests are susceptible to potential misinterpretations of test reactions and variations in interpretation between individuals. This is because the results are subjectively assessed through visual inspection of the agglutination reaction (Hamill et al., 2018).

Over the past few years, automated systems for RPR testing have emerged in clinical practice (Shukla et al., 2023). The implementation of automated diagnostic instruments diminishes the likelihood of technique-related errors, offers standardized data interpretation, introduces a digitalized sample tracking system, and decreases both the turnaround time for screening a large volume of samples and the hands-on time required.

The aim of this study was to assess the effectiveness of the fully automated AIX1000 instrument for RPR testing, comparing it with the standard manual VDRL test used by the National Reference Laboratory for the Diagnostics of Syphilis in the Czech Republic at the time of the research.

Our findings indicate that there is comparability between the results obtained from the AIX1000 and the manual VDRL tests. While acknowledging that the study's sample size is limited, it's worth noting that the samples encompass a wide range of syphilis infection states. This suggests that the AIX1000 is a suitable instrument for conducting robust and unbiased nontreponemal RPR screening across various samples within clinical settings.

METHODS AND MATERIALS

Clinical Material

The serum samples were sourced from the collection of the National Reference Laboratory for the Diagnostics of Syphilis in the Czech Republic. Among these samples, nineteen were obtained from patients without a diagnosed case of syphilis, fifty were collected from patients newly diagnosed with syphilis and without prior treatment, and the remaining fifty were acquired from patients who had received treatment for syphilis.

Study Design

A panel comprising 119 serum samples underwent testing using the AIX1000 Agglutination analyzer, employing the RPR method in accordance with the instrument's instructions. The AIX1000 RPR kit utilized for the testing had the number 902942773. Subsequently, the results obtained from this test were compared with those from the Syphilis VDRL DiaLab Gmbh test, which was conducted on the same set of samples following the standard procedures of the National Reference Laboratory for the Diagnostics of Syphilis in the Czech Republic. Moreover, to confirm the negativity of syphilis in the serum samples, additional tests such as TPPA, TPHA (in 19 of and in 12 of 19 samples respectively), FTA-Abs IgG (in 19 of 19), and 19S IgM SPHA (in 19 of 19) were performed. Serum samples obtained from patients with syphilis (untreated or treated) were also tested completely by Syphilis VDRL (in 100 of 100 samples), TP-PA, FTA-ABS IgG and 19S IgM SPHA (in 100 of 100), TPHA (in 35 of 100).

Data Interpretation

The raw data for the study can be accessed in the supplementary table. Test results were interpreted in accordance with the instructions provided for the AIX1000 Agglutination analyzer or the VDRL-micro DiaLab Gmbh test, as appropriate.

RESULTS

AIX1000 instrument measured correctly negative syphilis samples.

The study examined 19 samples that tested negative for syphilis using the AIX1000 instrument, aiming to assess the consistency in detecting sample negativity. These negative samples were sourced from newborns or individuals who had previously received false-positive diagnoses for syphilis infection via EIA or immunoturbidimetry. The syphilis negativity of these samples was evaluated through various tests, including the nontreponemal VDRL test, as well as specific treponemal tests such as TPPA and TPHA. Furthermore, negativity was confirmed through additional tests including the fluorescent treponemal antibody absorption test (FTA-Abs IgG) and the 19S IgM solid phase haemadsorption assay (19S IgM SPHA). The results revealed a 100% correlation in the negativity of syphilis samples as determined by the AIX1000 instrument (**Table 1**). Hence, the AIX1000 instrument exhibited precise detection of negative syphilis samples, demonstrating correlation with the Syphilis VDRL DiaLab and other methodologies.

AIX1000 succeeded in detection of syphilis in most of the syphilis positive serum samples.

While serologic tests are generally characterized by high sensitivity, their efficacy is believed to vary depending on the stage of syphilis. These tests tend to exhibit high sensitivity during the secondary and later stages of the disease. However, their ability to detect cases of primary infection is notably reduced. Several studies propose that between 14% and 46% of patients with primary syphilis may test seronegative, with the specific percentage varying depending on the testing method employed (Satyaputra et al., 2021).

We opted to assess syphilis samples encompassing a diverse range of patients, including those with confirmed primary or secondary infections, as well as those who had received treatment or remained untreated, using the AIX1000 instrument. The seropositivity of these samples had been clinically confirmed previously, along with confirmation via the VDRL assay (**Table 1**).

The AIX1000 instrument detected positivity in 92% of samples confirmed to be positive for syphilis (**Table 1**). In total, we tested 100 samples, with 50 samples originating from untreated syphilis patients and the remaining 50 samples obtained from patients who had received treatment for syphilis.

In the examination of 50 untreated syphilis samples, the AIX1000 instrument demonstrated a 94% correlation with the positivity detected by the SyphilisVDRL DiaLab assay (**Table 1**). The AIX1000 instrument showed a 100% correlation with positivity as determined by the VDRL test for recent cases, relapses/reinfections, and samples diagnosed with secondary syphilis. Slightly lower efficiency was observed for primary syphilis (detecting 4 out of 5 samples) and seropositive syphilis of unknown duration samples (detecting 4 out of 6 samples).

Nontreponemal antibody titers are used to monitor responses to treatment. The AIX1000 exhibited a 90% correlation with the positivity observed in samples from patients treated for syphilis. A 100% correlation was noted for samples from 11 out of 16 treatment conditions. However, in condition Z208, represented by only one sample, AIX1000 failed to detect syphilis entirely. Additionally, in condition Z017, 2 out of 3 samples tested negative for syphilis with the AIX1000. Among the treatment histories A510, A519, and B207, one sample from each (a total of 3 out of 20 samples) was inaccurately diagnosed by the AIX1000.

All samples that AIX1000 determined as negative, didn't exceed 1:2 titer number, measured by VDRL (**Supplementary Table**).

Six samples exhibited identical titers when measured by both the AIX1000 and Syphilis VDRL DiaLab assays. The titers of nontreponemal antibodies measured by the AIX1000 and the Syphilis VDRL DiaLab assay in syphilis-positive samples, irrespective of their history, showed a correlation of 41% within the +/-1 titer dilution range and 38% within the +/-2 titer dilution range (based on 100 samples) (**Table 2**). Overall, 85% of samples fell within the 0 to +/-2 titer dilution range between the AIX1000 and Syphilis VDRL DiaLab assays.

In all instances, the titers measured by the Syphilis VDRL DiaLab assay were higher than those measured by the AIX1000, except for six samples which exhibited titers identical to those measured by the AIX1000 (**Supplementary Table**).

While the AIX1000 exhibited lower sensitivity than the Syphilis VDRL DiaLab assay in titer measurements, changes in RPR titers of less than two serial dilutions (equivalent to a fourfold change) between two independent RPR assays are considered acceptable.

AIX1000 was 100% consistent in quantitative syphilis analysis.

In the end, the repeatability and reproducibility of the qualitative and quantitative results of the reference syphilis-positive sample were evaluated using the AIX1000 instrument (**Table 3**). The sample chosen had identical titers (1:16) as measured by both the AIX1000 and Syphilis VDRL DiaLab assays. Three independent measurements were conducted on 10 batches of the same sample using the AIX1000 instrument on different days and by different operators to mitigate potential errors due to time and operator variations. Across all experiments, there was 100% repeatability and reproducibility in both the qualitative and quantitative results of the reference syphilis-positive sample as measured by the AIX1000 instrument.

This underscores the AIX1000's consistent performance in precisely identifying positive syphilis samples, highlighting its effectiveness in accurately detecting positivity in syphilis samples.

DISCUSSION

Traditional serological screening for syphilis has typically relied on nontreponemal tests like the rapid plasma reagin (RPR) and the Venereal Disease Research Laboratory (VDRL) tests (Luo et al., 2021). It is also very important mentioned, that nontreponemal antibodies are used for monitoring of the activity of infection. The interpretation of manual nontreponemal tests results are subjective and can vary between individuals (Hamill et al., 2018).

The AIX1000 instrument offers a fully automated process for both qualitative and quantitative RPR screening. In this study, we assessed the efficacy of the AIX1000 in conducting proper RPR screening and estimating RPR titers in syphilis samples previously confirmed as either positive or negative. Overall, the AIX1000 system demonstrated sensitivity and specificity comparable to that of the manual VDRL test, which is routinely employed in the National Reference Laboratory for the Diagnostics of Syphilis in the Czech Republic.

The AIX1000 exhibited a sensitivity of 94% when compared to the VDRL assay. This aligns with previously reported data indicating reactivity rates with sensitivities of 96.4% and 100%, and specificities of 99.2% and 96.8% for both automated and manual RPR methods (Sanfilippo et al., 2020). Our findings are also consistent with studies conducted by the manufacturer, which reported precision and reproducibility rates of 98.8% and 100%, respectively (AIX1000 Rapid Plasma Reagin (RPR) Automated Test System manual). However, the sensitivity observed in our study appears slightly lower, potentially attributable to the implementation of more stringent experimental conditions.

In a previous study, the AIX1000 system was prone to generating false positive results during the screening of syphilis-negative samples, particularly in cases of biological false positives (Palavecino et al., 2018). However, our study did not observe this phenomenon. All syphilis-negative samples, including biological false positives, tested negative on the AIX1000. Additionally, it was noted in the previous study that the AIX1000 often reported higher titers compared to those obtained from manual RPR tests. However, our findings contradicted this observation. On the contrary, the titers obtained from samples tested with the AIX1000 were consistently 1 or 2 titers lower than those obtained from VDRL results.

AIX1000 is not the sole automated system available for syphilis diagnostics (Shukla et al., 2023). Recently, the performance of three automated RPR tests—AIX1000, ASI Evolution (Arlington Scientific), and BioPlex 2200 Syphilis Total & RPR assay (Bio-Rad Laboratories)—was evaluated for syphilis testing. Examination of the qualitative panel revealed sensitivities and specificities of 97.5% and 99.5% for AIX1000, 91.1% and 98.5% for ASI Evolution, and 90.1% and 99.0% for BioPlex, respectively, in comparison to manual RPR tests, confirmed by TPPA. Analysis of the quantitative panel demonstrated that 94% of specimens tested by AIX1000 yielded results within range (±2-fold to manual RPR titer), compared to 48% for ASI Evolution and 68% for BioPlex. Overall, this data suggests that while all automated platforms are suitable for syphilis testing, AIX1000 yielded superior results.

In clinical settings, various parameters of the AIX1000 system offer advantages. These include: results interpretation by pattern recognition software, ensuring objectivity and consistency and minimizing human-related variations in data interpretation; complete traceability from sample to result, ensuring accountability; and archiving images of results linked to samples, facilitating comprehensive record-keeping.

The primary limitation of this study lies in the restricted number of tested samples. Therefore, further evaluation is needed to assess how effectively the AIX1000-based diagnosis can be utilized for serum sample screening and monitoring treatment effectiveness.

Another drawback associated with the usage of the AIX1000 is the substantial requirement of serum (300ul) for each test. Although the test itself only necessitates 100ul of serum, the automatic pipetting system of the instrument demands a higher volume of the sample in the sample tube. This pipetting step can be omitted and assay programmed to start with the serum incubation with agglutination reagent, if the sample is pipetted manually. Additionally, it is challenging to ascertain whether the AIX1000 significantly reduces hands-on time compared to manual testing, if we do not exclude the time required for start-up and shut-down maintenance, which adds additional time both before and after each run.

Altogether, our data suggest that the AIX1000 system is suitable for the laboratory diagnosis of syphilis in clinical settings; however, each laboratory will need to carefully assess the workflow and cost benefits in comparison to manual RPR methods.

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Table 1. Syphilis positivity of the samples based on VDRL and AIX1000 tests. The generalized results are marked in bold. Specific conditions when AIX1000 result was negative, but VDRL was positive are underlined and in italic. Raw data is present Supplementary Table.

	VDRL (Sample Nr.)	**AIX1000 (Sample Nr.)**	**Correlation (%)**
Total Negative	**19**	**19**	**100**
Newborn	8	8	100
BFP	11	11	100
Total Positive	**100**	**92**	**92**
New /Untreated	**50**	**47**	**94**
Syphilis Recens	26	26	100
Syphilis seropositive of unknown duration	*6*	*4*	*67*
Relapse/Reinfection	7	7	100
Syphilis I	*5*	*4*	*80*
Syphilis II	5	5	100
Treated	**50**	**45**	**90**
A510	*5*	*4*	*80*
A515	11	11	100
A519	*5*	*4*	*80*
A528	1	1	100
A529	1	1	100
A539	4	4	100
A64	1	1	100
B207	*10*	*9*	*90*
B230	3	3	100
B232	1	1	100
B238	1	1	100
B353	1	1	100
F200	1	1	100
L309	1	1	100
Z017	*3*	*2*	*67*
Z208	*1*	*0*	*0*

Table 2. Number of samples with titer differences identified by AIX1000 and by Syphilis VDRL DiaLab.

	+/-0	+/-1	+/-2	+/-3	+/-4	ND
relaps/reinfection syphilis	1	4	3	0	0	0
syphilis I	1	2	0	0	1	1
syphilis II	0	3	2	0	0	0
syphilis recens	2	12	12	0	0	0
syphilis seropositive of unknown duration	0	4	0	0	0	2
Untreated Total (50)	**4**	**25**	**17**	**0**	**1**	**3**
A510	0	2	2	0	0	1
A515	0	2	8	1	0	0
A519	0	2	2	0	0	1
A528	0	1	0	0	0	0
A529	0	0	1	0	0	0
A539	0	2	0	2	0	0
A64	0	0	1	0	0	0
B207	0	4	3	2	0	1
B230	0	1	2	0	0	0
B232	1	0	0	0	0	0
B238	0	0	1	0	0	0
B353	0	1	0	0	0	0
F200	0	1	0	0	0	0
L309	0	0	1	0	0	0
Z017	1	0	0	1	0	1
Z208	0	0	0	0	0	1
Treated Total (50)	**2**	**16**	**21**	**6**	**0**	**5**
Total (100)	**6**	**41**	**38**	**6**	**1**	**8**

Table 3. Repeatability and reproducibility of the qualitative and quantitative result of the reference syphilis positive sample measured by AIX1000 instrument.

	Experiment 1		Experiment 2		Experiment 3	
Sample Number	**AIX1000**	**AIX1000 Titer**	**AIX1000**	**AIX1000 Titer**	**AIX1000**	**AIX1000 Titer**
36-22	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D1	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D2	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D3	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D4	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D5	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D6	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D7	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D8	Reactive	1:16	Reactive	1:16	Reactive	1:16
36-22D9	Reactive	1:16	Reactive	1:16	Reactive	1:16