Evaluation of a Digital Flocculation Reader for the Rapid Plasma Reagin Test for the Serological Diagnosis of Syphilis

Arnold R. Castro, PhD, David D. Binks, BS, MT(ASCP), MBA,† Danny L. Raymer, MS,† Susan E. Kikkert, BS,* Heather A. Jost, BS,* Mahin M. Park, PhD,‡ Benjamin D. Card, BSc,† and David L. Cox, PhD**

Abstract: We described the ASiManager-AT digital flocculation reader to demonstrate concordance between visual and digital readings of the rapid plasma reagin test for detection of antibodies in the serum of patients with syphilis. A qualitative and quantitative rapid plasma reagin was performed on each serum samples giving a concordance of 98.6% and 99.7%, respectively, for reactives and 100% for nonreactives.

From the *Division of STD Prevention, Centers for Disease Control and Prevention, Atlanta, GA; †Arlington Scientific, Inc, Springville, UT; and ‡Georgia Public Health Laboratory, Atlanta, GA

The authors thank the Georgia Department of Health Laboratories for supplying specimens for the study and Arlington Scientific, Inc for supplying all the necessary diagnostic reagents for this study.

Supported by the Centers for Disease Control and Prevention in the form of allowing the principal investigator and CDC coauthors to devote time and effort in testing and evaluating the ASiManager-AT digital flocculation reader instrument. Arlington Scientific, Inc funded the developmental phase of the final commercial instrument.

A.R.C. was the project microbiologist, developed the test protocol, designed the test parameters and functional components of the test, analyzed the data, and is the lead author for the paper. D.D.B. contributed to the design of the study. D.L.R. designed the resolution and parameters of the digital reader. S.E.K. assisted in performing the evaluation. H.A.J. assisted in performing the evaluation. M.M.P. provided the laboratory specimens and assisted in the data analysis. B.D.C. assisted in the data analysis. D.L.C. assisted in the data analysis. All authors contributed to the write up and critically reviewed the manuscript and approved the final draft.

Not commissioned; externally peer reviewed.

One of the functions of the Laboratory Reference and Research Branch (LRRB) of the Division of STD Prevention of the National Center for HIV/Aids, Viral Hepatitis, STD and TB Prevention of the Centers of Disease Control and Prevention is to evaluate new diagnostic reagents and/or instruments for the serological diagnosis of syphilis by providing unbiased assessment of the efficacy and relevance of reagents or instruments for the benefit of public health. Consequently there is no apparent conflict of interest between the CDC and Arlington Scientific, Inc.

There is no related paper or publications in reference to this work.

Arlington Scientific, Inc., is the sole proprietor of the ASiManager-AT digital flocculation reader instrument and ARC and CDC coauthors have no financial interest in the commercialization of this device.

This study was performed on archived sera from which all patient identifiers had been removed. Protocol for studies using these sera for the evaluation of new devices for the serological test for syphilis was reviewed by institutional review board of the CDC (protocol no 2018) and determined to be exempt from further review.

Correspondence: Arnold R. Castro, PhD, Division of STD Prevention, NCHSTP, Centers for Disease Control and Prevention, 1600 Clifton Rd, MS A-12, Atlanta, GA 30333. E-mail: acastro@cdc.gov.

Received for publication July 6, 2011, and accepted September 19, 2011.

DOI: 10.1097/OLQ.0b013e3182389ab9

The Centers for Disease Control and Prevention (CDC) recommends syphilis serologic screening with a nontreponemal test, such as the rapid plasma reagin (RPR) to identify persons with possible untreated infection.[1] The RPR is a macroscopic, nontreponemal flocculation card test used to screen for antibodies in patients suspected of having contracted syphilis.[2–4] The RPR test is highly subjective and lends itself to misinterpretation when performing the qualitative or quantitative tests. Different observers can arrive at different conclusions, especially when reading the endpoint of a quantitative test or the interpretation of a minimal reactive or slight roughness in the qualitative test. The ASiManager-AT digital flocculation reader is designed to read objectively each well of the RPR test. The purpose of the study was to demonstrate concordance between the visual and digital readings and to avoid any bias in reading the test.

METHODS

The antigen is prepared from a Venereal Disease Research Laboratory antigen suspension containing cardiolipin, lecithin, and cholesterol. Ethylenediaminetetraacetic acid is added to enhance the stability and choline chloride to eliminate the need to heat inactivate the serum. Finely divided charcoal particles are added as a visualizing agent.[5] In the RPR test, if antibodies are present, they combine with the lipid particles of the antigen causing them to aggregate trapping the charcoal particles and showing them as black clumps within the circle of a white card. If antibodies are not present, the test mixture appears as uniformly gray. The RPR antigen will detect IgG and IgM antibodies to lipoidal material released from damaged host cells as well as to lipoprotein-like material, and possibly cardiolipin released from the treponemes.[6] The antilipoidal antibodies are produced as a consequence of an active infection of syphilis and other treponemal diseases as well as in response to nontreponemal diseases in which tissue damage occurs.

The qualitative and quantitative RPR test is performed according to the procedures described in the manual of tests for syphilis.[5] The visual reading of the test is reported as reactive or nonreactive. Reactive is characterized by clumping, ranging from marked and intense to slight but definite, often called minimal reactive. Nonreactive is the absence of clumps or slight roughness in a smooth background. Minimal reactive and slight roughness is often misinterpreted as positive or negative because of the subjectivity of visually interpreting the results. This condition leads to variability in results from different operators reading the same test and lab-to-lab variability.

Serum samples (1091) that had been submitted to the Georgia Public Health Laboratory for serological testing for syphilis with all identifiers removed were sent to CDC for this study. The patterns of reactivity were determined at CDC by

TABLE 1. Performance Characteristics of the Visual Quantitative Titers and Qualitative Projected Endpoint Compared to the Digital Readings of the Rapid Plasma Reagin Test*

	ASiManager-AT Digital Reading No. (%) of Samples Tested					
	Quantitative Test†			Qualitative Endpoint Projected Test‡		
Visual Reading	Reactive	Nonreactive	Total	Reactive	Nonreactive	Total
Reactive	596 (54.6)	8 (0.73)	604 (55.3)	606 (55.5)	2 (0.18)	608 (55.7)
Nonreactive	0 (0)	487 (44.6)	487 (44.6)	0 (0)	483 (44.3)	483 (44.3)
Total	596 (54.6)	495 (45.4)	1091 (100)	606 (55.5)	485 (44.5)	1091 (100)

*One-doubling dilution difference between visual and digital reading was considered acceptable.
†The reactive and nonreactive concordance was 98.6% and 100%, respectively.
‡The reactive and nonreactive concordance was 99.7% and 100%, respectively.

using the quantitative RPR test (Arlington Scientific, Inc, Springville, UT) and confirmed with the*Treponamal pallidum* passive particle agglutination assay (Fujiribio, Tokyo, Japan).

The digital reader consists of a charge-coupled device camera that uses light reflectance to create a high-resolution image of the flocculation immunoassay. The image is then analyzed by a software algorithm to interpret the flocculation pattern. For this study, the information related to each sample was entered manually into the digital reader. If the samples would have been bar coded, the same information could have been scanned into the digital reader, saving time. The ASiManager-AT digital test card flocculation reader further enables the creation, storage, retrieval, and transmittal of the test results.

RESULTS

A quantitative RPR test was performed on each of the 1091 serum samples. Using a 10-well card, the samples were serially diluted from 1:1 to 1:16 in buffered saline and from 1:32 to 1:512 in a 1:50 dilution of nonreactive serum in buffered saline. Each of the test cards were read visually, and the last positive reaction was recorded as the endpoint. This was a blind study, and the person reading the test had no knowledge of the digital readings. At the end of the study, the visual and the digital results were collated and the data analyzed. Each card was inserted into the ASiManager-AT digital reader within 2 to 5 minutes of the visual reading, and the reaction of each well was analyzed on the basis of degree of flocculation and determined to be either reactive or nonreactive. A plus or minus 1 dilution difference between the visual and the digital reader was considered acceptable, giving a 98.6% concordance with reactive samples and a 100% concordance with nonreactive samples (Table 1) A 1 dilution difference is an acceptable range for equivalency because the minimal reactive endpoint titer is subject to interpretation between different observers.

The ASiManager-AT digital reader is also programmed to project the endpoint titer of each qualitative test by reading the undiluted serum (1:1) and determining its corresponding endpoint for reactive serums. A plus or minus 1 dilution difference between the visual and the digital reader was considered acceptable, giving a 99.7% concordance with reactive samples and 100% with nonreactive samples (Table 1).

Evaluation of this technology raises the question of whether the differences between the visual reading and the digital reading are because of the technology or to the interpretation. By applying the Kappa statistics analysis to correct for chance, the concordance of the quantitative and qualitative projected endpoint titer was 0.985 and 0.996, respectively.

However, the visual qualitative test lends itself to misinterpretation when a sample exhibits a prozone effect. An experienced observer can often differentiate the appearance of a true negative reaction from the assumed negative reaction of a prozone. It looks atypical because there is an appearance of microfloculation veiled as roughness. In these cases, it is advisable that a quantitative test be followed. The ASiManager-AT digital reader may also detect the microfloculation or roughness and call it minimal reactive. To avoid the misinterpretation from a prozone effect when using the projected endpoint titer assay, a 1:1 and a 1:16 dilution of the patient serum could be tested as an option of doing quantitative test.

The accuracy of the ASiManager-AT digital reader was determined by performing reproducibility studies using reactive and nonreactive samples that were tested qualitatively and quantitatively (Table 2). Reproducibility studies of the ASiManager-AT digital reader were performed by testing quantitatively 5 samples of known RPR titers ranging from R1 to R32. Each sample was serially diluted from 1:1 to 1:512, and the results of the RPR test were read visually and compared to the ASiManager-AT digital results. Each sample was tested quantitatively and repeated 10 times for a total of 50 repeats. A plus or minus 1 dilution difference between the visual and digital reader was considered acceptable, giving a 100% concordance.

The reliability of the ASiManager-AT projected endpoint titer for 5 qualitative samples with RPR titers ranging from R1 to R32 were compared to visual quantitative readings. Each sample with RPR titers of R1, R2, and R8 were repeated 100 times and with titers R4 and R38 were repeated 110 times for a total of 520 times. The percentage concordance of titers R1 through R8 was 100%. For RPR titer R32, 12 samples gave a 2-doubling dilution difference between the visual and projected titer readings for a 97.7% concordance.

Each of the 5 different nonreactive samples was repeated 100 times qualitatively for a total of 500 repeats. The percentage concordance between the visual and digital reader was 100%.

DISCUSSION

Visual readings of RPR tests lend itself to misinterpretation and variability of results because of the inherent subjectivity of the test. With the introduction of the ASiManager-AT

TABLE 2. Reproducibility of the ASiManager-AT Digital Reader Titer Confirmation and Endpoint Predictive Value of Reactive Sera and Predictive Value of Nonreactive Sera

Quantitative	Sample	Rapid Plasma Reagin Titer	Repeats Per Sample (No.)	Concordance of Repeats, No. (%)
Reactive endpoint confirmation	1	R1	10	10 of 10 (100)
	2	R2	10	10 of 10 (100)
	3	R4	10	10 of 10 (100)
	4	R8	10	10 of 10 (100)
	5	R32	10	10 of 10 (100)
			Total	50 of 50 (100)
Qualitative	Sample	Rapid Plasma Reagin Titer	Repeats Per Sample (No.)	Concordance of Repeats, No. (%)
Reactive endpoint predictive value	1	R1	100	100 OF 100 (100)
	2	R2	100	100 OF 100 (100)
	3	R4	110	110 OF 110 (100)
	4	R8	100	100 OF 100 (100)
	5	R32	110	98 OF 110 (89)
			Total	508 OF 520 (97.7)
Qualitative	Sample		Repeats Per Sample (No.)	Concordance of Repeats, No. (%)
Nonreactive	1		100	100 of 100 (100)
	2		100	100 of 100 (100)
	3		100	100 of 100 (100)
	4		100	100 of 100 (100)
	5		100	100 of 100 (100)
			Total	500 of 500 (100)

digital reader, an attempt was made to establish concordance between the visual and digital reading. The data generated in this study suggest that the digital reader could offer a high degree of objectivity when interpreting the result of RPR tests and could replace visual readings without compromising its accuracy. The ASiManager-AT digital test card flocculation reader offers a consistent objective result with all reactive or nonreactive specimens. A high resolution image of each well is recorded, compared with preset parameters, and the results reported as reactive or nonreactive. Each image is stored on the devices' hard drive for archival purposes and can be utilized for confirmation of equivocal reactions. According to the manufacturer, future research and development will offer a more unique solution by providing an upload capability to move the data and images to a local lab information system. There are also limitations of the effectiveness of the ASiManager-AT digital test card flocculation reader in interpreting the microfloculation or roughness of a suspected prozone effect. Human intuition or experience in detecting this phenomenon cannot be replaced and at this time; there are no efficient parameters that can be programmed into the reader to address this condition. However, the ASiManager-AT digital reader can aid in the standardization of RPR, avoiding the subjectivity and interpretation of visual reading and provides a tool for retrieval of documentation and archival of records. A digital reader can aid in the standardization of RPR, avoiding the subjectivity and interpretation of visual reading by reliably providing objective results.

REFERENCES

1. Radolf JD, Boland G, Park IU. Discordant results from reverse sequence syphilis screening—five laboratories, United States. MMWR 2011; 60:133–137.
2. Portnoy J, Brewer JH, Harris A. Rapid plasma regain card test for syphilis and other treponematoses. Public Health Rep 1962; 77: 645–652.
3. Portnoy J. Modifications of the rapid plasma regain (RPR) card test for syphilis, for use in large-scale testing. Am J Clin Pathol 1963; 40:473–479.
4. Portnoy J. A note on the performance of modifications of the rapid plasma regain (RPR) card test for syphilis, for use in large-scale testing. Public Health Lab 1965; 23:43.
5. Larsen SA, Pope V, Johnson EJ. A Manual of Test for Syphilis, 9th ed. Washington, DC: American Public Health Association, 1998: 194–207. Chapter 10.
6. Matthews HM, Yang TK, Jenkin HM. Unique lipid composition of *Treponema pallidum* (Nichols virulent strain). Infect Immun 1979; 24:713–719.
7. Catterall RD. Systemic disease and the biological false-positive reaction. Br J Vener Dis 1972; 48:1–12.