Qualitative Evaluation of the Reagin Screen Test

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After a preliminary study of 557 sera used as a procedural training exercise, the Reagin screen test (RST) for the macroscopic detection of reagin (as an aid to detecting syphilis) was qualitatively compared to the rapid plasma Reagin (RPR) (circle) card test and Venereal Disease Research Laboratory (VDRL) slide test on 435 random portions of sera using fluorescent treponemal antibody adsorption (FTA-ABS) results as a comparative base. A comparison of total agreement (positive and negative) with the FTA-ABS results led to the following order: RPRCT (85.5%), VDRL (79.8%), and RST (74.5%). Of the total samples shown to be reactive by the FTA-ABS procedure, the percentage of these interpreted as nonreactive (i.e., "false negative") by the procedures compared was considerably higher with the RST procedure (29.3%) than with either the RPRCT (10.9%) or the VDRL (7.1%) procedures. Minor problems encountered with procedural techniques are also mentioned.

A new macroscopic card test for syphilis has recently become commercially available. This test, the Reagin screen test (RST), is a nontreponemal macroscopic flocculation test for the detection and quantitation of reagin. The test methodology is similar in many respects to the Reagin card test previously described by Portnoy, Brewer, and Harris (4). The antigen (2, 3, 4, 5), instead of containing charcoal particles, has been dyed with a lipid-soluble blue dye and relies on the formation of blue agglutinate masses as a measure of reactivity.

The RST was initially described in a 1974 report of results obtained on 845 serum specimens (R. W. March, G. E. Stiles, and P. S. Forgiione, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1974, M160, p. 93). RST results were compared for reactivity and specificity with the Venereal Disease Research Laboratory (VDRL) slide test and the rapid plasma Reagin (RPR) card test. This report concluded that the RST, RPRCT, and VDRL tests appeared to have similar sensitivities and specificities. To further evaluate the RST relative to its reliability as a qualitative screening and clinical diagnostic tool, the following study was undertaken.

MATERIALS AND METHODS

RST kit. Three 500-specimen RST kits (Lederle Diagnostics NDC 0937-2300-76) were provided by the manufacturer for evaluation. Each kit contained antigen, control sera, reagent test cards, and sampling pipettes sufficient for 500 determinations. Additional support equipment that was necessary included a mechanical rotator capable of horizontally

circumscribing a circle $\frac{3}{4}$ inch (ca. 2.0 cm) in diameter and adjustable to 130 or 100 oscillations/min, humidifying covers, a source of adequate lighting for test reading, and a refrigerator for maintaining storage temperatures between 2 and 8°C.

Specimens. All specimens were portions of sera obtained from the Serology Branch, Bureau of Laboratories, Texas Department of Health Resources. The sera used were randomly selected from routine specimens that had been submitted to the laboratory either for confirmatory analysis, initial analysis, or problem analysis. Because clinical histories or previous laboratory results were not available on these specimens, the sensitive and specific fluorescent treponemal antibody adsorption (FTA-ABS) test (1) was arbitrarily used to classify the sera as reactive (probably syphilitic) or nonreactive (probably nonsyphilitic).

All specimens had been heat inactivated (a requirement not necessary for analysis by RST or RPRCT) and analyzed by the Serology Branch by using standard VDRL and FTA-ABS techniques (6). These specimens were subdivided (see below) and RPR card test (14 mm) results were obtained concurrently with RST results in a completely independent fashion (i.e., tests were not read simultaneously and all specimens were coded for anonymity). All sera and antigens were kept refrigerated at 2 to 8°C and were analyzed within 1 week of their receipt in the laboratory. All tests were run using specimens and antigens that had been allowed to equilibrate to room temperature.

Method. A total of 557 portions of sera were initially analyzed as a training exercise for the technologists involved. Strict adherence to procedures on the RST package insert (revision dated 1/75) was maintained. The two investigators conducting all RST analyses had previously demonstrated compe-

tendency at reading the RPR card test on not less than 100,000 specimens each. Because all specimens had been analyzed by both VDRL and FTA-ABS techniques before being obtained from the Serology Branch, the results of these analyses were available for review in cases where subjective RST determinations were necessary. During this preliminary training, RST readings were compared closely with the VDRL and FTA-ABS test results whenever necessary until each technologist had become competent and confident of her procedural techniques.

While conducting the initial training exercise, it was found that card rotation at 100 oscillations/min caused a light blue ring to form about the periphery of the specimen circle. This ring formation was so similar to the agglutinate formed with reactive specimens that it led to extreme difficulty in analyzing weakly reactive specimens accurately. Card rotation at 130 oscillations/min was found to eliminate this problem almost completely and the final study was conducted using only this rate of oscillation.

For the qualitative study, a group of 435 specimens were analyzed by both RST and RPRCT (14 mm) techniques. These specimens included portions randomly obtained from sera without regard to reactivity (to maintain statistical credibility) as follows: 48 sera were divided four ways, 121 sera were divided two ways, and one serum specimen was not divided (no specimens were used in this study if they were considered borderline by FTA-ABS techniques). This division process was performed to check the interpretation of results between the technologists performing the testing, and to look briefly at the testing sensitivity on the same specimen when analyzed at different times.

RESULTS AND DISCUSSION

Several minor RST procedural difficulties were encountered during the course of these studies. One such difficulty concerned the dyed antigen that is supplied in a dispensing bottle equipped with a diaphragm and spring-loaded needle. For dispensing, the needle is rotated to pierce the diaphragm, thus allowing an exit pathway for the antigen. In one instance, a malfunction of the penetrating apparatus caused the diaphragm not to be punctured and antigen delivery from the supplied dispenser was impossible.

Additionally, it was found that reproducible sample delivery from the disposable sampling pipettes was quite difficult. Indeed, a number of commercial micropipettors (Drummond, SMI, etc.) are available which proved to be most satisfactory as alternatives. Use of the open end of the sampling pipette also presented some difficulty in spreading the sera without sample loss; however, toothpicks served adequately in this respect.

Reading and interpretation of the test results in cases where subjective determinations were necessary was by far the greatest difficulty. Rarely were any problems encountered in reading samples of strong reactivity. These strong reactive cases were characterized by blue clumping, easily recognized against the white card background. However, in cases of weak reactivity, the clumping was considerably less pronounced, as expected, but recognition against the background, which still contained a bluish tint, was extremely difficult. A review of the data in the tables provides an indication as to how this reading difficulty manifested itself.

Table 1 includes a compilation of the varying combinations of results possible when analyzing the 435 specimens by four different techniques. Although FTA-ABS and VDRL results were run only on the specimens before their subdivision, these results were listed in this table for each of the 435 portions should the reader wish to make further calculations. Tables 2 through 4 serve to simplify the comparisons of the various analyses made by drawing direct comparisons between each analytical procedure and the FTA-ABS technique.

TABLE 2. *Qualitative comparison of RST and FTA-ABS^a*

Test	Agreement		Disagreement	
	R	N	R	N
RST				
FTA-ABS	R	N	N	R
Total	169	155	41	70

^a N, Nonreactive; R, reactive.

TABLE 1. *Total tabulation of qualitative testing results^a*

Test		Classification of test result ^b															
FTA-ABS	R		R		R		R		N		N		N		N		
RST	N		R		R		N		R		N		N		R		
RPRCT	N		N		R		R		R		R		N		N		
VDRL	N	R	N	R	N	R	N	R	N	R	N	R	N	R	N	R	
Totals	10	13	0	3	1	165	3	44	2	25	4	6	111	34	5	9	

^a FTA-ABS and VDRL results were obtained on 170 specimens before subdivision into 435 portions and are listed here on each of the 435 portions for completeness.

^b N, Nonreactive; R, reactive.

TABLE 3. *Qualitative comparison of RPRCT and FTA-ABS^a*

Test	Agreement		Disagreement	
RPRCT	R	N	R	N
FTA-ABS	R	N	N	R
Total	213	159	37	26

^a N, Nonreactive; R, reactive.TABLE 4. *Qualitative comparison of VDRL and FTA-ABS^a*

Test	Agreement		Disagreement	
VDRL	R	N	R	N
FTA-ABS	R	N	N	R
Total	91	44	28	7

^a N, Nonreactive; R, reactive.

As previously mentioned, we have elected to define a probable syphilitic infection as one in which the FTA-ABS results are positive. On this basis, a calculation of positive agreement shows the VDRL (92.9%) and RPRCT (89.1%) to agree with the FTA-ABS results much more closely than the RST (70.7%); (i.e., significantly fewer false negatives occurred with the VDRL and RPRCT than with the RST). Conversely, similar calculations with negative agreement show considerably fewer false positives with the RPRCT and RST than with the VDRL. In terms of total agreement with the FTA-ABS, the following order was established: RPRCT (85.5%); VDRL (79.8%); and RST (74.5%).

A comparison of results between the two technologists on portions of subdivided specimens showed some differences in interpretation. However, the percentage overall agreements with both the RST and the RPR card test when compared to the FTA-ABS results were

virtually identical for both investigators. Similarly, subdivided specimens run on successive days showed no change in qualitative results by either procedure (a detailed time study was not done).

The tabulations of data that we have obtained at this time tend to indicate a degree of sensitivity for the RST less than that of both the RPRCT and VDRL tests. Furthermore, the extreme difficulty in analyzing weakly reactive specimens leads us to consider this under sensitivity of a degree likely to result in a significant number of false-negative results when used as a screening procedure.

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