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THE USR TEST AS A SCREENING TEST IN A PUBLIC HEALTH LABORATORY

EVERY public health laboratory may be confronted with two problems with respect to syphilis serology: mounting costs and the biologic false positive reaction. The New York City Department of Health is attempting to solve these problems simultaneously.

For several years the procedure in the syphilis serology laboratory has been to screen test annually about 700,000 specimens by the VDRL slide test. All reactive serums are further tested by the Kolmer complement fixation test and titrated by the VDRL slide technique.

The major problem in performing such large numbers of tests is in the routine preparation of the specimens. This preparation requires space and the time of personnel for centrifugation of the blood, decanting of the serums, relabeling of the thousands of serum tubes, and finally the heating of the serums for 30 minutes.

The rapid plasma reagin (RPR) test (*1*) suggested the possibility of eliminating many of the time-consuming features of conventional serology. With the adaption of the test for use with unheated serum (*2,3*) instead of plasma, it appeared useful to study the value of the unheated serum reagin (USR) test as a screening procedure in a public health laboratory. Serum does not require special tubes with anticoagulants and is suitable for use in standard tests for syphilis as well.

If we could substitute the USR test for the VDRL slide test in our screening procedure, our laboratory would save space, personnel, and time. Some of these savings could be used to amplify our syphilis serology with treponemal testing.

A large-scale study of the USR test was undertaken in comparison with the VDRL slide test. Treponemal testing of a smaller sample of specimens was also done to obtain information that would aid in the selection of a treponemal test for confirmatory purposes. As a result of this study a suggested routine for a public health laboratory is presented.

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Methods

One hundred thousand blood specimens routinely received by the syphilis serology laboratory were screen tested by both USR (*2,3*) and VDRL slides tests (*4*). The USR test was performed on slides with paraffin rings 14 mm. in diameter, using 0.06 ml. of serum and 0.02 ml. of antigen suspension. All serums reacting (reactive or weakly reactive) by either test were further tested with the Kolmer complement fixation test (*4*), substituting plastic trays for test tubes (*5*), and in addition a selected number of specimens were examined with Reiter protein and tpcf-50 complement fixation tests (*4*).

Reiter protein antigen was supplied by the Public Health Service Venereal Disease Research Laboratory, Chamblee, Ga., and the test was performed in the serology laboratory of the New York City Department of Health.

The tpcf-50 test was performed at the Public Health Service Venereal Disease Experimental Laboratory, Chapel Hill, N.C.

Clinical diagnoses on the 1,885 patients who were tested with treponemal antigens were obtained through the New York City bureau of preventable diseases, division of social hygiene. The clinician was aware only of the results of the VDRL and Kolmer tests at the time the diagnosis was considered.

Results

Table 1 presents the results of simultaneous testing of 100,000 specimens of blood by both the USR and VDRL tests. Essentially similar percentages of reactivity were obtained with each procedure, and agreement between the two was 98.2 percent. Less than 1 percent of the specimens were reactive with USR and nonreactive with VDRL, and vice versa.

Split blood specimens on 1,885 patients for whom diagnoses were available were tested with the USR and VDRL techniques. Both tests were reactive and results were in agreement with the diagnosis in 92 percent of the cases of clinical syphilis (table 2). Somewhat lesser agreement was noted in latent syphilis. Both tests were reactive in about 62 percent of the cases diagnosed as biologic false positive. It must be emphasized that the clinician knew only the VDRL and Kolmer test results when making the diagnosis.

In order to determine the value of performing multiple tests on any one serum, the results of five tests were correlated with clinical opinion. The percentage of reactors was determined for each test when one or more of the other tests were reactive. When the clinician made a diagnosis of clinical syphilis, and the USR test was reactive, 91.7 percent of the serums were also reactive by the VDRL tests; 74.5 percent by the Kolmer test; 71.1 percent by the RPCF test; 71.2 percent by the tpcf-50 test; and only 58.7 percent by both RPCF and tpcf-50 tests. Similarly, if the USR, VDRL, and Kolmer tests were all reactive, 84.2 percent of the RPCF tests, 83.7 percent of the tpcf-50 tests, and 73.2 percent of both the RPCF and tpcf-50 tests were reactive.

Thus various combinations of test results obtained in this laboratory make possible determination of the probability of other tests being reactive in clinical syphilis, in early latent syphilis, in late latent syphilis, and in those cases considered as not syphilis by the clinician (tables 3–6).

Table 1. Results of testing 100,000 blood specimens by the unheated serum reagin (USR) and VDRL techniques

Reactors	Number	Percent
USR [1]	10,047	10.04
VDRL [1]	9,966	9.96
USR, VDRL, or both +	10,884	10.88
USR+, VDRL+	9,129	9.1
USR−, VDRL+	837	.84
USR+, VDRL−	918	.92
USR−, VDRL−	89,116	89.1

[1] Reactive or weakly reactive.

NOTE: Plus sign (+), reactive or weakly reactive; minus sign (−), nonreactive.

Table 2. Estimated agreement [1] between results of tests of 1,885 split blood specimens tested by unheated serum reagin (USR) and VDRL techniques, by stage of syphilis

Stage of syphilis	Number specimens tested	Percent USR and VDRL results in—	
		Agreement	Disagreement
Clinical	129	91.7	8.3
Primary	26	81.6	18.4
Secondary	37	95.5	4.5
Congenital	40	94.5	5.5
Late	26	93.7	6.3
Early latent	124	87.2	12.8
Late latent	1,106	89.8	10.2
Undetermined	343	80.8	19.2
No case (BFP) [2]	183	61.8	38.2
Total	1,885	84.4	15.6

[1] Tests were considered to be in agreement when a reactive or weakly reactive reaction in one test was accompanied by a reactive or weakly reactive reaction in the other test.

[2] Biologic false positive.

Discussion

It is evident that the USR test, employing unheated serum, may be substituted for the

VDRL test as a screening procedure. It must be emphasized that no screening test will detect all reactive serums. A certain percentage of serums will be reactive by one test and nonreactive by another, and vice versa. However, the USR test will select at least as many reactors as does the VDRL slide test. The advantage of using the USR rather than the VDRL test lies in the lower cost of processing the serums when large numbers of specimens are tested.

In the New York City Department of Health serology laboratory, by eliminating the necessity for long centrifugation, pouring of serums, labeling of serum tubes, and inactivating of serums, we have freed one laboratory room and several technicians for other purposes. In addition we are able to start processing the "negative" (nonreactive) reports earlier in the day.

The determination of biologic false positive results is facilitated by the use of treponemal tests. Of the many treponemal tests (*3–6*) the complement fixation test with Reiter protein antigen seemed the most likely choice because of its low cost, sensitivity, specificity, and adaptability. The results obtained with treponemal antigens were not too revealing since determination of the treatment status of each

Table 3. Serologic results on 129 patients with clinical diagnosis of primary, secondary, congenital, or late syphilis [1]

If serology result was—	Percent of specimens reactive [2] by—					
	USR	VDRL	Kolmer	RPCF	tpcf-50	RPCF and tpcf-50
USR +	-------	91. 7	74. 5	71. 1	71. 2	58. 7
VDRL +	91. 7	-------	76. 6	72. 0	74. 1	65. 1
USR +, VDRL +	-------	-------	74. 4	73. 2	76. 5	72. 0
USR +, VDRL −	-------	-------	[3]	[3]	[3]	[3]
USR −, VDRL +	-------	-------	[3]	[3]	[3]	[3]
USR +, VDRL + Kolmer +	-------	-------	-------	84. 2	83. 7	73. 2
USR +, VDRL + Kolmer −	-------	-------	-------	62. 8	55. 9	45. 4

[1] Clinician was aware of results of VDRL and Kolmer tests only.
[2] Includes weakly reactive.
[3] Numbers too small to determine percentages.

NOTE: Plus sign (+), reactive or weakly reactive; minus sign (−), nonreactive.

Table 4. Serologic results on 124 patients with clinical diagnosis of early latent syphilis [1]

If serology result was—	Percent of specimens reactive [2] by—					
	USR	VDRL	Kolmer	RPCF	tpcf-50	RPCF and tpcf-50
USR +	-------	94. 7	84. 5	71. 4	76. 5	63. 6
VDRL +	82	-------	84. 9	64. 7	69. 6	58. 0
USR +, VDRL +	-------	-------	83. 1	61. 9	62. 8	55. 4
USR +, VDRL −	[3]	[3]	[3]	[3]	[3]	[3]
USR −, VDRL +	-------	-------	90	32. 2	32. 1	23. 0
USR +, VDRL + Kolmer +	-------	-------	-------	74. 7	78. 3	69. 4
USR +, VDRL + Kolmer −	-------	-------	-------	58. 3	72. 6	50. 0

[1] Clinician was aware of results of VDRL and Kolmer tests only.
[2] Includes weakly reactive.
[3] Numbers too small to determine percentages.

NOTE: Plus sign (+), reactive or weakly reactive; minus sign (−), nonreactive.

case was not made and the diagnosis of biologic false positive was made to a great extent on the results of VDRL and Kolmer tests. Tables 3–6 indicate that the RPCF test was similar in reactivity to the more expensive tpcf-50 test, though it was not equally sensitive or specific on all serums. In the present state of our knowledge it appears advisable to perform additional treponemal tests when the RPCF test is found to be nonreactive (*7,8*). A test using the Nichols strain of *Treponema pallidum* would be desirable. Although it would be desirable to perform the TPI test (*9*), because of the many years of clinical experience with it, its expensiveness and complexity limits its usefulness in a public health laboratory. For this reason it is planned to use the fluorescent treponemal antibody (FTA) test (*10*).

Table 6 shows serologic results for the 183 patients in table 2 diagnosed as "no case (BFP)." It will be recalled that the physicians were aware of results of the VDRL and Kolmer tests, in addition to the clinical findings, but had no information on results of the treponemal tests. Of the group of serums whose serology was reactive by USR, VDRL, and Kolmer tests, 40 percent were RPCF reactive; 43.3 percent, tpcf-50 reactive; and 30 percent were both RPCF and tpcf-50 reactive. Thus a certain number of patients were considered as

Table 5. Serologic results on 1,106 patients with clinical diagnosis of late latent syphilis [1]

If serology result was—	Percent of specimens reactive [2] by—					
	USR	VDRL	Kolmer	RPCF	tpcf-50	RPCF and tpcf-50
USR +	-------	82. 4	69. 8	76. 4	70. 9	60. 0
VDRL +	88. 6	-------	72. 1	74. 5	70. 5	55. 1
USR +, VDRL +	-------	-------	73. 5	76. 3	71. 8	60. 3
USR +, VDRL −	-------	-------	26. 4	77. 4	62. 6	56. 8
USR −, VDRL +	-------	-------	60. 8	59. 8	52. 2	46. 2
USR +, VDRL + Kolmer +	-------	-------	-------	85. 8	83. 1	76. 3
USR +, VDRL + Kolmer −	-------	-------	-------	49. 8	40. 1	35. 9
USR +, VDRL − Kolmer −	-------	-------	-------	72. 0	62. 8	55. 3
USR −, VDRL + Kolmer −	-------	-------	-------	50. 0	50. 0	33. 3

[1] Clinician was aware of results of VDRL and Kolmer tests only.
[2] Includes weakly reactive.
NOTE: Plus sign (+), reactive or weakly reactive; minus sign (−), nonreactive.

Table 6. Serologic results on 183 patients clinically diagnosed as "not syphilis" [1]

If serology result was—	Percent of specimens reactive [2] by—					
	USR	VDRL	Kolmer	RPCF	tpcf-50	RPCF and tpcf-50
USR +	-------	57. 8	11. 1	44. 5	40. 1	30. 6
VDRL +	78. 9	-------	14. 6	39. 5	39. 1	27. 0
USR +, VDRL +	-------	-------	18. 0	44. 5	41. 9	31. 3
USR +, VDRL −	-------	-------	2. 8	44. 4	39. 2	29. 0
USR −, VDRL +	-------	-------	21. 5	20. 0	29. 1	10. 6
USR +, VDRL + Kolmer +	-------	-------	-------	40. 0	43. 3	30. 0
USR +, VDRL + Kolmer −	-------	-------	-------	45. 2	41. 2	31. 1
USR +, VDRL − Kolmer −	-------	-------	-------	43. 5	35. 8	27. 5

[1] Biologic false positive. Clinician was aware of results of VDRL and Kolmer tests only.
[2] Includes weakly reactive.
NOTE: Plus sign (+), reactive or weakly reactive; minus sign (−), nonreactive.

not syphilitic even though the USR, VDRL, Kolmer, RPCF, and tpcf-50 all were reactive. Similarly, we have indication of serums that were reactive by the USR, VDRL, RPCF, and tpcf-50 tests but were negative by the Kolmer technique, and these cases too were considered as not syphilis. It is reasonable to suspect that a certain number of these cases might have been considered as latent syphilis, instead of as biologic false positives, if the clinician had been aware of the results of the treponemal tests.

The clinician needs the results of a quantitative test to enable him to follow the patient under treatment. The simplest method in our hands is the titration of the serums by the VDRL slide test. With this technique we are simultaneously cross-checking our USR reactors by another cardiolipin slide test. This cross-checking is important, since it enables the serology laboratory to identify mechanical laboratory errors occurring while culling the serums in the screening procedure and rechecking if discrepancies are found.

The present laboratory practice in New York City is summarized as follows:

The USR test, in which unheated serum is pipetted directly from the supernatant above the blood clot, is employed as a screening test (*1*). Enough antigen is prepared for 1 week and preserved in the refrigerator (4°–6° C.). A quantity sufficient for the needs of the day is allowed to reach room temperature and is tested against serums of known reactivity. Tests are read microscopically at 100× magnification, and recorded as:

Reactive=large clumps
Weakly reactive=small clumps
Nonreactive=no clumping

All nonreactors are reported to the physicians as "Slide test—negative." USR reactors are further tested by the quantitative VDRL slide test and by the Kolmer complement fixation test (*4*), substituting the plastic trays for test tubes (*5*). The RPCF may be substituted for the Kolmer test. The RPCF and the FTA tests will be used by this laboratory on specimens requiring further testing. We are giving serious consideration to incorporating the RPCF as a routine to be performed simultaneously with the Kolmer test.

Reports to physicians include the results of each of the tests performed, including the VDRL titer.

Summary

In the New York City Department of Health serology laboratory, 100,000 specimens of blood were tested simultaneously by the unheated serum reagin (USR) and VDRL slide tests. All reactors were further tested by the Kolmer complement fixation test.

About one-third of the reactors to the USR or VDRL tests, or to both, were also tested by the Reiter protein complement fixation (RPCF) and the *Treponema pallidum* complement fixation 50 test (tpcf-50). Clinical diagnoses were correlated with the serologic results.

The USR test may be used as a screening test, with a resultant saving of time, space, personnel, and money.

A public health laboratory procedure may consist of: (*a*) use of the USR as a screening test, all negative serums to be reported immediately, all USR reactors to be further tested; (*b*) further testing to consist of VDRL titration, Kolmer complement fixation test, and Reiter protein complement fixation test; and, (*c*) if the additional testing is required, employment of the Nichols strain of *T. pallidum* in an approved test, the fluorescent treponemal antibody (FTA) test, for example.

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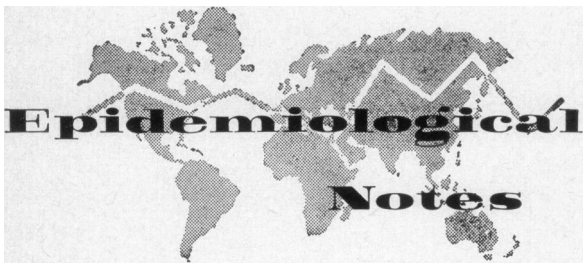

Accidental Drownings at Home

Deaths from accidental drowning in the United States in 1958 totaled 5,605, almost 11 percent of the 47,300 deaths from all nontransport accidents. Drownings at home accounted for 407 of these deaths. The estimated distribution by place of accident based on a sample of 85 death certificates, is shown below.

Place at home	*Number*
Bathtubs	140
Swimming pools	62
Wells, cisterns, or cesspools	57
Other open bodies of water	62
Miscellaneous specified	38
Unspecified	48
Total	407

About three-fifths of the decedents were less than 5 years old, with half no more than 2 years old. In every eight drownings, one decedent was 5 to 19 years old, and one was 20 to 44 years old.

By place of drowning, children under 5 years accounted for 39 percent of the deaths in swimming pools; 49 percent in bathtubs; 60 percent in wells, cisterns, or cesspools; 93 percent in other open bodies of water; and 100 percent in miscellaneous specified places. Of an estimated 38 drownings of infants, 29 occurred in bathtubs.

Deaths in specified places numbered an estimated 96 persons aged 20 years or over: 55 percent in bathtubs, 25 percent in swimming pools, and 20 percent in wells, cisterns, or cesspools.

The comparatively low percentage of deaths occurring in swimming pools to the under 5 age group may indicate that these places are more likely to be protected against accidental entry by very small children. In the sample, other open bodies of water included four ditches, five fishponds, a lake, a creek, a hole filled with water, and a pool. In only one instance in the sample was construction of the swimming pool specified, that being a plastic pool for which no size was stated.

How seemingly innocent objects can be hazardous to very small children is illustrated by the eight deaths of children aged 2 years or younger which were assigned to miscellaneous specified places in the sample. Three of these drownings were in tanks and one in a tub of unstated size or purpose. The others happened to a 10-month-old child in a 5-gallon bucket and to 1-year-olds in a 5-gallon can, a bucket, and a 20-gallon crock.—WARREN W. MORSE, *analytical statistician, National Office of Vital Statistics, Public Health Service.*