EXPERIENCE WITH VDRL FLOCCULATION ANTIGEN IN THE KOLMER COMPLEMENT FIXATION TEST

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IN RECENT years interest has been concentrated on treponemal antigens and little attention has apparently been given to improvement of the currently available nontreponemal antigens. This is unfortunate, since an easily available lipoidal antigen with improved specificity would be of extreme value in the laboratory.

In the United States, with its premarital and prenatal laws, millions of serologic tests for syphilis are performed on presumably healthy individuals. All too frequently, particularly with cardiolipin antigens commercially available, reactive laboratory findings are obtained which are not supported by clinical findings.

Various estimates of biologic false positive reactions with routine procedures have been advanced by various authors. Variation in these figures is wide. They, of course, are influenced by the type of population group evaluated, the serologic procedures and type of antigen employed, and the definition of positivity used.

Deservedly or not, the current criterion of the value of a serologic test for syphilis is its sensitivity and specificity in comparison with the sensitivity and specificity of the *Treponema pallidum* immobilization (TPI) test of Nelson and Mayer.

Using the TPI test as a reference, rather high false positive figures have been found with standard lipoidal antigens.

MacPherson, Ledbetter, and Martens (*1*) reported results of 21 to 40 percent of routine reactive tests as biologic false positives, and a number of other authors, including Moore and Lutz (*2*), reported 40 percent false positives,

In a comparative study of 572 "problem serums" which were reactive with nontreponemal tests, Wilkinson (*3*) found that the Kahn test gave 17 percent nonspecific reactions, the Wassermann test with lipoidal antigen 13 percent, and the Wassermann test with cardiolipin antigen 23 percent. In 244 patients found to be seropositive on routine testing during pregnancy, blood tests of 27.5 percent gave presumed nonspecific reactions.

In our own laboratory, of 796 reactive VDRL slide tests, 435, or 54.6 percent, were assumed to be biologic false positives, with the TPI test used as the reference test. Of 724 reactive cardiolipin Kolmer tests, 385, or 53.2 percent, were in the same category. These figures were obtained on specimens submitted to us for TPI tests, and these cases can consequently be placed in the "problem" category.

VDRL Flocculation Antigen as CF Antigen

There would be considerable advantage in being able to use the same antigen for both flocculation and complement fixation tests. In the Dickman Laboratories the VDRL antigen with its current composition seems to fall into this category. Our preliminary studies indicate that the VDRL antigen might profitably be evaluated in a future national survey such as the Serology Evaluation Research Assembly study recently completed by the Public Health Service. There might be further advantage in determining whether the cardiolipin-lecithin-cholesterol ratio of other commonly used antigens is the optimum when used with the TPI test as the reference test.

For many years we have performed a considerable number of serologic tests for syphilis, and since we are located in Pennsylvania, a State whose premarital law requires both a flocculation and a complement fixation test on each specimen of blood, we have routinely carried out both procedures. Most of the speci-

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mens examined in our laboratory are submitted for premarital tests, and we can assume that the majority of them come from a young, generally healthy group of individuals of satisfactory social and economic background. In a series of 64,206 premarital blood tests employing lipoidal antigens, 1.08 percent were reported as reactive in both flocculation and complement fixation techniques.

Having run both flocculation and complement fixation tests on each specimen of blood over a period of more than 20 years, and having at various times during this period employed the VDRL, Eagle flocculation, Hinton, Mazzini, Eagle complement fixation, and Kolmer complement fixation tests with crude lipoidal and cardiolipin antigens, we have been able to observe the behavior of the various tests on groups of specimens predominantly from presumably healthy nonsyphilitic individuals. Furthermore, we have had ample opportunity to evaluate the various techniques on serums of known reactivity in the monthly State department of health evaluations.

Since cardiolipin antigens became available we have gradually restricted ourselves to the use of the VDRL slide test and the Kolmer complement fixation test using cardiolipin antigens. In the monthly State evaluations we have consistently obtained excellent agreement between results of the two techniques on the pooled serums submitted, but in the higher dilutions of the positive test serums we have found the Kolmer test consistently the more strongly reactive.

Over the years, among the blood specimens submitted to us for diagnostic tests on treated or suspected syphilitic persons, the agreement in findings of the Kolmer and VDRL slide tests has been generally close. Since our change to cardiolipin antigens, however, and particularly since employment of commercially available antigens, we have been considerably concerned with what appears to us to be an inordinate number of weak reactions with the Kolmer complement fixation technique, coupled with nonreactive VDRL findings among our premarital and prenatal specimens. We have investigated every possible factor affecting performance of the tests and have gained the impression from questioning other laboratories that they are having similar experiences with commercial antigens employed at the recommended dilutions.

In 1948, Kolmer stated that six different antigens employing various ratios of cardiolipin, purified lecithin, and cholesterol might be employed in the Kolmer tests in doses of 0.5 ml. of a 1:150 dilution. The acceptable formulas are given in table 1. Kolmer also stated that in general an antigen prepared with 0.03 percent cardiolipin, 0.05 percent lecithin, and 0.6 percent cholesterol was preferred as a mixture having maximum sensitivity consistent with specificity in tests with serums and spinal fluids (*4*).

The currently available Kolmer antigen contains 0.03 percent cardiolipin, 0.05 percent lecithin, and 0.3 percent cholesterol. The optimum dose recommended has been in every case 0.5 ml. of a 1:150 dilution. In this study, we have in addition employed as an antigen for the Kolmer test one placed on the market as a VDRL flocculation antigen. It contains, in addition to lecithin, 0.05 percent cardiolipin and 0.9 percent cholesterol.

Although every commercial package of Kolmer antigen recommends a dilution of 1:150, a number of antigen titrations on lots from two major producers (Difco and Sylvana) gave a titer of 1:320 when carried out by the recommended procedure. The 1:320 dilution was found to be optimum for the VDRL flocculation antigen when it was used as a complement fixation antigen. In various sets of State evaluations of serums there seemed to be no significant loss in sensitivity when the 1:320 dilution of Kolmer antigens was used with either 0.9 percent or 0.3 percent cholesterol. There has been no evidence of increased anticomplemen-

Table 1. Formulas for antigens acceptable for use in Kolmer tests [1]

Antigen No.	Cardiolipin (percent)	Purified lecithin (percent)	Cholesterol (percent)
1	0.03	0.05	0.3
2	.03	.05	.6
3	.03	.05	.9
4	.06	.05	.6
5	.06	.05	.9
6	.175	.0875	.3

[1] Kolmer, J. A., and Lynch, E. R. (*4*).

tary activity in any of the dilutions employed. On the other hand, there does seem to be a decided difference in specificity of the two antigens when used on routine specimens submitted to the laboratory.

In our laboratory we have found that an antigen containing 0.03 percent cardiolipin, approximately 0.21 percent lecithin, and 0.9 percent cholesterol—the composition of standard VDRL flocculation antigen—when used in a test dose of 0.5 ml. of a 1:320 dilution in the standard Kolmer procedure, has a high degree of sensitivity when compared with various approved techniques, including the standard Kolmer test in State evaluations on positive serums of known activity. Compared with the standard Kolmer test, however, the complement fixation test with VDRL antigen gives fewer confusing weak reactions in presumably negative serums.

Weak reactions cannot be taken lightly, yet in those cases which we have been able to check with the TPI test there is an indication that the weakly reactive standard Kolmer reactions in the presence of nonreactive VDRL slide tests are very often nonspecific.

Table 2 shows that, in comparison with other standard procedures, use of an antigen having the composition of VDRL flocculation antigen in the Kolmer test, in a test dose of 0.5 ml. of a

Table 2. Comparison of reaction of VDRL flocculation antigen used as a complement fixation antigen in a test dose [1] with reference findings in a routine State evaluation

Test	Positive dilutions												Negative serums							
	1:4	1:4	1:8	1:8	1:16	1:16	1:16	1:16	1:24	1:24	1:24	1:24	N	N	N	N	N	N	N	N
VDRL slide [2]	R	R	R	R	R	R	R	R	R	R	R	R	N	N	N	N	N	N	N	N
VDRL tube [2]	R	R	R	R	R	R	R	R	R	R	R	R	N	N	N	N	N	N	N	N
Kline standard [2]	4	4	4	4	2	2	2	2	1	1	1	1	N	N	N	N	N	N	N	N
Kahn standard [2]	4	4	3	2	1	1	1	1	1	±	1	±	N	N	N	N	N	N	N	N
Mazzini cardiolipin [2]	4	4	4	4	2	2	2	2	N	N	N	N	N	N	N	N	N	N	N	N
Hinton flocculation [2]	R	R	R	R	R	R	R	R	R	R	R	R	N	N	N	N	N	N	N	N
Kolmer cardiolipin [2]	4	4	4	4	3	3	4	3	1	2	1	1	N	N	N	N	N	N	N	N
Kolmer lipoidal [2]	4	4	2	1	±	N	±	N	N	N	N	N	N	N	N	N	N	N	N	N
Kolmer using VDRL flocculation antigen [1 3]	4	4	4	4	4	4	4	4	1	2	1	1	N	N	N	N	N	N	N	N

[1] 0.5 ml. of a 1:320 dilution.
[2] Reference laboratory.
[3] Dickman Laboratories, Philadelphia, Pa.

NOTE: R—reactive; N—negative.

Table 3. Results of testing 86 blood specimens with the Kolmer standard, VDRL slide, Kolmer with VDRL antigen, and TPI tests

Number of tests	Kolmer standard test	VDRL slide test	Kolmer test using VDRL antigen (1:320)	TPI test
1	++++	NR	±	Negative.
1	++++	NR	+	Positive.
1	++++	NR	++	Do.
1	++++	NR	+++	Do.
1	++++	NR	++++	Do.
3	++++	NR	NR	Negative.
24	++++	NR	NR	Do.
11	+++	NR	NR	Do.
14	++	NR	NR	Do.
22	+	NR	NR	Do.
7	±	NR	NR	Do.

NR—nonreactive.

1:320 dilution, results in a very satisfactory performance. In our laboratory, in a series of 5,215 predominantly premarital blood specimens which were tested with the VDRL slide test and the Kolmer technique with the VDRL flocculation antigen, 51, or 0.98 percent, were reported reactive by both techniques and 35, or 0.67 percent, were reported as doubtful. The doubtful group consisted of specimens in which there was disagreement between the findings of the two tests. In 5,129 of the 5,215 specimens, both tests were nonreactive.

We have further checked at random some specimens submitted to us primarily for premarital or prenatal tests in which there were reactive findings in the standard Kolmer test employing standard antigen in a dilution of 1:150 and nonreactive findings in the VDRL slide test. Complement fixation tests, employing the Kolmer technique with a VDRL flocculation antigen containing 0.03 percent cardiolipin and 0.9 percent cholesterol in a dilution of 1:320, and a TPI test were performed.

The results of testing a series of 86 specimens, of which 68 were submitted for premarital tests, are given in table 3. The four tests in this series which were positive by the TPI test were all reactive to the Kolmer test using VDRL flocculation antigen. In the presence of nonreactive VDRL flocculation tests 82 of the 86 standard Kolmer tests gave presumably biologic false positive reactions.

Most of these blood specimens came from a group in which a low incidence of reactivity is to be anticipated. The significance of a questionable reaction probably varies considerably with the social and economic group from which the individual comes. A reactive finding is less likely to be a biologic false positive in an individual from a group in which the syphilis rate is high than in an individual from a group in which the incidence of syphilis is extremely low.

Kolmer Test in Problem Cases

In our experience, there seems to be considerable evidence of the advantage of using the VDRL flocculation antigen as a complement fixation antigen in analyzing routine specimens.

On 827 of the more than 1,100 "problem specimens" sent to us for TPI determinations, we were able to perform, in addition to the TPI test, both the standard Kolmer and the Kolmer complement fixation technique employing VDRL flocculation antigen in a 1:320 dilution. The results are shown in table 4.

Table 4. Comparison of results of TPI tests on 827 "problem specimens" with results of the Kolmer test with VDRL antigen and the standard Kolmer test

TPI test	Kolmer test with VDRL antigen [1]		Standard Kolmer test	
	Reactive	Nonreactive	Reactive	Nonreactive
Negative	190	322	331	181
Positive	252	63	285	30

[1] 0.5 ml. of 1:320 dilution.

In this group of 616 tests reactive with the standard Kolmer test 331, or 53.7 percent of the reactions may be presumed to be nonspecific. With the use of VDRL antigen as a complement fixation antigen in a Kolmer technique, of the 442 reactive specimens, 190, or 43.0 percent, may be considered nonspecific. Of the 63 TPI-positive specimens giving negative reactions with the experimental VDRL antigen, 28 were nonreactive and 35 were reactive in the VDRL slide test.

Summary

Of 796 reactive "problem specimens" of blood submitted to the Dickman Laboratories for *Treponema pallidum* immobilization tests, 54.6 percent were probably biologic false positive by the VDRL slide flocculation test and 53.2 percent by the standard Kolmer complement fixation test.

In "problem cases" there seems to be no relationship between the degree of reactivity in the standard lipoidal tests and TPI positivity. In a considerable number of routine laboratory specimens, however, weak Kolmer reactions in the presence of negative VDRL slide tests are nonspecific.

The VDRL flocculation antigen in a test dose of 0.5 ml. of a 1:320 dilution would appear to offer significant advantages as a complement fixation antigen in the Kolmer technique. In

general practice VDRL flocculation antigen has a high degree of sensitivity and specificity and, as confirmed by TPI tests, gives fewer questionable weak reactions in the average presumably negative specimens submitted to the laboratory. In problem cases there are fewer nonspecific reactions with the VDRL antigen than with the standard Kolmer antigen and, when run in conjunction with a VDRL slide test, the VDRL antigen should prove to be a desirable substitute for the current standard Kolmer antigen in the laboratory.

It would be profitable to study further experimental compositions and modifications of our currently employed lipoidal antigens in future serologic studies.

REFERENCES

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(*4*) Kolmer, J. A., and Lynch, E. R.: Cardiolipin antigens in the Kolmer complement fixation test for syphilis. J. Ven. Dis. Inform. 29: 166–172, June 1948.

VD Educational Materials

Educational materials pertaining to venereal disease and teenagers are available for meetings and discussion groups, free of charge, from the Venereal Disease Branch of the Communicable Disease Center, Public Health Service. The branch also performs special services, such as providing current data, studies, and reprints. Some of the materials offered are:

Films. "The Innocent Party" shows how a teenage boy contracts venereal disease from a pickup and its consequences. "The Invader" traces man's efforts since the 15th century to cope with syphilis.

Exhibit. "Who has VD?" demonstrates an actual outbreak of syphilis and gonorrhea as it occurred in a small community.

Literature. "Strictly for Teenagers" provides factual answers about venereal disease to teenage questions. "About Syphilis and Gonorrhea" explains briefly the cause, spread, and cure of these diseases. "Public Pressure Hinders Work of VD Eradication" challenges responsible individuals to remember their community obligations and prods community leaders to aid in public enlightenment. "VD in Children and Youth" describes venereal disease and its control problems, particularly as related to teenagers. This 32-page booklet contains advanced information for discussion leaders and writers.

For further information or to be placed on a mailing list for announcements or samples of new materials write to William J. Brown, M.D., Chief, Venereal Disease Branch, Communicable Disease Center, Public Health Service, Atlanta 22, Ga.