Reactivity of VDRL Antigen Suspensions Made at Various Temperatures

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WHENEVER a study is conducted to evaluate the performance of serologic tests for syphilis, the final analysis usually reveals a considerable "spread" in the levels of sensitivity and specificity attained by the participating laboratories, irrespective of the test procedure or the kind of antigen employed. As a matter of fact, wide differences in results are obtained even when all participants use the identical test material in the same test procedure. For example, in a recent National Serologic Laboratory Evaluation all interested participants were given the same VDRL test material to use in the studies. When the results were tabulated it was evident that the reactivity levels shown for the VDRL test varied to essentially the same degree as those shown for other test procedures in which few if any of the participants used the same test material. It appears, therefore, that the reproducibility of serologic test performance is dependent upon the standardization of other factors (besides the antigen) which have not been well enough defined in the literature, and which are therefore poorly or insufficiently controlled in practice. Experience has shown that environmental temperature ("room temperature" so called) is one such factor.

The present work was undertaken to obtain data on the effect of variations in environmental temperature on the physical appearance and reactive nature of a flocculation test antigen suspension.

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Method

The VDRL test was used for this study. In order to avoid features involving differences of opinion, it seemed well to keep the study as objective and tangible as possible. To avoid further complications in interpretation of results, the study was limited to temperature variations introduced only at the point in the procedure where the saline-antigen suspensions are prepared. To simulate conditions comparable to variations in room temperature, all the materials required for preparation of the suspensions (except the antigen) were placed in the refrigerator or in the incubator for periods of time sufficient to impart different temperature levels to them. (The bottle of VDRL antigen was left in the environment of the laboratory: 22.4° C.) Using these various materials and following the stipulations set forth in the serologic test manual (*1*), 10 saline-antigen mixtures were made. Immediately after each suspension was made, a thermometer was placed in the solution to determine the temperature of the resulting antigen suspension. These values were recorded, and for sake of convenience were designated as "suspension temperatures." Then the suspensions were allowed to establish equilibrium with the temperature of the laboratory (22.4° C.) before they were used in the comparative tests. From this point on, meticulous care was taken to as-

Table 1. Comparative sensitivity of VDRL antigen suspensions prepared at various "room temperatures"

[Tests with pooled reactive serum in series dilution]

Antigen No.	1	2	3	4	5	6	7	8	9	10
Suspension temperature, ° C.	15.3	18.2	19.4	21.2	21.8	22.7	23.6	24.8	29.6	37.0
Serum dilution	Plus readings									
1:4	2	3	3′	4	3′	3	3	3	2′	1′
1:8	1	1′	2	2	2	2	1′	1′	1	±
1:16	±	1	1	1	1	1	1	±	±	—
1:32	—	±	±	±	—	—	—	—	—	—
1:64	—	—	—	—	—	—	—	—	—	—
Total pluses	3. 5	6. 0	7. 0	7. 5	6. 5	6. 0	5. 5	5. 0	4. 0	2. 0

Note: The mark (′) denotes a reaction slightly stronger than the plus reading shown.

sure identical treatment of the reagents. The serologic test results were read by a well-trained technician who had no previous knowledge regarding the nature and purpose of this study. The recordings were made in terms of pluses for convenience of comparison.

Results

The suspension temperatures, as observed and recorded immediately after preparation of the suspensions, ranged from 15.3° to 37° C. (table 1).

The first step in studying these preparations consisted in an examination of the suspensions themselves. To several chambers of a Kline test slide there was delivered 0.05 ml. of VDRL buffered saline. One drop of each antigen suspension was dispensed into a separate chamber of this slide, the slide was tapped gently to disperse the particles, and the material was examined under the microscope. The slide was then placed on a conventional agitating machine at 180 rotations per minute for 4 minutes, and the material examined again.

In this series of suspensions there was a wide range in particle size: very fine pinpoint at the low temperature extreme to very large needle-like particles at the high temperature extreme. With the exception of suspension No. 10 (in which the very large particles had a tendency to become entangled on agitation) there was no appreciable change in the appearance of the material on the slide after the 4-minute agitation.

The next step in the study of these suspensions was to test their sensitivity to reactive serum. For this purpose, a mixture of reactive serums was prepared; a series of saline dilutions was made by the double dilution method, and these dilutions were tested simultaneously in the usual manner with all 10 antigens. In table 1 are given the results obtained with each antigen on each dilution of serum.

Glancing over these results, we can see a definite reactivity curve having a peak in sensitivity at the level of antigen No. 3 or 4. The totals of the pluses shown at the bottom of the table are not necessarily significant in themselves, but are given as a means of expressing the sensitivity curve. As indicated here, the sensitivity was greatest for the antigen prepared to have a temperature of 21.2° C.

Finally, comparative tests were done on a group of 36 routine clinical specimens. These specimens were selected because they were weakly reactive in the Mazzini test. For this work, only 5 of the 10 suspensions were used.

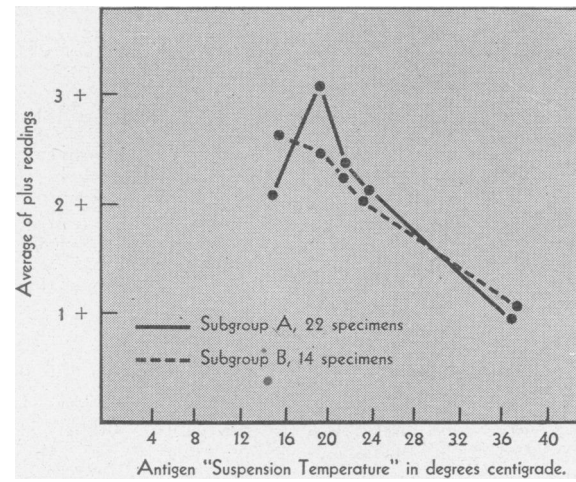
On analyzing the results obtained with this group of 36 specimens it was found that the data could be divided into two subgroups because of the two reaction curves represented (see chart): subgroup A, in which the paraboloid type of reaction was displayed, and subgroup B, in which the reaction seemed to be the semiparaboloid type. In order to show the uniform pat-

tern of reactivity displayed by this series of antigen suspensions when used on individual test serums, the entire protocol, separated accordingly, is given in table 2. The totals and averages of the pluses are also given as an expression of the over-all picture.

The reaction curves are better illustrated by the chart, in which the average plus readings (shown for both subgroups in table 2) are plotted against the respective "suspension temperatures."

The fact that the curves in the chart show a difference in elevation can only be considered a coincidence. On the other hand, the difference in the scope of the curves seems to be due to the nature of the serums under test. Irrespective of the type of reactive specimens involved, however, there was an obvious decrease in sensitivity of the suspensions as the "suspension temperatures" became elevated (above 22° or 23° C.). In addition to this, the suspensions prepared at the higher temperatures were rather coarse and more difficult to evaluate. Suspensions such as No. 10 would definitely be considered unsatisfactory, even by the inexperienced technician.

Variation in the sensitivity of VDRL antigen suspensions and temperature at which the saline-antigen mixtures were made.



Discussion

In considering the effect of temperature on serologic tests we must not fail to recall the work of Kahn, who has long shown with one form of "verification test" that, irrespective of its diagnostic value, differences in reactions are very often obtained under different environmental temperatures. All syphilis test antigens in present use are derived from essentially the same material and, whether we realize it or not, as long as one serologic test procedure is affected by environmental temperature, any procedure we may choose to employ is bound to be similarly affected. The only difference between the above-mentioned verification test and routine serology is that in the former each serum is tested in triplicate and the effects of various temperatures on the colloidal-chemical reaction can be seen side by side. In routine serology a given procedure is applied under only one undetermined and uncontrolled set of environmental temperatures and conditions. No comparative effects are seen; and there is no assurance that the test results would be duplicated on another day, or in another laboratory.

The philosophy advanced here is that "room temperature" is not a standard, designated by any particular line on a thermometer. The term itself serves only as a means of making the distinction between the main bulk of the laboratory space and the controlled thermal equipment, such as the refrigerator, incubator, and autoclave. It designates a place more than it does a condition. The thermometer reading in that place can vary over wide limits, and the fact that we ignore this in our routine work does not prevent it from exerting its influence on the test. Normal room temperature may range anywhere from near 0° C. to above 37° C. depending on the season of the year, geographic location of the laboratory, position of the room in relation to the rest of the building and the points of the compass, presence or absence of large electrical and steam equipment, air conditioning, windows, and many other features. Furthermore, it may vary on any one day from place to place in the room and from time to time. Reagents kept in such a laboratory must be expected to have the temperature of their immediate environment: cooler near the floor, warmer higher up. In winter they will be cooler in a cabinet on an outside wall, warmer in a cabinet on an inside wall—vice versa perhaps in the summer.

A good example of the type of situation that

Table 2. Comparative sensitivity of VDRL an tigen suspensions prepared at various "room temperatures"

[Tests with selected clinical specimens]

Subgroup A						Subgroup B					
Antigen No.	1	3	5	7	10	Antigen No.	1	3	5	7	10
Suspension temperature °C	15. 3	19. 4	21. 8	23. 6	37. 0	Suspension temperature °C	15. 3	19. 4	21. 8	23. 6	37. 0
Serum No.	Plus readings					Serum No.	Plus readings				
1	3	4	4	3	2′	3	3	3	2′	2	1
2	2′	3	3	1	1	6	2	2	1′	1	±
4	2′	3	2′	2	2	7	2′	2′	2	2	1
5	±	1	±	—	—	8	2′	2	2′	2	1
9	1	2	2	1	±	10	1	1	1	1	—
12	1′	2	1	1	—	11	2′	2′	1′	2	1
13	2	3′	2′	2	1′	16	2′	2′	2	2	1
14	3	3′	2′	NT	NT	17	2′	2	2	2	1′
15	2′	3	2′	2	1	18	3	2′	3	2	1
19	1′	2	1′	1	±	20	4	3	3	2′	1
21	2	3	3	2′	1	26	2	NT	1′	1′	±
22	2′	3	3′	3	1′	27	2	2	2	1′	±
23	1′	2′	1′	1′	±	29	4	4	4	4	2′
24	1′	2	2	1′	±	32	3	3	2′	3	2
25	2′	3	2′	2	1						
28	2	2′	2	1′	±						
30	2′	3	2′	2	1′						
31	3′	4	3′	3′	2						
33	1	1′	2	1	±						
34	2	2′	2′	1′	±						
35	3	4	3	2′	1						
36	2	3′	2′	2′	1						
Total pluses	46. 0	61. 5	52. 3	39. 0	20. 5	Total pluses	36. 5	32. 0	31. 0	28. 5	14. 5
Average	2. 09	3. 07	2. 38	2. 04	0. 97	Average	2. 61	2. 46	2. 22	2. 02	1. 03

NOTE: The mark (′) denotes a reaction slightly stronger than the plus reading shown. NT=Not tested.

actually obtains in laboratories is illustrated by an experience in a rather large, air-conditioned private laboratory which had a room temperature of 23° C. (75° F.) on a day when the out-of-doors temperature was −10° C. (14° F). The temperature in the cabinet hanging against an outside wall and containing the glassware, saline, and antigen was 8° C. (46° F). While the materials were used in the room at 23° C., the temperature of the reagents did not at all approach that of the room when the antigen suspension was made, not to mention the fact that the antigen itself had in reality been refrigerated for at least one period of approximately 12 hours prior to its use. Yet the technician considered his work satisfactory; he had complied with the instructions in the literature and had been using the material at room temperature.

The data given in this report are not presented to specify the suspension temperature at which the VDRL test antigen suspension should be made; for in the first place, the optimum suspension temperature might well differ for each alcohol preparation of the material (a phase which has not yet been studied); and second, the incorporation of such a stipulation in the test procedure is a matter for the consideration of the test authors. This report is intended only to record the observation that differences in the appearance of the microscope field as well as differences in sensitivity could be demonstrated to result from controlled variations in room temperature even though the variations were affected for only one of the many phases in test procedures where temperature can and does vary in actual practice.

The resulting differences in sensitivity of the antigens studied may not appear especially startling; and perhaps for practical purposes such variations can be considered negligible. However, if we carefully study serologic test procedures from the standpoint of the numerous combinations of conditions under which differences in temperature and other factors can easily be introduced, without violating the instructions given in manuals of procedures, we can readily understand that to a great extent inconsistencies in results are without doubt due to the "negligible" effect of one variable superimposed upon that of others.

The effects of all the negligible features are just as important to that serologic summation which we call the serologic test report, as seconds are to the accumulation of time.

Conclusions

Since temperature influences the colloidal make-up and behavior of syphilis test antigen suspensions, and since the temperature can be controlled for certain phases of serologic test procedures, the optimum temperature range for these phases should be determined and specified in the literature. The term "room temperature" has little or no meaning from a scientific standpoint, and should be deleted from serologic test descriptions.

Summary

In a laboratory with a "room temperature" of 22.4° C. it was possible, under controlled conditions, to prepare antigen suspensions under conditions of temperature ranging from 15.3° C. to 37° C. With the use of these antigens (brought to a room temperature of 22.4° C.) data were obtained showing that the microscopic appearance and the sensitivity of a flocculation test antigen suspension (VDRL) are in a degree determined by its temperature at the time it was made.

This report is offered as an indication that environmental temperature is one of the factors contributing to wide variations in serologic test performance.

REFERENCE

(*1*) U. S. Public Health Service: Manual of serologic tests for syphilis. Supplement No. 22, J. Ven. Dis. Inform., 1949.

Dr. Foard Retires

Dr. Fred T. Foard, chief of the branch of health, Bureau of Indian Affairs of the Department of the Interior, retired as an officer of the Public Health Service on October 31, 1952, after 36 years of service. He is now director of the division of epidemiology for the North Carolina State Department of Health.

Dr. Foard began his public health career by assisting in control of malaria, typhoid, and other environmental diseases in the West and Southwest. In 1920 in Montana he organized the first full-time county health unit in the Rocky Mountain tier of States, and assisted health officers in 10 other western States to organize district and local units when funds became available under the Social Security Act.