The Kahn Reaction.

R. L. KAHN.

The Kahn reaction is a precipitation method for differentiating syphilitic from non-syphilitic serum or spinal fluid. The following outline of technique is necessarily brief. For complete details, the reader is referred to "The Kahn Text—A Practical Guide" (Bailliere, Tindall and Cox, London, 1928).

I. APPARATUS.

1. Test tubes for performing test are about 7·5 cm. in length and 1 cm. in diameter.

2. Vials (with straight wall and flat bottom) for preparing antigen suspension are about 5·5 cm. in length and 1·5 cm. in diameter.

3. Pipettes : 10c.c. graduated to 0·1 c.c. ; 1 c.c. graduated to 0·01 c.c. and 0·2 c.c. graduated to 0·001 c.c.

4. Test tube rack : Made of sheet copper, 3-in. wide, 11½-in. long, 2½-in. high. Consists of three shelves, upper and middle ones containing three rows of 10 holes, each of approximately ½-in. diameter. The centre row holes are offset ½-in.

5. Shaking apparatus may be of any construction which will hold the test tube racks employed. The required speed is 275 oscillations per minute, with a stroke of 1½-ins.

6. Water bath (56° C.), centrifuge and centrifuge tubes may be of any make which will be found convenient in the particular laboratory.

II. REAGENTS USED IN KAHN TEST.

1. *Antigen.*—Standard Kahn antigen may be obtained on the market, titrated and ready for use in (1) the routine test with serum ; (2) the quantitative procedure with serum and (3) the spinal fluid procedures. Only chemically clean and dry glass vessels should be used for storing antigen, and the cork stoppers should be covered with thin high grade tin foil. Antigen should be kept in the dark at room temperature. When subjected to cold, a precipitate may be thrown down which can be redissolved upon warming the antigen in water bath at 37° C.

2. *Serum.*—Centrifuge blood specimen to remove clot and cells. Make sure that the serum when used in the test is entirely free from cells and other particles. Previous to its use, the serum is heated in a water bath at 56° C. for 30 minutes. It is desirable to use the serum soon after

heating, if possible, within 30 minutes. When heated serum is kept for four hours or longer, it is reheated for ten minutes at 56° C. before using in the test.

3. *Normal Saline.*—Prepare a solution of 0·9 per cent. sodium chloride (chemically pure) in distilled water.

III. ROUTINE (DIAGNOSTIC) TEST WITH SERUM.

This is a three tube test. Each tube contains a different proportion of serum and antigen suspension according to the following outline :—

Tube	1	2	3
Antigen suspension, c.c.	0·05	0·025	0·0125
Serum, c.c.	0·15	0·15	0·15

It is well to have everything arranged before mixing the antigen with salt solution for the test. Have racks set up, tubes numbered, sera heated and pipettes ready for measuring antigen suspension and serum. For measuring the 0·05 c.c. quantities of antigen suspension, mark off these amounts on a 1 c.c. (graduated to 0·01 c.c.) pipette with a wax pencil. For measuring the 0·025 and 0·0125 c.c. quantities, use 0·2 c.c. (graduated to 0·001 c.c.) pipettes on which these amounts are also indicated with a wax pencil.

1. *Preparation of Standard Antigen Suspension.*—Mix antigen with salt solution according to required titer. Thus, if the titer is 1 c.c. antigen plus 1·1 c.c. normal saline, mix the antigen as follows: (*a*) Measure 1·1 c.c. saline into a standard antigen suspension vial. (*b*) Measure 1 c.c. antigen into a similar vial. (*c*) Pour the salt soution into the antigen and as rapidly as possible (without waiting to drain the vial) pour the mixture back and forth six times to insure thorough mixing. (*d*) Allow the antigen suspension to stand for ten minutes before using. The suspension should not be used after 30 minutes standing.

One may mix more than 1 c.c. of antigen with a proportionally larger amount of salt solution but not less than 1 c.c. This amount when mixed with saline will be sufficient for about 15 tests.

2. *Measuring Antigen Suspension.*—After the antigen suspension has stood for ten minutes, shake it well and measure 0·05, 0·025, and 0·0125 c.c. amounts for each serum, delivering the suspension to the bottom of the tubes. When employing the standard rack which contains 30 tubes, measure 0·05 c.c. amounts in the tubes of the first row;

0.025 c.c. amounts in the tubes of the second row and 0·0125 c.c. amounts in the tubes of the third row.

3. *Measuring Serum.*—The serum should be added as soon as possible after the antigen suspension has been pipetted to avoid undue evaporation of the suspension. When examining large numbers of sera, it is well for one worker to measure the antigen suspension and for another to follow with the sera. Add 0·15 c.c. serum to the 0·05, 0·025 and 0·0125 c.c. amounts of antigen suspension, and shake the rack of tubes vigorously for ten seconds to insure thorough mixing of the ingredients. The rack can now be set aside until the remaining tests are ready for the regular three-minute shaking period.

4. *Controls.*—At least one positive and one negative serum control should be included with each series of tests. Every serum giving a positive reaction should be examined to establish that it is free from red cells or foreign particles which might be confused with a specific precipitate. Dilute 0·1 c.c. serum with 0·3 c.c. saline. Shake well and examine for particles. If particles are present, the serum should be recentrifuged and retested.

5.—*Shaking.*—During the three-minute shaking period, it is important not merely to agitate the rack of tubes, but to see to it that the fluids within the tubes are vigorously agitated. When shaking by hand, one may shake three one-minute periods with short rest periods. When a shaking machine is employed, its speed should be from 275 to 285 oscillations per minute, with a stroke of 1·5 inches. Shaking by hand should approximate this speed.

6. *Addition of Saline.*—After the tests have been shaken, add 1 c.c. saline to each tube of the first row of the rack (containing the 0·05 c.c. amounts of antigen suspension) and 0·5 c.c. saline to the remaining tubes. Shake sufficiently to mix ingredients.

7. *Reading Results.*—The results are read after the addition of the salt solution. Optimum reading conditions in each laboratory should be determined by trial. The following points will be found helpful: (*a*) When utilising daylight for reading the tests it is well to have but one source of light coming from a single window immediately in front of the reader. It will be found satisfactory to shade the lower three-fourths of the window, narrowing the sourse of light to a small section at the top of the window. Light from any other windows near the reader should be dimmed by lowering

the window shades. (*b*) When holding the rack in front of the exposed section of the window, the definitely positive and the negative reactions are readily differentiated without lifting the tubes from the rack. (*c*) In case of weak reactions examine each tube individually, lifting it several inches above the eye level, and slanting it until the fluid is spread into a thin layer. The precipitate will then become readily visible.

Those preferring magnification will find the microscopic mirror helpful. Place mirror on reading table with concave surface upward. Hold the tube in slanted position two to three inches above the mirror and examine the image in the mirror. Both daylight and artificial light may be employed. One may also utilise an ordinary hand lens for reading the tests. A two or three-fold magnification will be found satisfactory. Some workers prefer the use of a slit-light arrangement, the source of light being an electric bulb enclosed in a box which is provided with a narrow slit.

As far as possible, workers should limit themselves to one method of reading. The occasional use of magnification by readers who usually do not resort to it might affect the uniformity of their reading scale. The magnification must be sufficiently low in order to assure opalescent and clear cut negative reactions, with entire freedom from visible particles.

8. *Interpretation of Results.*—A definite precipitate suspended in a clear medium is read four plus. Proportionally weaker reactions are read three, two and one plus and doubtful, respectively. The final result of the test in all cases is the average of the readings of the three tubes, an indicated in Table I.

9. *Recording Results.*—Make a permanent record of findings in all tubes of each test at time of reading. Preferably, the tests should be read independently by two separate workers. When two workers are not available, the original reading should be checked by the same worker after a short interval.

TABLE I. OUTLINE OF KAHN TEST AND INTERPRETATION OF RESULTS.

Tube No.	1	2	3	Completion of Test.
Serum : Antigen suspension..	3 : 1	6 : 1	12 : 1	Tests are shaken three minutes, 1 c.c. salt solution is added to first tube and 0·5 c.c. to other two tubes and results are read.
Antigen suspension, c.c.	0·05	0·025	0·0125	
Serum (heated at 56° C. for 30 min.) c.c. ..	0·15	0·15	0·15	

INTERPRETATION OF RESULTS.

Reaction No.	——	——	——	Final Result (Average of Reactions of Three Tubes.)
1.	++++	++++	++++*	
2.	+++	++++	++++	++++
3.	++	++++	++++	
4.	+	+++	++++	+++
5.	—	+++	++++	
6.	—	++	++++	++
7.	—	—	++++	
8.	—	—	+++	+
9.	—	—	++	+
10.	—	—	+	—
11.	—	—	—	

* Weakly positive sera show most marked precipitation in the third tube because a small amount of *reagin* reacts best with a small amount of antigen suspension, the relatively larger amounts of suspension in the first two tubes being inhibitory to precipitation.

Strongly potent sera show four plus precipitation in each tube, but due to the different amounts of antigen suspension employed, the precipitates are of unequal bulk, being greatest in the first tube and least in the last tube.

In rare instances an a-typical reaction is obtained in which precipitation is marked in the first tube and weak or negative in the second and third tubes. In such a case, a quantitative test should be made and if the result is 20 units or more, the qualitative reaction may be considered four plus; if less than 20 units, the results of the qualitative reaction should be averaged.

10. *Procedure with Less than Three Tubes.*—If there is insufficient serum for the three-tube test, examine and report as follows: (*a*) If enough serum for two tubes, employ the lesser amounts of antigen suspension. Report as a two-tube test. (*b*) If enough for one tube, employ the least amount of antigen suspension. Report as a one-tube test.

Types of Reaction in Individual Tubes.—(*a*) Four plus reactions. In these reactions, definitely visible particles are suspended in a transparent or opalescent medium. The individual particles are readily visible by direct examination without lifting the tubes from the rack. (*b*) Three plus reactions. In these, the particles are also definitely visible, but are less clear-cut than in four plus reactions. The particles may not always be distinguished until the tube is lifted from the rack and examined individually. (*c*) Two plus

reactions. In these, finer particles are suspended, frequently in a somewhat turbid medium. The particles cannot be distinguished until the tube is examined individually, usually by slanting. (*d*) One plus reactions. In these, still finer particles are suspended in a somewhat turbid medium. (*e*) Doubtful reactions. In these, extremely fine particles, just within the visible range, are suspended in a somewhat turbid medium. (*f*) Negative reactions. In these, the medium is transparent, opalescent and free from visible particles. In the rack, negative reactions are readily distinguished from weakly positive reactions by the fact that the latter appear turbid.