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Method of titrating antigen for Kahn precipitation test.

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This method aims to overcome variations in antigens employed in the precipitation test for syphilis proposed by the author. Two antigens prepared under identical conditions from two different lots of beef heart, will be likely to show variation in sensitiveness when tested with syphilitic sera. This variation in sensitiveness may be considerably lessened if each antigen is first mixed with salt solution in such proportion as to bring forth its maximum power for producing precipitates with syphilitic sera. Generally speaking, this is accomplished by mixing antigen with *minimum* amounts of salt solution which will produce opalescent mixtures. Since the mode of adding salt solution to antigen markedly affects the final product, it is obviously important to render all conditions which are likely to affect this titration as constant as possible.

The test tubes used in this titration should not differ from those used in the regular tests. In our experience, tubes of 0.8 to 1 cm. diameter give best results. The set up of the titration is as follows:

Six test tubes receive 0.5 c.c. antigen each. Six similar tubes receive respectively 0.25, 0.5, 0.75, 1, 1.25 and 1.5 c.c. salt solution (0.85 per cent NaCl). The 0.25 c.c. salt solution is now poured into the first antigen tube and the mixture immediately poured back and forth several times. The next antigen tube receives the 0.5 c.c. salt solution in a similar manner. The remaining antigen tubes receive the four increasing amounts of salt solution under similar conditions. It will soon be observed that the first two or three tubes show varying degrees of precipitation, the middle tubes, clouding and the last few tubes, opalescence.

The salt solution-antigen mixture in which the smallest amount of salt solution is capable of producing opalescence is the salt solution-antigen titre for procedure I.¹

¹ PROC. SOC. EXP. BIOL. AND MED., 1923, XX, 325.

With most antigens, three parts of salt solution represent the minimum amount which will bring about an opalescent mixture. In some cases, however, 2.5 and even 2 parts of salt solution may still produce an opalescent mixture and may therefore be used with safety. An antigen-salt solution mixture must show no signs of turbidity when used in the tests.

Those tubes showing precipitation are now centrifuged for about 10 minutes, supernatant fluid poured off and replaced with an amount of salt solution (1 c.c.) equivalent to twice the amount of antigen originally used. After thorough shaking, it will be observed that whereas the tube which originally contained 0.5 c.c. antigen and 0.25 salt solution shows a precipitate, the next tube which contained equal quantities of antigen and salt solution, although milky, may be opalescent and entirely free from any suggestion of a precipitate. If this tube shows some turbidity instead of opalescence then the next tube which contained 0.5 c.c. antigen and 0.7 c.c. salt solution will be found to be opalescent. Under such conditions one is also likely to find that an antigen-salt solution proportion of 1:1.25 (instead of 1:1.5) will also produce opalescent mixtures and could therefore be used in the tests.

The salt solution-antigen proportion in which the smallest amount of salt solution produces a precipitate capable of forming an opalescent mixture on resuspension in salt solution is the antigen-salt solution titre in procedure II.

Table I illustrates a typical titration of antigen.

TABLE I. ANTIGEN TITRATION.

Tube	1	2	3	4	5	6
Antigen c.c.	0.5	0.5	0.5	0.5	0.5	0.5
Salt Solution c.c.....	0.25	0.5	0.75	1.0	1.25	1.5

The antigen and salt solution are mixed in each case by pouring the latter into the antigen tube and immediately pouring the mixture back and forth several times.

Results	Precipitate	Precipitate	Precipitate	Cloudy	Opalescence	Opalescence
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Tubes 1, 2 and 3 are now centrifuged for about 10 minutes, supernatant fluid poured off, replaced with 1 c.c. salt solution (twice the amount of antigen originally employed) and mixed thoroughly.

Results	Precipitate	Opalescence	Opalescence			
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This titration shows that in Procedure I two and a half parts of salt solution added to one part of antigen represent approximately the smallest amount of salt solution which will produce an opalescent mixture. The antigen-salt solution titre of this particular antigen, therefore, is

Antigen: Salt Solution = 1:2.5

In Procedure II, on the other hand, one part of salt solution added to one part of antigen appears to represent approximately the smallest amount of salt solution which produces a precipitate capable, after being freed from supernatant fluid, of forming an opalescent suspension in salt solution. The titre, therefore, is

Antigen: Salt Solution = 1:1

It is evident that the antigen titration outlined gives only approximate amounts of salt solution which produce opalescent mixtures. Occasionally one will find on trial that these amounts of salt solution can be slightly lessened and still obtain opalescent mixtures. Under these conditions, the smaller amount of salt solution should be employed in the tests.

After determining the smallest amounts of salt solution to mix with antigen in Procedures I and II, the next step is to test the opalescent mixtures with at least six positive and six negative sera, and particularly establish that there is no tendency for weak non-specific reactions.

One will not encounter non-specific reactions when employing opalescent mixtures. One should not confuse occasional sediments or precipitates settled on the bottom of the tube for specific precipitates which are almost invariably suspended in the medium. The employment of serum controls (0.3 c.c. serum + 0.05 c.c. salt solution) will overcome the uncertainty regarding

TABLE II. RESULTS OF ANTIGEN TITRATIONS

	PROCEDURE I		PROCEDURE II	
	Proportion of antigen and salt solution producing opalescent mixtures			
	Antigen:	Salt Sol'n.	Antigen:	Salt Sol'n.
Non-Cholesterinized antigen	1	: 1.75	1	: 0.85
Same antigen with 0.4 per cent. cholesterin..	1	: 2.5	1	: 1.1
Same antigen with 0.8 per cent. cholesterin..	1	: 3.5	1	: 1.4

these bottom precipitates, as they will be found to be present in the controls as well.

It may be of interest in this connection to give the titration results of an extract antigen prepared as outlined in a previous paper of these Proceedings,¹ and the same extract containing 400 and 800 mgm. of cholesterin per 100 c.c., respectively.

This titration would indicate that cholesterin plays an important rôle in necessitating proportionally larger amounts of salt solution to bring about opalescent antigen-salt solution mixtures. It might be added also that in a general way, cholesterin proportionally increases the sensitiveness of the antigen after mixing with syphilitic serum. The problems involved in the cholesterinization of antigen are reserved for further studies.

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Employment of different antigens in Kahn precipitation test.

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Employing the antigen titration outlined in the previous paper, the question arose to what extent the preparation of antigen may be varied without affecting the final results. The following antigens were accordingly prepared, titrated with salt solution and smallest amounts of the latter used which produced opalescent antigen-salt solution mixtures. The tests were carried out with positive and negative sera according to procedures I and II.

Antigen 1. This was prepared as described in a previous paper of these *Proceedings*.¹ Dried beef heart was freed from ether extractives and subsequently extracted in 95 per cent. alcohol for 9 days in the ice box and overnight at incubator temperature. Color approximated potassium bichromate color standard.

Antigen 2. After extracting the dried heart with ether in the usual manner, boiling alcohol was poured on the dried material, shaken and extraction continued for 1 day in the incubator. Color approximated potassium bichromate standard.

¹ Kahn, R. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, **xx**, 325.