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Preparation of Relatively Stable Emulsions With Kahn Antigen Base.

B. S. KLINE.

From the Laboratory Department, Mount Sinai Hospital of Cleveland.

In a previous article[1] describing a purified antigen for serum tests for syphilis, a formula was given for the preparation of most satisfactory precipitation test emulsions that retained their antigenic properties undiminished for 3 days. A further study of the Kahn antigen base has shown that by the following procedures, relatively stable emulsions can be prepared from it also:

The Kahn antigen base described by Kahn[2] (non-cholesterinized alcoholic extract of heart muscle powder previously extracted with ether) is evaporated at 45° C. to 50° C. on water bath (thermometer bulb within the extract). The resultant wax is weighed and 10 cc. of hot (at 50° C.) absolute alcohol (99+%) is added to each gram in glass stoppered bottle, shaken about 5 minutes, and the turbid solution placed in incubator at 50° C. for 1 hour. Bottle is shaken a few times to promote solution.

The turbid solution is then placed in refrigerator at 8° C. for 4 to 5 hours during which a whitish precipitate settles out. This is filtered off and the resultant filtrate is the antigen. The same formula as previously described[1] is then used as follows:

1. *Very Sensitive Antigen Emulsion.*	2. *Sensitive Antigen Emulsion.*
Distilled water, 0.85 cc.	0.85 cc.
Cholesterin (Merck)* 1.25 cc. of 1% sol. in	0.75 cc.
absolute ethyl alcohol	Same.
Antigen (concentrated, chilled Kahn extract), 0.1 cc.	Same.
NaCl (C. P. Merck), 2.2 cc. of 0.85%.	2.75 cc.

* The 1% cholesterin solution is prepared as follows: To 1 gm. of cholesterin (Merck), 100 cc. absolute ethyl alcohol is added and the bottle placed in an incubator at 50° C. to 56° C. The bottle is shaken at 15 minute intervals to promote solution (which is complete) in about 45 minutes. This stock solution is kept in the incubator at 37° C. and is perfectly satisfactory for the preparation of the emulsions for as long as 2 months.

[1] Kline, B. S., *J. Lab. and Clin. Med.*, 1928, xiii, 588.

[2] Kahn, R. L., 'Serum Diagnosis of Syphilis by Precipitation.'' Williams & Wilkins Co., 1925, 132.

The antigen emulsions are prepared as follows. Into 1 ounce bottle 0.85 cc. of distilled water is pipetted. The bottle is held at an angle and the 1% cholesterin (Merck) in absolute ethyl alcohol is allowed to run along the side of the bottle. The bottle is gently rotated from the neck for from 15 to 20 seconds. The bottle is held at an angle again and 0.1 cc. of antigen is pipetted against the side from a 0.2 cc. pipette (graduated in thousandths). The bottle is promptly stoppered with a cork covered with tin foil and shaken fairly vigorously (the fluid thrown from the bottom to cork and back) for 30 seconds. Lastly, the 0.85% sodium chloride (C. P. Merck) solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for 30 seconds.

The emulsions, when examined through the microscope at a magnification of about 75 times, show numerous exceedingly fine particles. They are satisfactory for use in precipitation tests immediately after preparation and at any time within 3 days (kept at room temperature).

The formula given above is based upon the following observations: Uniformly satisfactory emulsions result only when the cholesterin is precipitated before the antigen is added. Furthermore it has been found that the sensitivity of the precipitation test depends more upon the quantity of cholesterin than of the antigen present. Again, it was observed that alcohol interferes with the stability and cholesterin dispersion of antigen emulsions. This explains the use of a much more concentrated antigen than employed by other workers. As a result, the emulsions are relatively stable and as more cholesterin may be dispersed in a given total of emulsion, greater sensitivity is achieved. When prepared by the formula outlined above the antigen emulsions are superior to the Kahn antigen dilutions made from the same extract in the following ways:

Antigen Emulsions (Kahn Ext.)	*Kahn Antigen Dilutions.*
(a) Relatively stable, satisfactory for at least 3 days. (After this, at room temperature, emulsions are still exceedingly finely granular when examined through the microscope, magnification about 75 times, and contain no clumps or crystals.)	(a) Unstable, satisfactory for an hour and a half at most, usually less.
(b) May be used immediately after preparation.	(b) Must stand from 10 to 15 minutes before use.
(c) Satisfactory at low room temperatures as well as at ordinary ones.	(c) Majority unsatisfactory at low room temperatures.

(d) The very sensitive emulsion is more sensitive than the Kahn antigen dilution from the same extract and no less specific.

In addition it has been found that a Kahn extract giving unsatisfactory antigen dilutions (because of small granular particles not disappearing with greater saline dilution) yields satisfactory emulsions when concentrated, chilled and mixed as described above.

The following charts illustrate the advantages of the emulsions of modified Kahn antigen base in comparison with Kahn antigen dilutions from the same extract:

CHART I.

Comparison of stable antigen emulsion and Kahn antigen dilution in the microscopic slide precipitation test for syphilis.[3]

	1	2	3	4
			Sera retested 3 days later with	
Number of sera tested	Freshly prepared emulsion of concentrated chilled Kahn extract No. 4 by new formula	Freshly prepared Kahn antigen dilution (same extract as 1, 3, 4)	Three day old emulsion of concentrated chilled Kahn extract No. 4 by new formula	Freshly prepared emulsion of concentrated chilled Kahn extract No. 4 by new formula
11	—	—	—	—
1	+, ++	++	+, ++	+, ++
1	++	++	++	++
1	++	++	+	+
1	++	+	+	+, ++
1	+++	+++	++, +++	+++
3	++++	++++	+++, ++++	++++
3	++++	++++	++++	++++

Total sera tested, 22.

Tests for syphilis with the emulsions of Kahn extracts prepared as described in this paper, agreed closely with those in which a purified antigen was used and with Wassermann tests (Cleveland method) in which a cholesterinized antigen was employed. The preparation and necessary modification of the Kahn antigen base for most advantageous use is more laborious than the preparation of a purified antigen previously described.[1]

Conclusions. 1. Kahn antigen base when concentrated, chilled and used according to a formula described above yields thoroughly satisfactory emulsions that retain their antigenic properties undiminished in precipitation tests for syphilis for 3 days.

2. These emulsions are further superior to Kahn antigen dilutions in tests done at low room temperatures in that under these conditions they give no false clumping.

[3] Kline, B. S., and Young, A. M., *J. Lab. and Clin. Med.*, 1927, xii, 477; *Am. J. Syphilis*, 1927, xi, 290.

CHART II.

Comparison of stable antigen emulsion and Kahn antigen dilution in the microscopic slide precipitation test for syphilis.

Number of sera tested. Courtesy of Dr. H. J. Knapp, Cleveland Health Department	1 Three day old emulsion of concentrated chilled Kahn extract No. 4 by new formula	2 Freshly prepared emulsion of concentrated chilled Kahn extract No. 4 by new formula	3 Freshly prepared Kahn antigen dilution (same extract as 1 and 2)	4 Three day old emulsion of concentrated chilled Kahn extract No. 1 by new formula	5 Freshly prepared emulsion of concentrated chilled Kahn extract No. 1 by new formula
49	—	—	—	—	—
4	—	—	—	—	—
1	±	—	—	—?	±
1	±	±	—	—	—
2	±, +	±	—	—	—
1	+	±	±	—	—
1	++	++	++	++	++, +++
1	++	+	+	++	+, ++
1	+++	++	—?		++
1	++++	++++	++++	+++	++, +++
3	++++	++++	++++		
7	++++	++++	++++	++++	++++

Total sera tested, 73.

3. Because more cholesterin may be dispersed in a given total by the new formula, it is possible to make the emulsions more sensitive, yet no less specific, than Kahn antigen dilutions from the same extract.

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Bronchial Perfusion of Isolated Lung as a Method for Studying Pharmacologic Reactions of Bronchiolar Muscle.

TORALD SOLLMANN AND W. F. VON OETTINGEN.

From the Department of Pharmacology of the School of Medicine of Western Reserve University.

The methods for studying the changes of bronchiolar tone in intact animals are often complicated by changes in the circulation which make their interpretation uncertain. It would therefore be desirable to check them under conditions in which the circulation would be excluded, namely outside of the body. The use of ring preparations of tracheal or bronchial muscle for this purpose has not been altogether satisfactory, partly because these are confined to the trachea and larger bronchi which might react differently from