ADDENDUM B.

Memorandum by W. M. Scott, M.D. on the Technique of the Wassermann Test as proposed by 29 Pathologists.

This analysis is based on such details as have been submitted to the Board by 29 pathologists who proposed to perform laboratory diagnosis in connection with the V.D. organization. They have not in every case been approved by the Board but they represent to a fair extent the range of current practice in conducting the Wassermann test at the beginning of 1917.

Hemolytic Combination.

All use the original Wassermann combination consisting of Sheep's Blood Cells, Hemolytic Immune Body contained in the serum of rabbits injected with sheep's blood cells and Complement contained in the serum of guinea pigs.

Suspensions of Sheep's blood cells employed.

The strength of suspension stated by 25 pathologists is 5%; 3 pathologists use 10% or more and 3 use less than 5%, the lowest being 1½%.
The mode od calculation is not always stated; some pathologists state the percentage in terms of "full blood" while others take deposited cells at the basis; as a result in the case of two suspension, each stated as 5% one may represent approximately twice the strength of the other.

Hemolytic Immune Body.

Sensitization of the sheep's blood cells with this varies in degree from 10 times the minumem necessary (1) to 3 times (15) and less than 3 (7).
The method employed is now always stated; in most cases it consists in mixing equal volumes of the hemolytic immune serum (appropriately diluted) and the blood suspension; hence the latter is really half the strength stated when actually employed as indicator for the presence of complement

Complement.

A preliminary titration performed by 24 pathologists.

The acutal amount of guinea pig's serum found necessary varies from less than .005cc (4) to between.005 and .02cc (16) and between .02 and .05cc (9). These differences may be explained by the diffenences in the amount of blood cells (varying from .001cc to .05cc of neat cells per tube) and in the degree of sensitization of these.

Two patholigists proposed to perform the complement titration in presence of antigen, which would introduce an error in the estimate of total complement fixed in the final test.

Fresh guinea pig's serum(that is bled the same day as used) is employed by 17 pathologists while 7 state their preference for serum at least 24 hours old.

Serum from a single animal is employed by 17 pathologists while three state that they prefer to pool the serum from several animals.

Period of Incuabation.

One hour is chosen by 12 pathogists as the period of incubation required before reading results of hemolysis, while 17 pathologists read results after shorter intervals (in most cases half an hour.). A water bath is preferred by 13 of the 29.

Patient's Serum.

In all cases inactivation of the patient's serum is performed. Ten pathologists state that it should be done immediately beofre carrying out the test, the others made no reference to this point. The method of inactivation varies from 20 minutes at 55^{0} C. to an hour at 56^{0}C; one pathologist proposed 15 minutes at 60^{0}C.

Graduated amounts of the patient's serum are employed by 19 pathologists but 5 of these use only two different quantities. Ten pathologists employ only one quantity.

The Maximum quantities employed vary as follows: .5cc is used by 4 pathologists, between .4 and .2cc by 7, .1cc by 10 and .05 cc by 8.

Test for anti-complementary action of the patient's serum is performed

by 23 pathologists, of whom 2 determine it accurately in terms of doses of complement fixed, while 6 test the effect on the minimum complementing dose only and the remaining 15 employ a simple control with the amount of complement employed in the final test (2 to 5m.c.d.) Six pathologists neither estimate the anti-complementary action of the patient's serum nore employ a simple control. Most of these six, however, work with a considerable excess of complement in the final test, which somewhat lessens the need for this control.

Antigen.

A commercial preparation is employed by 7; the remaining 22 use alcoholic organ-extracts prepared by themselves.

Cholesterin is a constituent of the antigen as used by 25; only 4 pathologists prefer the organ-extract alone.

Standardization of the antigen is attempted by all. In 12 of the schemes submitted a test for possible hemolytic acitivity of the antigen itself is mentioned, while in 28 of the total 29 the non-specific anti-complementary action of the antigen is put down as requiring to be tested. Seven pathologists estimate it accurately in terms of doses of complement removed, while 17 put up a simple control with the doses of antigen and complement used in the final test. Four only estimate this non-specific action in presence of normal sera.

Specific activity that is the complement-fixing action in presence of known syphilitic sera, is tested by all, but only 8 patholigists estimate it in terms of doses taken up. The remainder employ a simple control with the quantities used for the final test, putting them up with one or more "known positive" sera.

Final test.

Only 3 pathologists rely on a single tube for the final test in which antigen, patient's serum and complemant are incubated together. Two tubes, containing different quantities of one or other of those

constituents, are used by five. The remainder (21) make some attempt to estimate more closely the range of the positive effect. Nine do so by increasing the amount of complement available for fixation by the given quantities of the patient's serum and antigen. Nineteen diminish the quantity of the patient's serum; 14 of these keep the other constituents constant while two also increase the amount of complement and three also diminish the amount of antigen.

Practically all these estimates are directed towards determing the height of "positiveness". Graduation in the opposite direction that is towards determining degrees of "positiveness" short of the standard taken as a diangostic positive, is rarely stated. This may be because the techniques submitted were supposed by the latter estimate becomes of importance.

The ratio of the amounts of antigen (expressed as undilated extract) and patient's serum varies much in the different schemes submitted. Seven pathologists use about equal amounts; 12 employ at least 10 times as much patient's serum as extract, while in 10 of the schemes the quantities differed to an intermediate degree.

The total volume of the combined fluids in the final test varies from 2.5cc in two of the schemes to .12cc in one; the commonest amounts are 1.5 and 2cc.

One hour at 37°C is allowed for fixation of complement in the final test by 23 pathologists; three gave 20 minutes and one five minutes only.

Interpretation of Results.

The report made on a given result of the test is now always precisely stated. Ten patholigists agree in taking the fixation of 2½ minimum complementing doses as the basis for a positive report, but the actual amounts of guinea pig's serum, patient's serum and antigen involved in the reaction differ considerably in the ten schemes and it is doubtful

if their standard is in reality even approximately the same.

Conclusions.

There is general unanimity as to the form of the reaction among the 29 schemes submitted but almost complete absence of uniformity in the details of conducting it. If concordant results are to be obtained uniformity would appear to be necessary inthe following matters :

(1) the limits in the amount of guinea pig's serum to form the minimum complementing dose and to be used in the final test for fixation (this would involve using the same amount of blood cells, the same amount of hemolytic serum, the same total volume of fluid and the same time limit for incubation; (2) the amount of antigen, its preparation and standardization; (3) the amount of patient's serum and its treatment as regards inactivation; and (4) the concentration in which the three constituents interact in the final test, when possible fixation of complement is taking place.

Such uniformity having been attained, the basis for a positive diagnosis would become standard and be expressed ad complete absence of hemolysis. Thereafter it would become possible to grade one or more of the constituents so as to give a quantitative estimate of the reaction as above or below standard.

The optima as regards (1) to (4) are matters involving research.