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## Standardization of the Wassermann Test.

If Wassermann work is to be standardized the principles below stated cannot be varied from to any great degree, inasmuch as the large majority of competent workers in this field have found by experience that the best results may be obtained by the simple application of fundamental principles such as are here outlined. Failure to observe these simple principles has caused discrepancies between the different laboratories with resulting annoyance and bewilderment to the clinician as well as unnecessary worry and inconvenience to the patients whose blood or spinal fluid is tested.

The Wassermann Laboratory recommends that the following reagents be used:

(1) Salt solution. .85% chemically pure sodium chloride in distilled water.

(2) Red blood corpuscles. These are obtained from a sheep, thoroughly washed three times with .85% salt solution in order to remove the serum and finally diluted with salt solution until the total volume is equal to the volume of the original <sup>defibrinated</sup> blood employed. For the tests and standardization of other reagents a 5% suspension of these washed corpuscles is made in .85% salt solution.

(3) Amboceptor. Is an anti-sheep immune substance obtained by injecting washed sheep's corpuscles into a rabbit. The amboceptor is diluted so that .25cc causes complete hemolysis of .5cc of a 5% suspension of washed sheep's corpuscles in the presence of .5cc of a 10% solution of complement. This quantity represents essentially one unit of amboceptor. Twice this amount is used in the test.

(4) Complement. Is a 10% solution of guinea pig's serum in .85% salt solution. The age of the complement employed is never less than three hours old nor more than twenty-four hours old and is never exposed to sunlight or room temperature for more than four hours during the maximum length of time that the complement is available for use. Twice the amount of complement necessary to hemolyze .5cc of a 5% suspension of sheep's corpuscles in the presence of two units of amboceptor is determined. This amount is used in testing unknown serums and spinal fluids.

(5) Patient's Serum. .1cc of serum which is not grossly contaminated bacterially nor decomposed as evidenced by excessive hemolysis is used after being heated to 56° for one-half hour, 1.0cc of unheated spinal fluid is used.



(6) Antigen. The antigens employed are accurately standardized cholesterinized, alcoholic extracts of human hearts. It is never safe to use less than three antigens separately in actual practice. This standardization is affected by well known Wassermann methods, and its validity is determined by testing hundreds of known positive and known negative serums and spinal fluids, before venturing its use in practical work. If the facilities for this method of standardization are not available it is necessary that a standard antigen be obtained from a laboratory which is in a position to fully standardize this reagent. The Wassermann Laboratory of the State Department of Health will gladly furnish such standardized antigens to maintain high standard of work upon request. The amounts above stated should not vary relatively.