WASSERMANN REACTIONS SIMPLIFIED

BY

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with the technical assistance of

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(RECEIVED FOR PUBLICATION SEPTEMBER 2, 1953)

Most techniques at present in use for the Wassermann reaction require a great deal of bench and water bath space, are very time consuming, and put a considerable burden on those responsible for the preparation of glassware. The use of moulded perspex plates (M.R.C. pattern)* in this laboratory has cut down immensely the space and labour involved in doing routine Wassermann reactions, and has added considerably to the pleasure in their performance. The plates are easy to handle, no racks are required and each plate, equivalent to 80 test tubes, is washed in a few seconds. This note is submitted in the hope that others may be persuaded to review their present techniques and to consider changing or adapting them for use with perspex.

The M.R.C. pattern perspex plates, which were originally designed for virus haemagglutination tests, consist of moulded sheets of perspex 7 x 5.7 x 0.55 in. in which there are 80 vertical-sided, round-bottom cavities 0.6 in. diameter. The cavities hold about 1.5 ml. when full; a convenient working final volume is, however, 0.4 to 1.0 ml. The plates are at their best when a method is used in which reagents are added by drops. They may, however, be used with almost equal convenience with any Wassermann reaction technique in which the final volume is not more than 1 ml. Apart from any adjustment of volumes used, it is only necessary to determine by trial the periods to be allowed for fixation of complement and for lysis of red cells in order to obtain results with the perspex plates identical with those obtained in test tubes.

The technique in use in this laboratory is adapted from those described by Wyler (1929) and Orpwood Price (1949). The following is an outline of the method which has proved consistently satisfactory with the perspex plates.

Method

All sera are first put through a one “tube” screen test and at the same time in one of the plates a titre is set up of a positive serum of known titre as a control. Sera giving a positive result in the screen test are then titrated in doubling dilutions beginning at 1 in 4 with a control tube at the first dilution (i.e., 1 in 4) containing saline instead of antigen.

Reagents.—All reagents are distributed by means of dropping pipettes, the neat sera with a hand pipette delivering a small drop = 0.02 ml.; cerebrospinal fluids and dilutions of sera in quantitative tests are distributed with a hand pipette delivering a large drop = 0.10 ml.; other reagents with a fixed vertical dropper with a reservoir, delivering a large drop.

QUANTITIES OF REAGENTS

<table>
<thead>
<tr>
<th>Screen W.R.s</th>
<th>Serum (neat)</th>
<th>1 small drop</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antigen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td></td>
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<tr>
<td></td>
<td>Complement</td>
<td></td>
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</tbody>
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<table>
<thead>
<tr>
<th>Quantitative Wassermann reactions and gonococcal complement fixation tests</th>
<th>Serum dilution</th>
<th>1 large drop</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antigen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Complement</td>
<td></td>
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</tbody>
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<table>
<thead>
<tr>
<th>C.S.F. Wassermann reactions</th>
<th>C.S.F.</th>
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<tbody>
<tr>
<td></td>
<td>Complement</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Antigen</td>
<td>1</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Gonococcal complement fixation screen tests</th>
<th>Serum</th>
<th>5 small drops*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complement</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Antigen</td>
<td>1</td>
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</tbody>
</table>

* This saves changing pipettes if sera for G.C.F.T. and W.R.s are put out at the same time.

Complement.—Complement is titrated according to the method of Orpwood Price (1949) and is so diluted that 1 large drop contains 1.25 M.H.D. for the test and 1 M.H.D. for controls without antigen.

Antigens.—Antigens are those supplied by the Venereal Diseases Reference Laboratory. Each batch of antigen before being taken into use is titrated chess-board fashion (Orpwood Price, 1950) to determine its optimal dilution. In our hands this is in the region of 1 in 200 for Wassermann antigen at which dilution the error using the same sized dropper for antigen and other reagents is negligible.

Period of Fixation.—We have found that incubating the plates in 37° C. incubator for 60 min. gives the same results as the 30 min. at bench temperature, followed by 30 min. in a 37° C. water-bath usually employed when glass tubes are used.

Period of Haemolysis.—After the addition of sensitized cells plates are incubated for one hour in a 37° C. incubator and the results then read (see below and illustration).
Use of Perspex Plates.—It adds enormously to the convenience of using these plates if a numbered sheet of plastic or cardboard is placed underneath while sera are being put out and when results are read. The figures on the card are so spaced that one is visible under each cup in the plate. It is advisable to mark one corner of the plate so that it may be correctly orientated on the numbered sheet. It will be seen from the illustration that the numbers make the putting out of sera very simple and the reading of tests almost fool-proof, since the number under a positive cup is obscured by either the suspended or settled unlysed cells. A reasonably accurate estimate of the amount of haemolysis in any cup can be made if the cells are left to settle completely before tests are read.

Sheets of perspex cut to the size of the plates make suitable dust covers during the test and periods of incubation. It is advisable to incubate the plates in stacks of not more than two in order to avoid delay in reaching incubator temperature, although, since 75 tests (and control titration) may be done in one plate, the average batch of Wassermann reactions will seldom require more than two plates.

After use plates are washed in tap water, rinsed in distilled water, and kept in 2% HCl. If washing is not unduly delayed there is no difficulty in removing all traces of reagents.

We have performed some 3,000 tests in duplicate with plates and glass tubes and in no case has there been a difference of more than half a tube and in almost all results have been identical.

The plates are in use in this laboratory also for Paul Bunnell and Rose's tests. Results have proved consistently good.

References
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C. L. Greenbury and J. Keningale

_J Clin Pathol_ 1954 7: 73-74
doi: 10.1136/jcp.7.1.73

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