A modified rubella HI test using prestandardized reagents

G. E. D. URQUHART, D. A. W. WORSWICK1, AND N. R. GRIST

From the Regional Virus Laboratory, Ruchill Hospital, Glasgow G20 9NB

SYNOPSIS  A modified haemagglutination inhibition test for rubella antibodies using prestandardized freeze-dried reagents was compared to a 'standard' method. Tests of 707 serum samples showed that the modified test was sensitive and reliable by both macrotitration and microtitration techniques. The minor disadvantages of some reduction in antibody level when rubella sera were tested within one week of the rash and of spontaneous sheep erythrocyte agglutination in 0·7% of sera were outweighed by the increased speed of the new test and the fact that it was carried out at room temperature.

The most commonly used technique for the diagnosis of rubella infection or immunity status is the haemagglutination inhibition (HI) test, of which there have been many modifications since the description by Stewart et al in 1967. Current methods require standardization of reagents from numerous sources as well as special incubation facilities. We evaluated a modified commercial13 HI test using prestandardized reagents available in kit form and carried out at room temperature with our 'standard' test procedure.

Material and methods

The 'standard' test was carried out as described by Grist et al (1974) using dextran sulphate, calcium chloride for the removal of non-specific inhibitors, and trypsin modified human erythrocytes as indicators of agglutination.

The prestandardized commercial (Organon) kit comprised:

1 a bottle of 25% kaolin suspension in borate-buffered saline, pH 9·0, for absorption of non-specific serum inhibitors;
2 formalinized freeze-dried sheep erythrocytes (5%) for serum absorption;
3 HEPES buffer (pH 6·6) five times concentrated;
4 freeze-dried rubella haemagglutinin (HA);
5 formalinized freeze-dried sheep erythrocytes (0·5%) for use as indicator cells;
6 freeze-dried control positive serum (supplied for this trial only).

The preparation of these reagents is described by van Weemen and Kacaki (1976), and before use they were reconstituted with distilled water or HEPES buffer.

A comparison of 'standard' and Organon test procedures is shown in table I. The main differences of the Organon test were as follows:

1 no prestandardization was required;
2 serum absorption and serum/HA incubation times were reduced;
3 test procedure was carried out at room temperature.

Apart from test serum controls, the only control for Organon tests was positive serum, whereas in the standard test haemagglutinin back titration was included as well as high, low, and negative control sera.

The 'standard' test by microtitration technique

<table>
<thead>
<tr>
<th>Stage</th>
<th>Standard test</th>
<th>Organon test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
<td>90+ min</td>
<td>15 min</td>
</tr>
<tr>
<td>Standardization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reconstitution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum absorption</td>
<td>30 min</td>
<td>0 min</td>
</tr>
<tr>
<td>Serum/HA incubation</td>
<td>60 min</td>
<td>15 min</td>
</tr>
<tr>
<td>Cell incubation/settling</td>
<td>4 h</td>
<td>3 h</td>
</tr>
<tr>
<td>Comparative time</td>
<td>7+ h</td>
<td>3+ h</td>
</tr>
<tr>
<td>Test temperature</td>
<td>4°C/37°C</td>
<td>Room</td>
</tr>
</tbody>
</table>

Table I  Comparison of 'standard' and Organon rubella HI techniques

Received for publication 17 May 1976
was compared with the Organon test by both microtitration and macrotitration in round-bottom glass tubes (35 × 9.75 mm, 0.1 ml volumes). Test sera were titrated from 1 in 8 to 1 in 128 in batches of 31 as this was the capacity of the two racks provided to hold tubes for the macrotitration test. The trial was organized in two phases:

phase I retrospective testing of a panel of sera with known HI antibody levels, at least 20% of them being antibody negative by the 'standard' test;

phase II prospective testing of single sera in which the presence of rubella antibodies was unknown.

Results

**PHASE I**

Single sera from 202 persons were selected for comparative testing, to represent as wide an antibody spectrum as possible, and a wide range of ages and clinical indications for rubella HI tests. Thirty-one percent of sera gave 'standard' HI titres less than 8; 47% gave titres between 8 and 64; and 22% gave titres equal to or greater than 128. The ages of patients ranged from less than 1 year to 45 years, 62% being between 21 and 30 years of age. Forty-seven and one-half percent were clinical rubella contacts in pregnancy; the remainder, in order of numerical importance, were (1) cases for susceptibility testing, (2) cases of rashes, arthropathy, or glandular syndromes, and (3) embroyopathy cases. Thirteen percent of sera were from males.

Table II summarizes the comparison of Organon with 'standard' tests: 92-1% of macrotitration (tube) and 95-5% of microtitre tests were the same or within two-fold of titres in the 'standard' test. Because the three tests were very similar in sensitivity and the 22 sera giving the 25 discrepant results (table II, four-fold or greater differences) were randomly distributed with respect to age, standard HI titre, and clinical category, repeat estimations were carried out on these sera including those where the 'standard' test showed a four-fold difference from the two Organon tests. On repeat all 22 sera gave HI titres within a two-fold difference of the other two tests, showing that the sensitivity and specificity of the Organon tests were identical with those of the standard, within the limits of technical error.

Readability was a little more difficult with the Organon microtitration test than the 'standard' test due to the brownish sheep cell button and the slightly lower concentration of cells in each cup (0.025 ml of 0.5% sheep erythrocytes compared to 0.05 ml of 0.3% human 'O' erythrocytes), but no significant observer errors were detected on blind reading by two observers.

**PHASE II**

In this phase single sera from 505 persons were tested concurrently by the 'standard' and Organon tests. Where two sera were available from one patient, the second serum only was comparatively tested, because if antibody rise were taking place this second sample would have the higher titre. Of these 505 sera, 17% gave 'standard' titres less than 8, 72% gave titres between 8 and 64, and 11% equal or greater than 128. The age range of patients was from 1 to 46 years, 63.4% being between 21 and 30 years. Of the sera, 71-7% were submitted for susceptibility testing; 22-2% were from clinical rubella contacts in pregnancy and 4.8% from patients with rashes, arthropathy or glandular syndromes. In none of the latter was an aetiology for the clinical syndrome established. There were also two cases of rubella and three of measles proved by standard serological tests, and two post-immunization sera from rubella vaccinees known to have been previously susceptible to rubella. In this phase 2-2% of sera were from males.

The comparison of Organon with standard tests (table II) showed that 96.4% of tube tests and 96% of microtitration tests were the same or within a two-fold difference of the 'standard' test, confirming the general similarity in sensitivity and reliability of the Organon tests.

The 38 tests where four-fold or greater differences were found on comparative testing (table II) represented 23 sera; repeat tests showed that most

<table>
<thead>
<tr>
<th>Organon test</th>
<th>Phase</th>
<th>Total sera tested</th>
<th>Same (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrotitration</td>
<td>I*</td>
<td>202</td>
<td>104 (51.5)</td>
</tr>
<tr>
<td>Microtitration</td>
<td>I*</td>
<td>202</td>
<td>121 (59.9)</td>
</tr>
<tr>
<td>Macrotitration</td>
<td>II*</td>
<td>505</td>
<td>265 (52.5)</td>
</tr>
<tr>
<td>Microtitration</td>
<td>II</td>
<td>505</td>
<td>235 (46.5)</td>
</tr>
<tr>
<td>Macrotitration</td>
<td>I + II</td>
<td>707</td>
<td>369 (52.2)</td>
</tr>
<tr>
<td>Microtitration</td>
<td>I + II</td>
<td>707</td>
<td>356 (50.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organon titres compared to standard titres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within 2-fold (%)</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>82 (40-6)</td>
</tr>
<tr>
<td>72 (35-6)</td>
</tr>
<tr>
<td>222 (43-9)</td>
</tr>
<tr>
<td>250 (49-5)</td>
</tr>
<tr>
<td>304 (43-0)</td>
</tr>
<tr>
<td>322 (45-6)</td>
</tr>
</tbody>
</table>

Table II Comparison of Organon and 'standard' rubella HI tests—phases I and II

*Phase I = retrospective tests of serum panel.
*Phase II = prospective tests of 'unknowns'.
of these sera gave titres within two-fold of the other two tests. However, one serum taken from a case of rubella seven days after onset of the rash, with an antibody rise by the 'standard' HI test, consistently showed eight-fold lower titres by the Organon tests. Because of this, sera from 13 additional cases of proved rubella infection were examined. Antibody rises were detected in 12 of these by the Organon tests although three of six sera taken within seven days of onset gave four-fold lower titres by the Organon techniques; one failed to give a rise by the Organon tests in paired sera taken three days before the rash and three days after. This slightly decreased ability to detect antibody in early rubella sera may be due to removal of 'early' specific antibody by the kaolin used in the Organon technique. This theory is supported by the finding that IgM antibody was detected by the Organon tests in appropriate fractions when suitable sera were subjected to sucrose density gradient ultracentrifugation without treatment to remove non-specific inhibitors. However, if sera in rubella cases are taken more than seven days apart, as is usual practice, antibody rises can be detected by the Organon tests.

One minor disadvantage of the Organon tests was spontaneous agglutination of indicator sheep erythrocytes in five (0.77%) sera, not included in table II, so that no rubella HI result was obtained in these cases. Repeated sheep cell absorption was not attempted with these sera. When 11 additional sera from six patients with proved glandular fever were tested by the Organon methods, only one gave no rubella HI result because of sheep cell agglutination.

van Weemen and Kakaki (1976) report that satisfactory HI results can be obtained for most of these sera by repeating the test after absorption with kaolin and erythrocytes for 15 minutes with each reagent.

In approximately 23 separate Organon test batches the positive control serum gave titres between 32 and 64, and the absence of negative control serum did not give rise to any difficulty in test control. Haemagglutinin back titration was not included as a routine test control but, when carried out, indicated that two units of haemagglutinin had been used in the Organon test.

Discussion

This evaluation of a new commercial rubella HI kit using prestandardized reagents showed that on a retrospective and prospective basis the commercial test was generally as sensitive, reliable, and reproducible as a standard method. The test, which uses kaolin and formalized sheep erythrocytes (5%) for absorption of non-specific inhibitors and formalized sheep erythrocytes (0.5%) to indicate haemagglutination, took half the time of the standard method to perform because of prestandardization of reagents and reduction in serum absorption and serum/haemagglutinin incubation times. Because all reagents except kaolin suspension and concentrated HEPES buffer were presented in freeze-dried form, storage deterioration was not a problem. The commercial test was conveniently carried out entirely at room temperature whereas the standard test requires 4°C and 37°C incubation facilities. The commercial test performed well by both macrotitration and microtitration, giving a 95.2% and 95.9% correlation respectively with the standard method (table II). Repeat testing showed that most apparent discrepancies were due to test error. The source of this error was investigated by repeating tests on 269 sera by the standard method. On repeat, 38% of sera gave titres which were four-fold or greater different from the first estimation. Fifty percent of sera with an initial titre of 16 or less gave discrepant results but only 19% of sera with initial titres between 32 and 128. When repeat tests were carried out using the Organon techniques, 22% of sera gave four-fold or greater differences irrespective of the first test result. This probably indicates the unreliability of the dextran sulphate method for removal of non-specific inhibitors which were adequately removed by kaolin in the Organon tests. The reproducibility error of between 19 and 22% is close to the 16% standard error observed by Geser et al (1970) for arbovirus HI tests, and probably accounts for most apparent discrepancies between the standard and Organon tests. The minor disadvantages of some reduction in detectable antibody levels in sera from rubella patients taken within seven days of the rash, and of spontaneous sheep erythrocyte agglutination with 0.7% of sera, were outweighed by the advantage of speed and ease of test performance. As well as finding a place in routine rubella serology this new commercial kit might provide the basis for collaborative interlaboratory rubella studies, including rubella HI quality control tests. The estimated cost of reagents for the standard kit is approximately 15p per test; the cost of the proposed Organon test is not yet available.

We should like to thank Dr E. Edmond, City Hospital, Edinburgh, for supplying sera from glandular fever patients and Dr M. Karmali for assistance.

References

Reports and Bulletins prepared by the Association of Clinical Biochemists

The following reports and bulletins are published by the Association of Clinical Biochemists. They may be obtained from The Publishing Department, British Medical Journal (ACB Technical Bulletins), B.M.A. House, Tavistock Square, London WC1H 9JR. Overseas readers should remit by British Postal or Money Order.

**SCIENTIFIC REPORTS** *(price £1.00/$2.00 each)*


4 An Evaluation of five Commercial Flame Photometers suitable for the Simultaneous Determination of Sodium and Potassium March 1970 P. M. G. BROUGHTON and J. B. DAWSON

**SCIENTIFIC REVIEWS** *(price £1.00/$2.00 each)*

1 The Assessment of Thyroid Function March 1971 F. V. FLYNN and J. R. HOBBS


3 Biochemical Tests for the Assessment of Fetoplacental Function May 1975 C. E. WILDE and R. E. OAKEY

**TECHNICAL BULLETINS** *(price £1.00/$2.00 each)*

9 Determination of Urea by AutoAnalyzer November 1966 RUTH M. HASLAM

11 Determination of Serum Albumin by AutoAnalyzer using Bromocresol Green October 1967 B. E. NORTHAM and G. M. WIDDOWSON

13 An Assessment of the Technicon Type II Sampler Unit March 1968 B. C. GRAY and G. K. MCGOWAN

14 Atomic Absorption Spectroscopy: an outline of its principles and a guide to the selection of instruments May 1968 J. B. DAWSON and P. M. G. BROUGHTON


16 A Guide to Automation in Clinical Chemistry May 1969 P. M. G. BROUGHTON

17 Flame Photometers: a comparative list of 17 instruments readily available in Britain August 1969 P. WILDER

19 Spectrophotometers: a comparative list of low-priced instruments readily available in Britain May 1970 C. E. WILDE and P. SEWELL

**20 Qua\ntities and Units in Clinical Biochemistry** June 1970 P. M. G. BROUGHTON

21 Filter Fluorimeters: A comparative list of 18 instruments September 1970 H. BRAUNBERG and S. S. BROWN

22 Bilirubin Standards and the Determination of Bilirubin by Manual and Technicon AutoAnalyzer Methods January 1971 BARBARA BILLING, RUTH HASLAM, and N. WALD

23 Interchangeable Cells for Spectrophotometers and Fluorimeters September 1971 S. S. BROWN and A. H. GOWENLOCK

24 Simple Tests to Detect Poisons March 1972 B. G. MEADE et al.

25 Blood Gas Analysers May 1972 K. DIXON

26 Kits for Enzyme Activity Determination September 1972 S. B. ROSALKI and D. TARLOW

27 Assessment of Pumps Suitable for Incorporation into Existing Continuous Flow Analytical Systems November 1972 A. FLECK et al.

28 Routine Clinical Measurements of Transferrin in Human Serum September 1973 K. DIXON

29 Control Materials for Clinical Biochemistry (5th edition) September 1973 J. E. STEVENS

30 Notes on the Quality of Performance of Serum Cholesterol Assays September 1973 S. S. BROWN

31 Determination of Uric Acid in Blood and in Urine July 1974 R. W. E. WATTS

32 A Survey of Amino Acid Analysers Readily Available in the United Kingdom September 1974 J. E. CARLYLE and P. PURKISS

33 Definitions of some Words and Terms used in Automated Analysis November 1974 A. FLECK, R. ROBINSON, S. S. BROWN, and J. R. HOBBS

34 Measurement of Albumin in the Sera of Patients January 1975 LINDA SLATER, P. M. CARTER, and J. R. HOBBS


36 Factors Influencing the Assay of Creatinine November 1975 J. G. H. COOK