A modified rubella HI test using prestandardized reagents

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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 commercial² 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

Stage	Time taken by	
	Standard test	Organon test
Preparation		
Standardization	90+ min	15 min
Reconstitution		
Serum absorption	30 min	0 min
Serum/HA incubation	60 min	15 min
Cell incubation/settling	4 h	3 h
Comparative time	7+ h	3½ h
Test temperature	4°C/37°C	Room

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

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²Reagents for this method were supplied by Organon Teknika, Oss, The Netherlands under the trade name Rubenosticon.

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) embryopathy 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

Organon test	Phase	Total sera tested	Same (%)	Organon titres compared to standard titres		
				Within 2-fold (%)	≥ 4-fold (%)	Same or 2-fold (%)
Macrotitration	I ¹	202	104 (51.5)	82 (40.6)	16 (7.9)	186 (92.1)
Microtitration	I	202	121 (59.9)	72 (35.6)	9 (4.5)	193 (95.5)
Macrotitration	II ²	505	265 (52.5)	222 (43.9)	18 (3.6)	487 (96.4)
Microtitration	II	505	235 (46.5)	250 (49.5)	20 (4.0)	485 (96.0)
Macrotitration	I + II	707	369 (52.2)	304 (43.0)	34 (4.8)	673 (95.2)
Microtitration	I + II	707	356 (50.4)	322 (45.6)	29 (4.1)	678 (95.9)

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

¹Phase I = retrospective tests of serum panel.

²Phase II = prospective tests of 'unknowns'.

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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 (0.7%) 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 Kacaki (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 formalinized sheep erythrocytes (5%) for absorption of non-specific inhibitors and

formalinized 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 per cent 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 inter-laboratory 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.

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