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The derivation of a minimum immune titre of rubella haemagglutination-inhibition (HI) antibody.* A Public Health Laboratory Service collaborative survey†

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SUMMARY

Ten laboratories collaborated in a study of minimum immune titre (MIT) of rubella haemagglutination-inhibiting (HI) antibody with one laboratory acting as a reference laboratory to provide a uniform basis for comparison of the HI results. The international unitage equivalent to the MIT used by the ten laboratories was found to vary from 24 to 98 units. Testing of the sera by immunofluorescence and by HI after flotation centrifugation indicated that residual non-specific inhibitors may interfere with HI antibody testing to an extent equivalent to 12–15 units. An acceptable MIT would therefore be equivalent to 24–48 units of rubella HI antibody. The single radial haemolysis (SRH) results on the sera indicate that this is a sensitive and specific test for rubella antibody.

INTRODUCTION

The rubella haemagglutination-inhibition (HI) test is widely used as a screening test for rubella antibodies. Each laboratory chooses an HI titre to distinguish between women who should be given vaccine and those who should not. Rubella immunization is recommended for persons with antibody below this titre which may be called the minimum immune titre (MIT). The MIT in a given laboratory will be influenced by two factors; first, by the sensitivity of the HI test used, and, secondly, by the allowance made for the possible occurrence of residual non-specific inhibitors of rubella haemagglutination (HA). Methods for the removal of these non-specific inhibitors vary from laboratory to laboratory and the two most

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commonly used, based on kaolin or manganous chloride/heparin, are known to fail with occasional sera (Haukenes & Blom, 1975).

This survey has attempted to determine whether the MIT used by different laboratories is the same and whether a firm recommendation can be made for an MIT in terms of international units of rubella antibody. In order to be sure that the low HI activity of certain sera was not due to residual non-specific inhibitors, the sera were also tested for rubella antibody by immunofluorescence, by HI after flotation centrifugation (FC), and by single radial haemolysis (SRH).

MATERIAL AND METHODS

Rubella HI tests

Each of nine laboratories sent to a tenth laboratory 11–20 coded sera which, when tested by their routine rubella HI test, had either (i) an MIT of rubella antibody, (ii) a titre 2 to 4-fold lower than the MIT, or, (iii) a titre 2 to 4-fold higher than the MIT. The tenth laboratory acted as a reference laboratory to provide a uniform basis for comparison of the HI titres of the sera and performed rubella HI tests on all 145 sera in a single batch. These results were sent to the Central Public Health Laboratory, Colindale for comparison with those of the referring laboratories.

The HI test used by the reference laboratory was similar to the standard test previously described by the Public Health Laboratory Service Standing Advisory Committee on Viral Reagents (Report, 1978). Non-specific inhibitors were removed by treatment of 0·1 ml of serum with 0·4 ml of 25% kaolin in borate–saline buffer pH 9·0. Tests for HI antibody were performed in microplates using four units of rubella haemagglutinin (Standards Laboratory, Central Public Health Laboratory, Colindale) and overnight reaction at 4 °C of virus/serum mixtures before adding 0·4% day-old chick cells (Tissue Culture Services, Slough, Bucks). The results were converted to units by comparing the titres of the test sera with those of the Colindale rubella reference serum which had a potency of 297 international units (i.u.) per ampoule (Report, 1978).

Rubella immunofluorescence (IF) antibody tests

All 145 sera were tested in Manchester at a dilution of 1/8 for specific IgG antibody using the immunofluorescent method previously described (Cradock-Watson, Bourne & Vandervelde, 1972). Sera which showed doubtful results were retested after preliminary absorption with BHK 21 cells.

Single radial haemolysis (SRH) tests

Sufficient remained of 130 sera for testing by SRH which was performed at Coventry. The method used was based on that of Strannegard, Grillner & Lindberg (1975). Briefly, 1 ml of 8% washed day-old chick cells was added to 9 ml of rubella haemagglutinin diluted in dextrose-gelatine-veronal buffer (DGV) to give eight HA units per 0·025 ml and incubated at room temperature for 20 min. Seven ml was added to 6·4 ml of 1·5% agarose warmed to 56 °C. The mixture was

then poured into a 10 cm square Petri dish, allowed to set and wells 2·5 mm diameter cut out of the gel at intervals of 14 mm. A control plate containing unsensitized cells was prepared at the same time. The sera were heated at 56 °C for 3 min, added to wells in test and control plates and allowed to diffuse overnight. Plates were then warmed to 37 °C for 20 min, flooded with preserved complement diluted 1/5 with DGV and incubated for 4 h at 37 °C. The presence of rubella antibody was indicated by a zone of haemolysis in the test plate only.

Flotation centrifugation (FC)

FC was performed by the reference laboratory according to the method described by Blom & Haukenes (1974). The density of 0·3 ml of serum was adjusted to 1·30 g/ml by adding 0·13 g sodium bromide (NaBr). The serum was then layered underneath 4·7 ml of NaBr density 1·25 g/ml at room temperature in an ultracentrifuge tube. The tube was centrifuged for 16–18 h at 40000 rev./min in an MSE Superspeed 75 Ultracentrifuge. The bottom of the tube was pierced, the first 0·4 ml removed and tested for rubella HI antibody from 1/2 to 1/64 using the rubella HI test described above.

RESULTS

Minimum immune titre (MIT)

Each of the participating laboratories had submitted 3–10 sera which they stated had an MIT of rubella HI antibody according to their local laboratory test. These sera were retested by the reference laboratory and the geometric mean titre (GMT) for each laboratory was calculated and converted into units. The distribution of the unitages found to be equivalent to the MITs is shown in Table 1. In the reference and four other laboratories the MIT was between 24 and 34 units, in four laboratories between 48 and 76 units, and in one it was 98 units.

In deriving an MIT each laboratory not only performs the HI test but also interprets the results. It is possible to evaluate the contribution that variation in interpretation makes to the variation in MIT by analysing those sera which, in HI tests in the local laboratories, had an end-point at a particular dilution. Thus each laboratory had submitted between five and ten sera which they stated had a rubella HI titre of 16 or 20. After testing by the reference laboratory, the GMT of these sera for each laboratory was calculated and expressed as units (Table 1). The results are almost uniform with the local HI titres of 16 or 20 in nine laboratories being equivalent to 24–48 units. The other laboratory appeared to be using a relatively insensitive HI test as a titre of 16 was equivalent to 98 units.

Rubella antibody titrated by immunofluorescence

Table 2 shows the results of testing all 145 sera for rubella specific IgG antibody by indirect immunofluorescence. All sera which had 24 or more units of HI antibody were also found to contain IF antibody. Of 12 sera with 12 units of HI antibody, nine contained detectable rubella IF antibody. However, of the 27 sera which had less than 12 units of HI antibody, only two had IF antibody.

Table 1. *Interlaboratory comparison of minimum immune titres and rubella HI titres of* 16 *or* 20

Laboratory	Unitage equivalent found by the reference laboratory for: Sera giving minimum immune titres at local laboratory (no. of sera tested)	Sera giving a rubella HI titre of 16 or 20 at local laboratory (no. of sera tested)
Reference	24 (9)	24 (9)
1	32 (5)	32 (5)
2	32 (5)	32 (5)
3	32 (5)	32 (5)
4	34 (6)	34 (6)
5	48 (5)	48 (5)
6	55 (5)	24 (5)
7	76 (3)	30 (6)
8	76 (3)	24 (7)
9	98 (10)	98 (10)

Table 2. *The detection of rubella antibodies in the test sera by HI, IF and SRH*

Reference laboratory rubella HI result (i.u.)	Number rubella antibody positive by IF (no. tested)	Number rubella HI antibody positive after flotation centrifugation (no. tested)	Number rubella antibody positive by SRH (no. tested)
Less than 12	2 (27)	2 (27)	2 (24)
12	9 (12)	10 (12)	8 (8)*
24	31 (31)	31 (31)	24 (24)
48	28 (28)	n.t.	28 (28)
96	29 (29)	n.t.	28 (28)
192	14 (14)	n.t.	14 (14)
384	4 (4)	n.t.	4 (4)

n.t., Not tested.

* These eight did not include any of the three sera which had 12 units of HI antibody but no detectable IF antibody.

Rubella HI antibody after flotation centrifugation (FC)

All 70 sera with 24, 12 or less than 12 units of rubella HI antibody were retested after removal of the non-specific inhibitors by FC (Table 2). All 31 sera found by routine HI testing to have 24 units of antibody were still positive after FC. Twelve sera had 12 units of HI antibody in the routine test. Only ten of these were positive after FC. The other two were HI antibody negative after FC and both were also negative in the IF antibody test. Of the 27 sera which were HI antibody negative (less than 12 units) in the routine HI test two were HI antibody positive after FC (Table 2) and both of these were positive in the IF test.

Rubella antibody by single radial haemolysis (SRH)

Sufficient material was available from 130 of the sera to perform SRH tests for rubella antibody. Of these, 106 contained HI and IF antibody. All these 106 were rubella antibody positive by SRH (Table 2). None of the three sera which were rubella antibody positive in the routine HI test but negative in the IF test was

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available for testing by SRH. Two of the 24 sera which were found to contain less than 12 units of antibody in the routine HI test were positive in the SRH test. These were the same two sera which were positive by IF and by HI after FC.

DISCUSSION

The results show that the difference in MIT of HI antibody between the ten laboratories is up to 4-fold even when all sera are tested by a single laboratory. Much of this variation is due to the interpretation that laboratories place on the HI results rather than variation in the HI results themselves. When the sera stated by individual laboratories to have HI titres of 16 or 20 were analysed by the reference laboratory then the unitage equivalent to these titres in nine laboratories is between 24 and 48 units. Thus the HI results obtained by laboratories 6, 7 and 8 are not significantly different from those obtained by the reference laboratory and laboratories 1, 2, 3 and 4. However, laboratories 6, 7 and 8 choose as their MIT a higher unitage than the reference laboratory and laboratories 1, 2, 3 and 4. It is therefore important to decide whether those using a relatively low MIT are always measuring HI antibody, or whether it is important to use a higher MIT in order to avoid problems due to non-specific inhibitors.

A discrepancy between the results of the HI test performed by the reference laboratory and the detection of specific rubella IF IgG was found with five sera. With two sera the rubella HI result was less than 12 units but rubella antibody was detectable by immunofluorescence. When tested by the local laboratory which submitted the two sera both were found to have 17 units of HI antibody. It is clear that these sera do contain rubella antibody but that it is of such low titre that in some HI tests it will be undetected.

A more important discrepancy was found with three other sera which were HI antibody positive (12 units) in the reference laboratory test but IF antibody negative. With two of these sera it was not possible to demonstrate HI antibody after removal of non-specific inhibitors by FC, although, in the third, HI antibody could be demonstrated by this means. It seems certain therefore that the HI activity found in at least two of these sera by routine HI testing was due to residual non-specific inhibitors. It was noted that the local laboratories which submitted these three sera also reported that low titre (9–15 units) HI antibody was detected. In the case of the two sera which had no detectable HI antibody after FC the local laboratory test used $MnCl_2$/heparin mixture to remove non-specific inhibitors as opposed to the kaolin used by the reference laboratory. With all three sera the local laboratory HI test used 1 h reaction of virus and serum as opposed to the 18 h used by the reference laboratory. Thus these sera gave false positive results whichever of the common methods was used to remove non-specific inhibitors although the effect of kaolin pretreatment followed by HI tests incorporating a 1 h virus/serum reaction time was not investigated by the reference laboratory. In addition, the false positive results were not simply due to long reaction time HI tests.

All sera with 24 or more units of rubella HI antibody contained rubella specific

IF IgG. Thus, if the HI test is used to select those patients who lack rubella antibody and therefore require rubella vaccine it is important that the MIT represents more than 20 units of antibody. If a lower unitage is selected with some sera the apparent HI antibody will be due to residual non-specific inhibitors. The majority of sera (nine out of 12 in the present series) with the lowest detectable HI activity do contain rubella antibody and therefore regarding them as suceptible leads to a certain amount of unnecessary vaccination. However, this is likely to occur with only a small proportion of the population; for example, with the rubella HI test used by the reference laboratory between 15 and 25 per thousand of the ante-natal clinic population have 12 units of rubella HI antibody.

In nine of the participating laboratories, a titre of 16 or 20 was found to be equivalent to 24–48 units of HI antibody and would be an appropriate MIT. With such an MIT each of the nine would have recommended vaccine for the three 'false-positives' without involving too great a proportion of truly sero-positive patients in an immunization schedule. Therefore, it is concluded that the minimum titre should be equivalent to 24–48 international units and that the sensitivity of any HI test should be adjusted so that this is equivalent to a titre of 16 or 20.

In the 130 sera in this series which were tested by SRH there is an absolute correlation between the presence of rubella antibody by immunofluorescence and SRH. SRH detected as sero-positive any serum with 12 units of HI antibody and therefore if the SRH test was used for rubella antibody screening all patients with such low titre antibody would be regarded as immune. This is acceptable since there is no interference from non-specific inhibitors in the SRH test. A positive control serum would have to be included in each SRH test, and a serum with an MIT of HI antibody would be suitable.

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