*J. Hyg., Camb.* (1976), **77**, 31
*Printed in Great Britain*



# A modified haemagglutination inhibition test for rubella antibodies, using standardized, freeze-dried reagents. Report of a comparative multi-centre trial

By BAUKE K. van WEEMEN and JOVAN KACAKI
*Organon Scientific Development Group, Oss, The Netherlands*

(*Received* 30 *October* 1975)

SUMMARY

A modified haemagglutination inhibition test for rubella antibodies, using standardized freeze-dried reagents, was developed and compared with haemagglutination inhibition tests using fresh erythrocytes. This comparison was made in collaboration with six European laboratories. A total of 4205 serum samples were tested. The results show that:

(1) Sensitivity and reliability of the modified test are good;

(2) the modified test can be performed in glass tubes as well as in polystyrene microtitration plates.

## INTRODUCTION

The need for reliable and convenient methods for the determination of antibodies against rubella virus has been clear ever since Gregg (1941) discovered the teratogenicity of rubella infection in early pregnancy.

Several methods have so far been developed (for a review see Meyer, Parkman & Hopps, 1972). Haemagglutination inhibition (HAI) (Stewart *et al.* 1967) has found the widest application, owing to its reliability and relative ease of performance. However, the need for a regular supply of fresh bird erythrocytes and for strict, daily standardization of the reagents have limited the use of this technique to the specialized virological laboratory.

To overcome these drawbacks, we have developed a method for the detection of rubella antibodies using stable freeze-dried erythrocytes,* based on the finding of Gupta & Harley (1970) that formalinized sheep erythrocytes can be used for the rubella HAI reaction. In this paper we describe the test procedure and present the results of a multi-centre trial performed to compare our method with that of Stewart *et al.* (1967), as modified by the participating centres.

## MATERIALS AND METHODS

### *Materials*

Sheep erythrocytes were treated with formalin according to the procedure described by Wide (1962), with some modifications (Schuurs, de Jager & Homan,

* Reagents for this method are produced by Organon Teknika, Oss, The Netherlands, under the trade-name RUBENOSTICON®.

1968). The erythrocytes were washed and freeze-dried either as a 10% suspension (for use as absorption erythrocytes), or as a 5% suspension (for use as indicator erythrocytes).

Rubella haemagglutinin was prepared by growing BHK-21 (C-13) cells, infected with rubella virus strain HPV-77,* in a suspension culture, followed by inactivation by a Tween/ether treatment, according to the method of Norrby (1962). It was freeze-dried after the addition of 0·5% (w/v) BSA (bovine serum albumin, Cohn fraction V, Armour, Eastbourne, UK).

Kaolin (Merck, Darmstadt, W. Germany) was used as a 25% (w/v) suspension in borate-buffered saline pH 9·0 (Inouye & Kono, 1972).

The test was performed in 0·025 M HEPES (*N*-2-hydroxy-ethyl-piperazine-*N'*-2'-ethane sulphonic acid) buffer pH 6·6, also containing 0·14 M-NaCl, 0·01 M-$CaCl_2.2H_2O$ and 0·5% (w/v) BSA. Combinations of erythrocytes and haemagglutinin were standardized

(1) to give a constant haemagglutination titre of 4 and

(2) to give constant haemagglutination inhibition titres with an internal panel of positive and negative test sera.

The reagents were stable for at least 1 year at 2–8° C.

*Test procedure*

The test was performed in round-bottom glass tubes, measuring 35 × 9·75 mm. (external measurements), as well as in disposable polystyrene V-shaped microtitration plates (Microtiter M25A plates, obtained from Nutacon, Schiphol, The Netherlands). Both tubes and plates were previously tested for suitability with corresponding batches of reagents.

Using glass tubes, the test was performed as follows:

(1) 0·1 ml. of test serum and 0·6 ml. of kaolin suspension were mixed thoroughly in a centrifuge tube.

(2) 0·25 ml. of a 5% suspension of the reconstituted freeze-dried absorption erythrocytes was added to the tube, its contents were mixed thoroughly, and centrifuged for 5 min. at 1500 *g*.

This absorption treatment leaves the test serum in a 1/8 dilution.

(3) A dilution series of the supernatant, ranging from 1/8 to 1/128 with a dilution factor of 2, was prepared in settling tubes (0·1 ml. of diluted serum per tube). If necessary, the dilution series was extended beyond 1/128. A control tube containing 0·1 ml. 1/8 diluted absorbed serum was added to the series.

(4) 0·1 ml. of a solution of the freeze-dried haemagglutinin was added to each tube, except the control tube; 0·1 ml. of buffer was added to the control tube. The contents of the tubes were thoroughly mixed, and left to stand for 15 min.

(5) 0·1 ml. of a 0·5% suspension of the reconstituted freeze-dried indicator erythrocytes was added to each tube. After thorough mixing, the erythrocytes were allowed to settle in an appropriate test rack for 3 hr.

(6) The settling patterns were read. When haemagglutination occurs, an even

* Kindly made available by Professor R. Gispen and Ir. A. L. van Wezel, National Institute of Public Health, Bilthoven, The Netherlands.

Table 1. *Characteristics of rubella HAI tests as used in the centres participating in the comparative trial*

| Centre | Absorption procedure | Type of erythrocytes | Source of haemagglutinin | Test dilutions |
|---|---|---|---|---|
| Bilthoven | Heparin/$MnCl_2$ + $Na_2CO_3$ | Pigeon | Own production | 1/8, 1/16, etc. |
| Bordeaux | Kaolin | Pigeon | Mérieux | 1/8, 1/16, etc. |
| Copenhagen | Kaolin | Chicken | Own production | 1/10, 1/20, etc. |
| Glasgow | Dextran sulphate/ $CaCl_2$ | Trypsinized human O | Flow | 1/8, 1/16, etc. |
| Würzburg | Heparin/$MnCl_2$ | Chicken | Behringwerke | 1/8, 1/16, etc. |
| Zagreb | Heparin/$MnCl_2$ | Chicken | Behringwerke | 1/8, 1/16, etc. |

light-brown layer forms on the bottom of the tube. When the haemagglutination is inhibited, a dark-brown ring appears on the bottom. The reciprocal value of the highest serum dilution giving complete inhibition of haemagglutination was taken as the HAI titre of the sample. The control tube should show a ring, indicating complete absorption of non-specific agglutinators.

The whole procedure was carried out at room temperature (15–25° C.).

Using microtitration plates, the test was performed in a similar way, using the matching Microtiter droppers and dilutors. All volumes were accordingly reduced from 0·1 to 0·025 ml.

We will refer to the test performed in glass tubes and in microtitration plates as macromethod and micromethod, respectively.

*Set-up of comparative, multi-centre trial*

The trial was organized in collaboration with six research centres:

National Institute of Public Health, *Bilthoven*, The Netherlands (Professor R. Gispen).

Laboratory for Medical Analyses, *Bordeaux*, France (Dr S. Verdaguer).

State Serum Institute, *Copenhagen*, Denmark (Dr J. Leerhoy).

Regional Virus Laboratory, *Glasgow*, Scotland (Dr G. E. D. Urquhart, Professor N. R. Grist).

Virological Institute of the University of Würzburg, *Würzburg*, W. Germany (Dr R. Kibler, Professor V. ter Meulen).

Institute of Immunology and Institute of Public Health, *Zagreb*, Yugoslavia (Dr S. Smerdel, Dr A. Hrabar).

In each centre, our method was compared with a modification of the original technique of Stewart *et al.* (1967). All investigators used only microtitration plates for their own test. The main characteristics of the techniques used in the participating laboratories are given in Table 1.

In view of the differences in sensitivity between the HAI tests as performed in the collaborating centres, it was decided to base the comparison between our method and the comparative tests primarily on agreement between tests with regard to positivity (titre $\geqslant 8$) and negativity (titre $< 8$) of serum samples rather than on titre differences. Sera giving contradictory results with different test



https://doi.org/10.1017/S0022172400055480 Published online by Cambridge University Press

methods were sent to our laboratory and tested in the indirect immunofluorescence (IF) test, performed as described previously for poxvirus (Gispen, Huisman, Brand-Saathof & Hekker, 1974). The outcome of this test was used to decide which HAI test result was correct.

The trial was organized in two phases:

Phase I: testing of panels of sera with known HAI titres. At least 20% of the sera were negative.

Phase II: use in daily routine, either for susceptibility screening, or for the investigation of paired serum samples from patients, contacts and/or vaccinees, or for both.

Four different batches of reagents were sent to the investigators. The centres at Bilthoven and Glasgow received Batch A, that at Bordeaux Batch B, those at Copenhagen and Würzburg Batch C, and that at Zagreb Batch D.

The investigators were also provided with a freeze-dried serum pool to be used on each test day. In our hands, this serum pool had a HAI titre of 16-32.

## RESULTS

### *Phase I of the comparative trial*

Table 2 shows the results obtained in Phase I of the trial to compare our modified HAI test in glass tubes (macromethod) with the comparative tests.

In 29 out of 1469 sera the two tests gave contradictory results with regard to positivity and negativity; in 25 of these 29 sera the IF test was in agreement with our method. In 2 of the centres, our test performed significantly better than the comparative ones,* whereas in the other centres no significant differences were found.

To investigate the possibility of using the freeze-dried reagents in a micro-titration system (micromethod), all 1469 sera were also tested in this system and the results compared with those of the macromethod. There were 15 sera giving discrepant results, i.e. 1·0% of the total number of sera. The analysis of the discrepancies is given in Table 3. Judged against the results of the IF test, the macromethod was correct in 12 of the 15 sera, and the micromethod in the remaining 3. These results were significantly in favour of the macromethod in 2 centres, whereas in the other centres no significant differences were found.

### *Phase II of the comparative trial*

In this phase, a total of 2698 sera were tested.

Table 4 shows the results obtained comparing our macromethod with the existing test, in those centres where single serum samples were tested for susceptibility screening.

The results with paired serum samples of rubella patients, contacts or vaccinees are given in Table 5.

In this group, Bilthoven tested sera from acute rubella patients, Copenhagen

* In one of these centres (Copenhagen), an initial serum dilution of 1/10 was used for the comparative test.

https://doi.org/10.1017/S0022172400055480 Published online by Cambridge University Press

Table 2. *Results obtained in Phase I of the trial comparing the modified rubella HAI test macromethod with the comparative tests*

| | | Agreements | | | | | | Discrepancies | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | − in both tests | | + in both tests | | | | Mod. test +, comp. test − | | | Mod. test −, comp. test + | | | |
| | | | | | | | | | | | IF result | | | IF result | | Statistical significance |
| Centre | No. of sera | No. | % | No. | % | No. | % | No. | % | No. | + | − | No. | + | − | |
| Bilthoven | 297 | 296 | 99·7 | 65 | 21·9 | 231 | 77·8 | 1 | 0·3 | 0 | 0 | 0 | 1 | 0 | 1 | N.S. |
| Bordeaux | 203 | 202 | 99·5 | 63 | 31·0 | 139 | 68·5 | 1 | 0·5 | 0 | 0 | 0 | 1 | 0 | 1 | N.S. |
| Copenhagen* | 299 | 285 | 95·3 | 89 | 29·8 | 196 | 65·5 | 14 | 4·7 | 12 | 12 | 0 | 2 | 2 | 0 | S.+ |
| Glasgow | 202 | 191 | 94·6 | 52 | 25·8 | 139 | 68·8 | 11 | 5·4 | 10 | 10 | 0 | 1 | 1 | 0 | S.+ |
| Würzburg | 240 | 238 | 99·2 | 69 | 28·8 | 169 | 70·4 | 2 | 0·8 | 1 | 1 | 0 | 1 | 1 | 0 | N.S. |
| Zagreb | 228 | 228 | 100 | 78 | 34·2 | 150 | 65·8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| Total | 1469 | 1440 | 98·0 | 416 | 28·3 | 1024 | 69·7 | 29 | 2·0 | 23 | 23 | 0 | 6 | 4 | 2 | |

* Initial serum dilution of 1/10 used in comparative test.

IF, immunofluorescence; N.S., not significant; S.+, significant ($P = 0{\cdot}01$) in favour of modified test.

Table 3. *Discrepancies between macro- and micromethod of the modified rubella HAI test*

| Centre | Macromethod +/ micromethod −: No. | IF result + | IF result − | Macromethod −/ micromethod +: No. | IF result + | IF result − | Statistical significance |
|---|---|---|---|---|---|---|---|
| Bilthoven | 5 | 5 | 0 | 0 | 0 | 0 | S. macro |
| Bordeaux | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| Copenhagen | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| Glasgow | 5 | 5 | 0 | 0 | 0 | 0 | S. macro |
| Würzburg | 1 | 0 | 1 | 1 | 1 | 0 | N.S. |
| Zagreb | 3 | 2 | 1 | 0 | 0 | 0 | N.S. |
| Total | 14 | 12 | 2 | 1 | 1 | 0 | |

IF, immunofluorescence; N.S., not significant; S. macro, significant ($P = 0{\cdot}05$) in favour of macromethod.

screened rubella contacts and Würzburg and Zagreb tested vaccinees for seroconversion.

The total percentage of discrepancies found in Phase II was 2·7, i.e. 74 sera. The result of our test agreed with that of the IF test in 27 sera and disagreed in 32, whereas 15 discrepant sera were not available for investigation by IF. There was no significant difference between our test and the comparative ones, except in the 100 sera taken within 1 week after exanthema in Bilthoven, where the modified test failed to pick up early antibody in 18 cases, whereas the comparative test was already positive. However, our test detected a significant rise in antibody titre in all second serum samples from these series.

All serum samples were also tested using our micromethod. As in Phase I, this version of our method was slightly less reliable than the tube version.

*Titre differences between the modified and comparative tests*

The titre differences between the modified and comparative tests were evaluated by plotting the distribution of the ratio 'titre modified test:titre comparative test' for the macro- as well as for the micromethod of our test. This was done for each centre separately. All sera from Phase I and Phase II of the trial giving a titre $\geqslant 8$ in both tests were included. The results are shown in Fig. 1, which also gives the mean ratios. In general, the titres found with different techniques were rather close, except in Bilthoven (comparative test about 5 times higher titre than the modified test) and Copenhagen (comparative test about 2 times lower titre than the modified test). There was also a close agreement between the titres found with the macro- and micromethods of our test, except in Zagreb.

Table 4. *Results obtained in Phase II of the trial comparing the modified rubella HAI test (macromethod) with the comparative tests when used for susceptibility screening*

| | | Agreements | | | | | | Discrepancies | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | − in both tests | | + in both tests | | | | Mod. test +, comp. test − | | | | Mod. test −, comp. test + | | | | |
| | | | | | | | | | | | IF result | | | | IF result | | | Statistical significance |
| Centre | No. of sera | No. | % | No. | % | No. | % | No. | % | No. | + | − | ? | No. | + | − | ? | |
| Bordeaux | 441 | 441 | 100 | 43 | 9·8 | 398 | 90·2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| Copenhagen* | 38 | 38 | 100 | 6 | 15·8 | 32 | 84·2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| Glasgow | 231 | 213 | 92·2 | 49 | 21·2 | 164 | 71·0 | 18 | 7·8 | 13 | 8 | 5 | 0 | 5 | 2 | 2 | 1 | N.S. |
| Würzburg | 100 | 99 | 99·0 | 7 | 7·0 | 92 | 92·0 | 1 | 1·0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | N.S. |
| Total | 810 | 791 | 97·7 | 105 | 13·0 | 686 | 84·7 | 19 | 2·3 | 14 | 8 | 5 | 1 | 5 | 2 | 2 | 1 | |

* Initial serum dilution of 1/10 used in comparative test.
IF, immunofluorescence; N.S., not significant.

Table 5. *Results obtained in Phase II of the trial comparing the modified rubella HAI test (macromethod) with the comparative test when used for testing of paired sera*

| Centre | No. of serum pairs | Sample code | Agreement | | | | | | Discrepancies | | | | | | | | | | Statistical significance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | − in both tests | | + in both tests | | | | Mod. test +, comp. test − | | | | Mod. test −, comp. test + | | | | |
| | | | | | | | | | | | | IF result | | | | IF result | | | |
| | | | | % | No. | % | No. | % | No. | % | No. | + | − | ? | No. | + | − | ? | |
| Bilthoven | 100 | 1 | 81 | 81·0 | 43 | 43·0 | 38 | 38·0 | 19 | 19·0 | 1 | 1 | 0 | 0 | 18 | 18 | 0 | 0 | S.⁻ |
| | | 2 | 100 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| Copenhagen* | 382 | 1 | 363 | 95·0 | 74 | 19·4 | 289 | 75·7 | 19 | 5·0 | 16 | 6 | 3 | 7 | 3 | 0 | 2 | 1 | n.d. |
| | | 2 | 366 | 95·8 | 59 | 15·4 | 307 | 80·4 | 16 | 4·2 | 14 | 6 | 4 | 4 | 2 | 0 | 2 | 0 | n.d. |
| Würzburg | 149 | 1 | 148 | 99·3 | 145 | 97·3 | 3 | 2·0 | 1 | 0·7 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | N.S. |
| | | 2 | 149 | 100 | 0 | 0 | 149 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| Zagreb | 332 | 1 | 332 | 100 | 184 | 55·4 | 148 | 44·6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| | | 2 | 332 | 100 | 2 | 0·6 | 330 | 99·4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N.S. |
| Total | 963 | | 1871 | 97·1 | 507 | 26·3 | 1364 | 70·8 | 55 | 2·9 | 32 | 13 | 7 | 12 | 23 | 18 | 4 | 1 | |

* Initial serum dilution of 1/10 used for comparative test

IF, immunofluorescence; N.S., not significant; S.⁻, significant ($P = 0{\cdot}00008$) in favour of comparative test; n.d., not done.

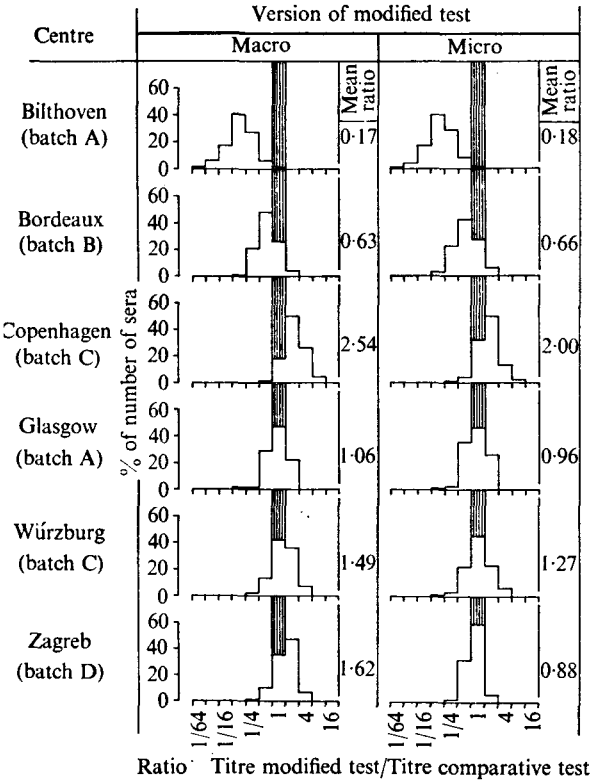


Fig. 1. Comparison of antibody titres found using the modified HAI test with those found using the comparative tests.

*Titre of a serum pool with the modified test*

The participating centres determined the HAI titre of a serum pool supplied by us, on every day test samples were run. The distribution of the titres found during the whole trial period (Phase I and Phase II) is shown in Fig. 2.

*Efficacy of absorption procedure*

The fast, combined absorption procedure (kaolin and erythrocytes) gave satisfactory results in about 98% of the test sera (ring in control tube). Most of the sera for which the absorption procedure was inadequate gave a satisfactory control reaction when the absorption procedure was modified in the following way:

(*a*) the sample was mixed with kaolin suspension and left to stand at room temperature for 15 min.

(*b*) the erythrocyte suspension was then added, mixed and left to stand at room temperature for 15 min.

https://doi.org/10.1017/S0022172400055480 Published online by Cambridge University Press

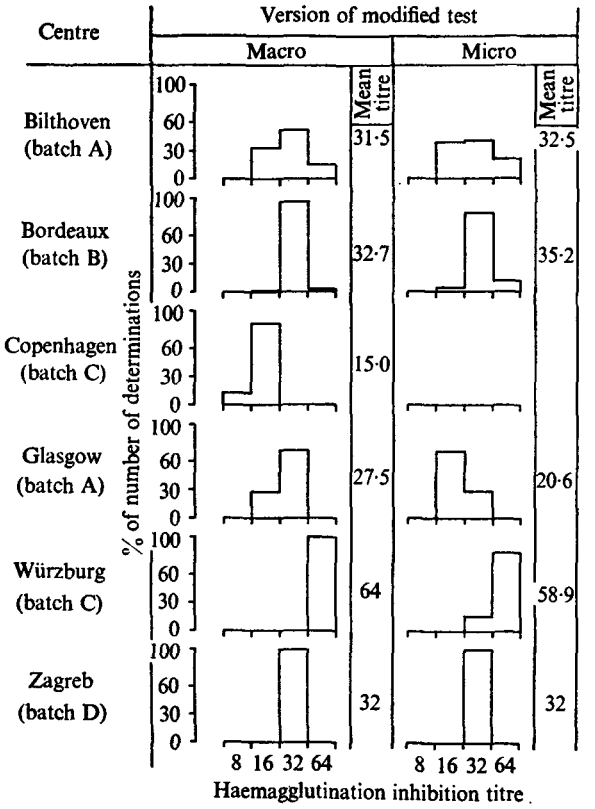


Fig. 2. Distribution of the HAI-titres of a freeze-dried serum pool, as determined in different centres with the modified HAI test.

## DISCUSSION

The conventional haemagglutination inhibition test for rubella antibodies, although very useful for screening and diagnostic purposes, presents some difficulties:

(*a*) the need for a regular supply of fresh erythrocytes.

(*b*) the need for a daily standardization of the sensitivity of the erythrocyte/haemagglutinin combination, due to the instability of the erythrocytes.

With careful control of these factors, it is perfectly feasible to establish an assay with satisfactory intra-laboratory reliability and reproducibility. The characteristics of assays performed in different laboratories, however, may vary widely. For example, it can be seen in Fig. 1 that the conventional test as performed in Bilthoven gives antibody titres which are 5–6 times higher than those obtained with our modified test, whereas a similar test performed in Copenhagen gave antibody titres which were 2–2·5 times lower than those obtained with the modified test. One approach to avoid such problems is the use of a standard positive serum which is tested in parallel with every test. In our test modification, we have used freeze-dried, formalinized erythrocytes in an attempt to overcome these problems. The intra-laboratory reproducibility of the test method, as assessed by regular

https://doi.org/10.1017/S0022172400055480 Published online by Cambridge University Press

assays of a standard serum pool, is very satisfactory (Fig. 2), although differences in the distribution patterns occur (compare for instance Bilthoven with Zagreb). The inter-laboratory reproducibility is also satisfactory with three out of four reagent batches whereas the fourth one, used in Copenhagen and Würzburg, gives somewhat inconsistent results. Because the difference in sensitivity between our modified test and the comparative tests varied from centre to centre, the main comparison between the performance of the tests was made on the basis of positive (titre $\geqslant 8$) and negative (titre $< 8$) outcomes, using immunofluorescence results as decisive for the correctness of the HAI results.* In spite of the sensitivity differences, the number of contradictions between our modified test and the comparative tests was low (103 sera, i.e. 2·4%), indicating that the concentration of antibodies which is normally present in a state of immunity is above the detection threshold of our test modification. Of the 103 discrepant sera, 88 were available for further study. In 52 (59%), our test was in agreement with the IF test. Of the remaining 36 sera, 18 were early sera from acute phase rubella patients. It is not clear whether these discrepancies arose from the high sensitivity of the Bilthoven test (see Fig. 1), or from a possibly reduced ability of our modified test to detect antibodies of the IgM class.

Our reagents can be used in glass tubes as well as in polystyrene microtitration plates. The sensitivity of the two versions is about the same (Figs. 1 and 2). The number of discrepancies is slightly higher in the micromethod, although the test still performs satisfactorily (Table 3). This may be due to the fact that the micromethod patterns are more difficult to read than the macromethod patterns. On the other hand, the micromethod requires smaller amounts of the reagents and can be handled faster than the macromethod. Which version is preferable depends on the individual needs of the laboratory.

We conclude that the modified HAI test for rubella antibodies is as reliable as the comparative tests, and is, moreover, easier to perform because it uses stable reagents and a daily standardization is not required. In view of these advantages, the test appears suitable for use in both virological and general clinical laboratories.

We thank the investigators participating in the multi-centre trial for their excellent contributions; Mr T. H. A. Janssen, Miss M. Mantingh and Miss J. C. M. van de Berg for their technical assistance in development of the test method; Dr O. T. Schönherr and Mr P. Nelissen for producing the haemagglutinin and performing the immunofluorescence test; Professor R. Gispen and Mrs B. Brand-Saathof for advice and support in learning the immunofluorescence technique; and Ir. J. W. A. Voerman for the statistical evaluation of the trial results.

* Copenhagen used a serum dilution series starting at 1/10 instead of 1/8 for its comparative test. This has probably increased the number of discrepancies in this centre, viz. the sera with a titre between 8 and 10, which are positive in our test and negative in the comparative test.

## REFERENCES

Gispen, R., Huisman, J., Brand-Saathof, B. & Hekker, A. C. (1974). Immunofluorescence test for persistent pox virus antibodies. *Archiv für die gesamte Virusforschung* **44**, 391–5.

Gregg, N. M. (1941). Congenital cataract following German measles in the mother. *Transactions of the Ophthalmological Society of Australia* **3**, 35–46.

Gupta, J. D. & Harley, J. D. (1970). Use of formalinized sheep erythrocytes in the rubella haemagglutination inhibition test. *Applied Microbiology* **20**, 843–4.

Inouye, S. & Kono, R. (1972). Effect of a modified kaolin treatment on serum immunoglobulins. *Applied Microbiology* **23**, 203–6.

Meyer, H. M. Jr., Parkman, P. D. & Hopps, H. E. (1972). The clinical application of laboratory diagnostic procedures for rubella and measles (rubeola). *American Journal of Clinical Pathology* **57**, 803–13.

Norrby, E. (1962). Haemagglutination by measles virus. 4. A simple procedure for production of high potency antigen for haemagglutination inhibition tests. *Proceedings of the Society for Experimental Biology and Medicine* **111**, 814–18.

Schuurs, A. H. W. M., de Jager, E. & Homan, J. D. H. (1968). Studies on human chorionic gonadotrophin. *Acta endocrinologica* **59**, 120–38.

Stewart, G. L., Parkman, P. D., Hopps, H. E., Douglas, R. D., Hamilton, J. P. & Meyer, H. M. Jr. (1967). Rubella virus haemagglutination inhibition test. *New England Journal of Medicine* **276**, 554–7.

Wide, L. (1962). An immunologic method for the assay of human chorionic gonadotrophin. *Acta endocrinologica* Suppl. **70**, 20–27.