Simplified method for the rubella haemagglutination inhibition screening test

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SUMMARY A modification of the rubella haemagglutination inhibition (HAI) test in which both pretreatment and serum titration are carried out in wells of the same microtitre plate saves time, labour, and materials and gives results comparable to a conventional HAI procedure.

Although over the last five years diagnostic laboratories have faced a growing demand for rubella HAI screening tests few attempts have been made to simplify the procedure. We have been looking for ways to reduce the number of manipulations required so that larger batches of sera may be handled. Our findings may be of interest.

Materials

The sera used were specimens sent for rubella haemagglutination inhibition (HAI) tests. A 25% kaolin suspension in borate buffer pH 9.0 (Flow Laboratories) was diluted for use with an equal volume of borate buffer pH 9.0, to give a 12.5% suspension. The diluent for the HAI procedure was dextrose gelatin veronal buffer with 0.2% bovine plasma albumin pH 7.2. Erythrocytes were collected by heart puncture from day-old chicks hatched out in the laboratory. Rubella haemagglutinin was kindly supplied by Dr C. M. P. Bradstreet, Director, Standards Laboratory, Central Public Health Laboratory, Colindale. A working bromocresol green solution was prepared by adding 2.1 ml of a stock solution (7 g of bromocresol green in 1 litre of 0.02 M sodium hydroxide solution) to 100 ml of citrate buffer pH 3.8. Both stock solution and buffer were supplied by Clin. Tech., Ltd.

Methods

Conventional 0.10 ml of each serum and 0.90 ml of kaolin suspension were added to 3 x ½ in. (7.5 x 1.25 cm) test tubes using a 'selectapette' automatic pipette. The tubes were shaken by hand and incubated at room temperature for 30 minutes. They were then spun at 1800 rpm for 10 minutes in a Mistral 4L centrifuge (MSE) and the supernatants decanted into fresh tubes. To each tube 0.02 ml of 20% chick cell suspension was added. After incubation at 4°C for one hour the tubes were centrifuged at 1000 rpm for five minutes. The supernatants (serum dilution 1 in 10) were used to prepare doubling dilutions of from 1 in 10 to 1 in 80 for the HAI test and of 1 in 10 and 1 in 20 for the cell agglutinin control by the microtitre technique described below.

Simplified 0.02 ml of each serum was drawn into the probe of an automatic dispensing apparatus (Compu-pet®) (Cremer et al., 1975) and discharged with 0.18 ml of 12.5% kaolin suspension into the first well of the first short row of a new 8 x 12 'V' well microtitre plate. The exterior of the probe was then wiped with a paper tissue. The next specimen was added with kaolin to the first well of the following row, the probe wiped, and the procedure repeated until the plate was full (12 specimens). Each completed plate was shaken on a microshaker (Cooke AM 69) for 15 seconds, incubated at room temperature for 15 minutes, and reshaken. The plates were then centrifuged for 15 minutes at 1500 rpm using microtitre plate carriers (Dynatech). To carry out the HAI screening tests 0.025 ml volumes of supernatant were transferred from the first well of each row to the second, third, sixth, and seventh well using a microtitre dropping pipette. Dilutions up to 1 in 80 for the test and up to 1 in 40 for the serum control were then prepared with a 12-loop automatic microdiluter (Titertek). The procedure is shown in the figure. A 0.025 ml volume of antigen containing four complete units of rubella haemagglutinin was then added to each test well and the same volume of diluent to

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conventional
subsequent
been with each of between test, HAI
agglutinins.
10 025
hour 0 025
incubated
15 minutes
room temperature, read over an x-ray
film illuminator. Patients whose sera had a titre of
\( \geq 20 \) were reported as immune to rubella.

The following control sera were tested in triplicate
with each batch of tests: (1) a local working standard
matched with the British standard for the rubella
HAI test, (2) a rubella HAI negative serum, and
(3) an aliquot from a serum pool made up to give a
titre of between 10 and 20 when examined by our
conventional test procedure.

When the simplified method was used a few sera
had subsequently to be absorbed to remove chick
cell agglutinins. This was done by adding 0-02 ml of
10% chick cells to the supernatants after the plates
had been centrifuged to sediment the kaolin. The
plates were agitated for two seconds on the micro-
shaker, incubated for one hour at 4°C, and re-
centrifuged for five minutes at 1000 rpm.

Results

EVALUATION OF COMPU-PET\textsuperscript{100}
To discover if there was carry-over between specimens
sera with titres of \( \geq 1280 \) and < 10 were tested
alternately. In testing 48 sera no instance of carry-
over of inhibitory activity from a positive into the
lowest dilution of the following negative serum was
seen. We concluded that the relatively large volume
of kaolin suspension used effectively flushed out the
probe of the apparatus between specimens.

COMPARISON OF CONVENTIONAL AND SIMPLIFIED METHODS
Two batches, respectively of 150 and 160 sera for
screening tests, were examined in parallel by the
conventional and simplified methods. Identical
results were obtained by the two methods for all but
five sera. Four of these had a titre of 40 by one and
of \( \geq 80 \) by the other method. The fifth serum had
titres of 20 and 40. A further batch of 32 sera from
patients in contact with rubella were examined after
dilution from 1 in 10 to 1 in 2560 for the HAI test
and 1 in 10 and 1 in 20 for the cell agglutinin control.
Results obtained by the two methods for these sera
did not differ by more than one dilution in any case.

EFFECT OF OMITTING ROUTINE ABSORPTION OF CELL AGGLUTININS
In the course of using the simplified method to test
eight batches of sera, totalling 1242 specimens, there
were seven sera in which the concentration of
agglutinins was too high for the HAI titre to be read,
and which therefore had to be retested after absorp-
tion with chick cells.

Discussion

The capacity of the wells in a microtitre plate is
sufficient to allow in-situ treatment with kaolin
suspension of small samples of serum. Up to 0-20 ml
of a serum/kaolin mixture can be dispensed into the
wells of a new ‘V’ plate and shaken without spilling,
and this volume may conveniently be delivered by
the Compu-pet\textsuperscript{100} apparatus. However, workers
who do not have this apparatus may use microtitre
dropping pipettes to make up a mixture of 0-025 ml
of serum, 0-025 ml of borate buffer, and 0-10 ml of
25% kaolin suspension in the wells of new or used
plates. This mixture yields a serum dilution of about
1 in 5 which can be further diluted and tested as
described above.

Chick erythrocytes are commonly used as indicator
cells in the rubella HAI test. Titres of chick cell
agglutinins in human sera are relatively low, and,
provided that the HAI test includes control dilutions
of each serum up to 1 in 40, we have found that less
than 1% of specimens need to be retested with
preliminary red cell absorption. We have therefore
felt justified in omitting the routine absorption of
agglutinins from our simplified procedure.

Two sources of error may be encountered in the
simplified procedure: firstly a failure to pick up
serum from the specimen bottle with the probe of
the Compu-pet\textsuperscript{100} and, secondly, a failure to transfer
the treated serum from the first well of each row.
These omissions may be recognised by the following
means. When the serum dilutions have been prepared
the plates are held at eye level and the volume of
supernatant remaining in the first well of each row
is checked. At the end of the test the presence of
serum in these wells is verified by adding a drop of
the working bromocresol green solution. If serum is

Figure Preparation of serum dilutions.
Simplified method for the rubella haemagglutination inhibition screening test

present a green-blue colour develops within 30 seconds. If the wells hold only kaolin the suspension remains yellow.

The cost of adopting this simplified rubella HAI test need not be great. The Compu-pet100 apparatus is complex and expensive (about £800) but is not essential to the procedure. The microtitre plate carriers, which are made to fit most of the centrifuge heads in common use, cost less than £30 a pair. The microshaker costs £90 (November 1976 prices).

The simplified method halves the labour of carrying out a large batch of rubella HAI tests. It also has the advantage that specimen numbers can be marked on the plates and sera dispensed directly into them. In this way large numbers of specimens can be handled without their falling out of order, as may happen during the manipulations of conventional HAI procedures. We therefore recommend this method, particularly for large scale antenatal rubella screening and for epidemiological inquiries in which only small volumes of serum may be available.

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References