Use of the direct haemolysis-in-gel test for rubella antibodies in the Icelandic prevention programme for congenital rubella

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SUMMARY Untreated earlobe capillary blood samples from 5958 girls were screened for rubella antibodies by placing them directly onto haemolysis-in-gel (HIG) test plates. The method is in our experience quick, reliable and also acceptable to young girls. The method is convenient for testing for natural immunity before vaccination and in most but not all cases satisfactory as a test for seroconversion after vaccination. A comparison was made between naturally infected girls and a group of vaccinated girls. Vaccinated girls had lower values and most of the low or doubtful positives were in this group. Serum samples from 51 low positive girls were tested by the haemagglutination inhibition (HI) test and the serum HIG test and their results compared with their respective direct HIG values.

The rubella vaccination programme of teenage girls in Iceland involves testing for rubella antibodies and vaccination only of the negatives. We find this worthwhile as during the last four years only 13–24% of 12-year-old girls have been seronegative and have needed vaccination. The method we use was developed in this laboratory and is called the direct HIG test. It is a modification of the haemolysis-in-gel test (HIG) or single radial haemolysis, using untreated capillary blood instead of serum and placing it directly onto the test plate. This method was described in a previous publication.¹ During the period from autumn 1979 to spring 1981 we performed 5958 tests on capillary blood from 5777 girls using the direct HIG test. This includes samples collected from vaccinated girls, in most cases taken one year after vaccination. The purpose of this report is to describe the practical use of this method.

Material and methods

COLLECTION OF BLOOD SAMPLES

Included in this study are 5958 capillary blood samples, taken from schoolgirls over a period of two years, beginning shortly after a severe rubella epidemic. The HIG plates were brought to the schools and samples from girls mostly between the

ages of 12 and 15, but some up to 20 years old, were collected by two people from the laboratory in co-operation with school nurses. Some of the negative cases were retested after vaccination (RA 27/3 Wellcome, Beckenham, Kent, England) to test for seroconversion. The second sample was taken a year after the vaccination. The samples were taken from the earlobe and placed directly onto the test plates. Recording and collection of capillary blood takes two people 1–1.5 min per girl. All samples were taken during the afternoon to allow for a diffusion time of 16–18 h at 4°C in the laboratory before treatment with complement the next morning. There was never any need to visit the same school twice because of failure in the test. One visit to each school per year has enabled us to collect samples from more than 95% of girls in the relevant age groups. The few girls who are not present when we visit a school are asked to attend a neighbouring school when we are sampling there or to come to the laboratory. In this way we have obtained samples from virtually all girls in these age groups. Furthermore we collected venous blood samples from 51 girls with low direct HIG values. These samples were collected less than a month after the collection of the original capillary blood sample. Venous blood samples were collected into vacutainers. The sera were separated and kept in sterile vials at –20°C until tested. These samples were tested in the conventional serum HIG test and the HI test and the

results compared with their respective direct HIG values.

THE HI TEST AND THE HIG TEST

The haemolysis-in-gel test plates were prepared as previously described.^{1,2} Briefly, rubella HA antigen (1/5) was reacted with a 1.3% suspension of pigeon red blood cells (total 5 ml) at 4°C for 30 min. The RBCs were centrifuged and resuspended in the same volume of RPMI-1640 at 37°C, mixed with 5 ml of molten agarose and the mixture poured into a square Petri dish (Intergrid-1012, Falcon). Control plates were made the same way, without antigen. Samples were either serum samples (the serum HIG test) or capillary blood samples (the direct HIG test). In the serum HIG test values of ≥ 8.5 mm are considered positive, in the direct HIG test zone sizes tend to be slightly smaller and diameters of ≥ 8.0 mm are regarded as positive. These diameter values were found to correspond to a HI-titre of $\geq 1/20$.¹ In our hands test-to-test variation has been negligible.

The haemagglutination inhibition (HI) test was done as previously described.^{1,3} Serum samples were pretreated with kaolin and absorbed on pigeon red blood cells. Serum dilutions were incubated with 4 units of HA antigen for 1 h at room temperature, the pigeon red blood cell suspension was then added (0.25% susp) and the plates kept at room temperature until the cells had settled. Control sera with high, low and negative titres were included in each run. Titres of $\geq 1/20$ are considered positive.

Results

A total of 5958 direct HIG tests were performed over a period of 18 months (autumn 1979 to spring 1981). Of these, 5300 (88.9%) were positive with a diameter of ≥ 8.0 mm. There were 504 (8.5%) negative samples (a diameter of less than 6.0 mm). One hundred and fifty four (2.6%) had diameters between 6.0 and 8.0 mm. Included are 923 girls known to have been vaccinated and a small number of girls who have probably been vaccinated but the records were not available. The Table compares the

direct HIG diameters of the whole group and the 923 vaccinated girls. When the negative samples are excluded the most marked difference is the low direct HIG values in the vaccinated group. Samples with values between 6.0 mm and 8.0 mm are 7.6% compared with 2.8% in the whole group and 59.9% have a diameter of 9.0 or more compared with 82.0% in the whole group.

Our main use of this method is to measure rubella antibodies in 12-year-old girls, as this is the age group that is vaccinated. In the winter of 1979–1980 antibodies were measured in samples from 947 girls born in 1967. Of the 128 (13.5%) who had no measurable antibodies, 102 were vaccinated and retested a year later.

Clear seroconversion (HIG ≥ 8.0 mm) was achieved in 97 cases, three girls gave a retest value of 7 mm and two failed to respond. The positive values were again lower than for naturally immune girls, 64% of the vaccinees had a HIG diameter of ≥ 9.0 mm.

In order to analyse the low direct HIG values further, 51 venous blood samples were collected from a group of girls with direct HIG diameters between 6.0 and 8.0 mm. Of these, 32 girls were known to have been vaccinated 2–3 years earlier. Thirty-three girls (including 22 vaccinees) had ≥ 8.5 mm in the serum HIG test; of these, 12 gave 1/20 in the HI test but the remaining 21 were negative (HI titre $\leq 1/10$). Eighteen of the 51 girls gave a diameter of 8.0 mm or less in the serum HIG test and these were all also negative in the HI test except for one who had a titre of 1/20.

Approximately 6500 direct HIG tests have been performed so far in this laboratory, and of these we have checked 750 cases with either an HI test or a serum HIG test. We have discovered two false positives in the direct HIG test. One girl was tested during a school test programme and the result was a HIG value of 8.0 mm. More than a year later a serum sample from the girl came to this laboratory. It was tested in the HI and HIG tests repeatedly and was always negative. The other girl had 10.0 mm in the direct HIG test, but her serum always gave negative results on repeated testing. She was then

Comparison of the direct HIG values of the whole group and the 923 vaccinated girls (Negative samples are excluded when percentages are calculated).

No tested		Direct HIG values (mm)												
		6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0
5958	504	5	6	73	70	377	449	1400	959	1338	309	300	74	87
%		0.1	0.1	1.3	1.3	6.9	8.2	25.7	17.6	24.5	5.7	5.5	1.4	1.6
923	4	5	5	30	30	141	158	298	121	103	15	11	1	1
%		0.5	0.5	3.3	3.3	15.3	17.2	32.4	13.2	11.2	1.6	1.2	0.1	0.1

HIG = haemolysis-in-gel.

vaccinated and tested three months later showing 9.0 mm in the serum HIG test. We cannot say whether these are true examples of a false-positive reaction or due to an operator fault as the original capillary blood sample cannot be retested.

Discussion

The direct HIG test was designed for the screening of large groups of schoolgirls before vaccination. The test has proved very useful for that purpose; it is easy to perform in schools and acceptable to young girls. Similar tests have been performed elsewhere, that is, using capillary blood in the HIG test. Those tests involve some pretreatment, centrifugation or heparinisation and heat inactivation. When compared with the HI test or the conventional HIG test with venous serum samples the capillary blood tests show good correlation.^{1 4 5}

We find the direct HIG test very convenient as a test for natural immunity and in most but not all cases satisfactory when testing for seroconversion after vaccination. We feel that we can safely consider those samples with ≥ 8.0 mm positive but it is more difficult to decide whether 7.5 mm or less indicates presence of antibody. We have at present no sensitive tests such as RIA or ELISA available in the laboratory to determine whether this is a non-specific reaction or indicates low levels of antibody. In such cases we therefore ask for a venous blood sample and do a serum HIG test. If the serum HIG test is < 8.5 mm we recommend vaccination and seroconversion is often observed in such cases.

In Great Britain where the rubella vaccination was first directed towards 11–14-year-old schoolgirls without prior screening, 8–10% of the children are not vaccinated because of parental refusal.⁶ We find that in Iceland very few parents (less than 1%) refuse vaccination but all parents approve of the screening and show interest in the prevention programme. By screening all girls in the relevant age groups we know which ones require vaccination. Most girls stay at least three more years at school after rubella HIG screening and negative unvaccinated girls are offered rubella vaccine each year.

The lower titres in the vaccinees compared with the naturally infected girls reflect a slightly inferior antibody response after vaccination. This is in

agreement with other reports on this vaccine (RA 27/3).^{7–9}

The screening procedure described has been very successful during the last few years as it enables us to test all 12-year-old girls in Reykjavík and its surroundings in 3 months, by visiting schools twice a week. The number of girls tested by this method is just under 1000 each year and screening reduces the cost of vaccination considerably as only the known seronegatives are vaccinated. Future follow-up of vaccinated girls is also made much easier.

Although this work has been carried out in a small, well defined community we feel that the procedure is sufficiently simple and economical for it to be applied on a larger scale.

We are most grateful to Professor Margrét Guðnadóttir and Dr Helga Ógmundsdóttir for encouragement and advice. We would like to thank Valgerður Sigurðardóttir for performing the HI tests.

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