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Comparison of Four Enzyme Immunoassays for Detection of Immunoglobulin M Antibodies against Rubella Virus

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We evaluated four tests for the detection of rubella virus-specific immunoglobulin M antibodies. Primarily, consecutive serum samples were tested by two different assays. Selected panels of sera from patients with proven or likely recent rubella and false-positive and true-negative results in the two primary assays were further tested with two recently developed, fully automated techniques. The four tests were comparable in overall accuracy, but their dynamic ranges may differ considerably. Ways to optimize the predictive values are discussed. We conclude that automated assays may be used without causing significant changes in diagnostic accuracy or distortions in notifications of the incidence of rubella compared with the use of established tools.

Detection of immunoglobulin M (IgM) antibodies against rubella virus becomes a reliable marker for recent infection within 10 days of the appearance of the first signs of primary infection. IgM antibodies usually persist for 1 to 4 months but may remain detectable for much longer intervals in a minority of patients (5, 8, 16, 17, 20). Certain tests are more likely to produce false-positive results than others or may produce positive results for longer periods of time following primary infection (6). Weakly reactive IgM tests may also occur following reinfection or vaccination (13, 14). Although a μ capture radioimmunoassay (15) has been considered a reference technique, there has been no valid standardization of these tests and in diagnostic laboratories they have been largely replaced by enzyme-linked immunoassays because of longer shelf life of the reagents and to avoid radioactive waste (2, 5). Since laboratory ascertainment of rubella is based predominantly on IgM tests, performance characteristics of these assays may have considerable influence on individual diagnoses, as well as on the notification of rubella incidence based on laboratory reports. Therefore, the comparability of commercial rubella IgM tests is relevant for the surveillance of this infection. With the decreasing incidence of rubella due to the mass vaccination strategies pursued in most industrialized countries, it is increasingly difficult to maintain sufficient quality and expertise with in-house methods and the availability of reliable but technically less demanding assays is desirable for efficient surveillance of vaccination programs.

We selected sera from consecutive patients which were tested for anti-rubella virus IgM antibodies by two established tests. This provided panels of sera from patients with proven and likely recent infections, as well as negative and false-positive controls. These panels were then used to evaluate two recently developed automated assays in a comparison with established techniques.

MATERIALS AND METHODS

Patients and sera. A total of 3,437 consecutive serum specimens obtained for diagnostic anti-rubella virus IgM testing from January 1991 through May 1993 were frozen within 8 h of receipt at the laboratory and kept at −20°C for up to 2.5 years, until additional tests were performed. Sera from 82 of 143 patients whose sera had been reactive with one of the primary assays were available for further testing. Group 1 consisted of 24 serum samples from 15 patients who had a seroconversion or an anti-rubella virus IgG concentration increase of ≥1.8-fold or who excreted rubella virus. Group 2 consisted of 50 serum samples from 50 patients with a clinical diagnosis of rubella and IgM reactivity in both primary assays. Group 3 comprised 23 serum samples from 17 patients who were unlikely to have recent rubella virus infection (see the comments in Table 1). Group 4 contained 128 serum samples from 128 consecutive patients which tested negative in both primary IgM assays.

IgM. As primary tests, we used the μ capture enzyme-linked immunosorbent assays (ELISAs) Platelia Rubéole IgM (Diagnostics Pasteur, Marnes-la-Coquette, France) (test A) and the ETI-Rubek-M test (Sorin Biomedica, Saluggia, Italy), which uses unlabelled cell culture-derived rubella virus antigen and horseradish peroxidase-labelled mouse monoclonal antibodies for detection (test B). The test from Diagnostics Pasteur used infected and uninfected cells to detect nonspecific reactions until July 1992 (test A1 [see Table 1]); later, the manufacturer omitted the uninfected cell control and changed the interpretation of the results by expanding the indeterminate range from sample/cutoff ratios of 0.8 to 1.0 to 1.0 to 2.0 (test A2 [see Table 1]). We interpreted the results accordingly. As additional tests, we performed the Rubella IgM IMx test (Abbott Laboratories, North Chicago, Ill.) (test C) and the VIDAS Rubéole IgM test (bioMérieux sa, Marcy-l'Etoile, France) (test D). Test C is based on automated microparticle enzyme immunoassay technology; it uses microparticles coated with purified rubella virus (strain HPV-77), which bind to a glass fiber matrix after reaction with diluted serum, with alkaline phosphatase-conjugated anti-human IgM and 4-methylumbelliferyl phosphate as detecting reagents; in this procedure, all sera were absorbed with the IMx rheumatoid factor neutralization reagent in accordance with the manufacturer's instructions. Test D is an automated μ capture ELISA using inactivated wild virus antigen and alkaline phosphatase-labelled Fab′ fragments of an anti-rubella virus hemagglutinin monoclonal antibody and 4-methylumbelliferyl phosphate as detecting reagents.

IgG. Rubella virus-specific IgG was measured by the Rubella IgG IMx test or the VIDAS Rubéole IgG test. Consecutive

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TABLE 1. Patients and laboratory results

Category[a] and patient/ sample	Sex[b]	Age (yrs)	Anti-rubella virus IgM test result (sample/cutoff ratio)					Anti-rubella virus IgG level (IU/ml)	Comment(s)
			A/1 (0.8–1.0)[c]	A/2 (1.0–2.0)[c]	B (0.9–1.1)[c]	C (0.8–0.999)[c]	D (0.8–1.199)[c]		
Group 1									
1/A	M	20	0.8		1.0	0.2	0.9	0	Lymphadenopathy, conjunctivitis, 7 days
1/B			5.2		6.1	3.5	15.9	356	23 days after beginning of illness
2/A	M	30		1.5	1.1	1.2	1.3	2	Rash, fever
2/B				6.5	4.6	7.3	16.3	131	20-day interval
3/A	F	10	0.7		0.9	0.4	0.4	0	Rubella
3/B			>6.5		5.5	5.1	14.1	199	12-day interval
4/A	M	7	0.0		0.0	NA[d]	NA	1	Cough for 2–3 wks
4/B			1.9		2.2	1.1	2.8	7	18-day interval
5	F	10	1.5		1.4	1.3	1.17	12	Rubella virus isolated from throat swab
6/A	F	31	2.0		2.2	2.2	3.0	12	Screening in 13th wk of pregnancy
6/B			6.7		4.5	1.2	7.2	239	5-day interval
7/A	M	26	0.1		0.2	0.2	0.1	1	Rash, fever
7/B			6.1		4.7	4.1	8.5	185	14-day interval
8/A	M	29	3.2		2.8	0.5	3.1	6	
8/B			6.4		5.1	1.3	8.7	205	16-day interval
9/A	F	11		0.7	0.9	0.9	0.4	2	Rash for 4 days, occipital lymphadenopathy
9/B				4.9	3.3	1.8	5.3	>400	27 days after beginning of illness
10/A	M	15	0.0		0.0	NA	NA	4	Rash, conjunctivitis, lymphadenopathy
10/B			1.5		1.3	0.3	1.6	>500	36-day interval
11	M	0	>5.3		3.7	1.8	10.9	331	Congenital rubella syndrome, rubella virus isolated
12	F	30	2.2		1.4	1.5	1.7	970	Mother of patient 11 in 16th wk of pregnancy
13/A	F	19	>6.3		6.8	4.0	13.2	165	Rash, fever, unvaccinated
13/B			>6.3		>3.5	4.4	6.9	297	16-day interval
14/A	F	11	0.1		0.3	0.3	0.3	3	Rash, fever, cough for 10 days, measles?
14/B			>3.6		4.5	0.1	11.6	>500	27-day interval, measles tests negative
15/A	F	14	0.0		0.0	NA	NA	0	Rash, lymphadenopathy, splenomegaly, unvaccinated
15/B			5.6		4.1	3.5	6.7	175	10-day interval
Group 2									
16	F	11		6.5	5.4	4.8	13.2	136	
17	F	24	5.2		2.1	1.7	2.8	NA	26th wk of pregnancy
18	F	21		2.2	1.2	1.3	1.5	89	
19	M	3		6.1	3.8	5.6	10.1	111	
20	M	32	5.4		>4.2	0.1	5.2	348	CMV[e] infection
21	F	22	2.2		2.7	2.0	4.4	484	Pregnancy wk 30, premature labor
22	M	21	1.8		2.3	0.3	3.0	18	
23	F	26		3.1	2.3	1.0	2.2	>400	
24	M	36	>4.4		>5.0	6.7	19.2	NA	
25	F	11	3.2		2.6	2.5	3.8	159	Rubella outbreak in school
26	M	20	1.7		1.2	2.1	1.4	355	
27	F	13	>7.0		>4.3	6.9	13.1	151	Rash for 8 days
28	F	9		4.0	2.5	2.0	4.0	178	Sister of patient 27
29	F	14	4.0		>5.6	5.4	16.7	151	
30	F	43		3.5	2.1	2.4	2.9	2	
31	F	5	>3.6		5.0	1.7	11.3	416	Illness for 13 days
32	F	7	6.0		4.8	2.5	10.0	113	Illness for 6 days
33	F	1	1.8		1.3	0.8	2.2	>400	Recurrent febrile convulsions
34	F	8	5.4		5.2	2.2	12.1	313	
35	F	15	5.1		4.1	0.8	9.6	>500	Rash for 2 wks, unvaccinated
36	M	6		>6.7	8.2	6.3	17.8	10	Illness for 7 days
37	M	13	>7.0		>4.3	7.0	15.7	148	Illness for 7 days
38	M	45		2.4	1.3	1.3	1.4	30	Thrombopenia
39	M	46	2.3		1.7	2.6	3.0	359	
40	F	67		2.4	3.1	0.8	2.2	39	Fever, 1 mo after cardiac surgery, CMV negative
41	F	28		2.4	1.3	1.0	1.9	176	
42	F	10		8.4	4.8	6.0	13.5	NA	
43	M	3		5.8	3.6	3.1	5.9	NA	
44	F	22	3.9		3.9	7.1	8.9	NA	Pregnancy
45	M	19	5.7		4.5	2.7	9.1	53	
46	F	13	1.5		1.7	1.5	1.1	31	Rubella contact
47	F	39		3.3	1.6	2.1	2.8	8	

Continued on following page

TABLE 1—*Continued*

Category[a] and patient/ sample	Sex[b]	Age (yrs)	Anti-rubella virus IgM test result (sample/cutoff ratio)					Anti-rubella virus IgG level (IU/ml)	Comment(s)
			A/1 (0.8–1.0)[c]	A/2 (1.0–2.0)[c]	B (0.9–1.1)[c]	C (0.8–0.999)[c]	D (0.8–1.199)[c]		
48	F	25	5.1		4.7	1.9	1.1	>400	
49	F	41	6.3		7.1	9.0	18.3	36	Rubella contact
50	F	49	>6.8		>4.2	0.77	15.8	93	
51	M	29		5.5	3.3	1.8	4.1	NA	
52	F	29		2.6	1.5	0.3	2.8	124	Caesarean section
53	F	29		4.8	2.5	2.4	5.7	>400	
54	F	18		12.2	10.2	7.4	15.9	135	
55	F	17	>6.2		4.5	3.1	14.1	NA	
56	M	29		7.0	>8.3	4.5	12.6	187	
57	F	26	1.8		0.9	1.2	1.6	144	
58	M	1	3.1		1.8	1.2	3.1	NA	Hepatitis
59	F	9	>6.8		>3.0	6.1	13.0	NA	Cystic fibrosis
60	F	9	6.0		>3.0	5.2	15.3	NA	Twin of patient 59
61	F	17		5.4	2.4	1.2	3.6	NA	
62	F	22	2.1		1.1	2.1	7.3	530	Pregnancy
63	M	0	2.6		2.5	1.2	4.5	499	Fetal death at 39 wks of gestation, maternal rubella in 1st trimester
64	M	24	0.9		1.2	0.1	0.9	4	Rash and fever, anti-measles virus and anti-parvovirus B19 IgM negative
65	F	35	3.9		1.3	1.8	3.3	139	
Group 3									
66	M	16		1.3	0.5	0.4	0.6	54	Non-Hodgkin's lymphoma, no increase in anti-rubella virus IgG 3 mo later
67/A	F	28	1.9		0.4	1.0	0.9	1,150	Orthotopic liver transplant
67/B				2.2	0.5	0.7	1.0	221	Reactive anti-rubella IgM for >1.5 yrs
68	F	54		0.5	1.2	0.3	0.3	NA	Hepatitis, multiple IgM reactivities (CMV, toxoplasma)
69	F	32		1.5	0.1	0.3	0.1	33	Pregnancy, borderline anti-rubella virus IgM for >500 days
70/A	F	24		1.8	0.4	2.1	1.5	92	
70/B				1.5	0.5	2.0	1.0	98	Persisting IgM for >150 days without evolving IgG
71/A	F	30		1.2	1.6	NA	NA	326	
71/B				2.5	1.7	NA	NA	307	
71/C		31		2.4	1.9	0.3	1.21	323	Pregnancy wk 13, unchanged IgM and IgG for 6 weeks
72/A	M	32	2.0		1.0	0.8	2.3	165	Acute toxoplasmosis
72/B			0.4		0.6	0.6	1.3	167	Recent (14 wks ago) toxoplasmosis
73	F	24		0.4	1.3	0.2	0.1	184	Pregnancy wk 12, multiple IgM reactivities (CMV, toxoplasma)
74	F	30	0.2		>5.7	1.6	0.1	NA	Bell's palsy, nonspecific reaction with uninfected cells
75/A	F	20	1.5		0.8	0.7	0.9	98	Pregnancy wk 30
75/B			1.5		0.8	0.7	0.9	107	Unchanged borderline IgM without evolving IgG within 24 days
76	F	24	0.1	0.1	>7.0	0.2	0.2	NA	Pregnancy wk 20, nonspecific reaction with uninfected cells, multiple IgM reactivities (CMV, EBV[f], toxoplasma)
77/A	F	29		2.4	0.9	0.7	1.5	241	Pregnancy wk 8, anti-rubella virus IgG positive >2 yrs ago
77/B				2.3	0.9	0.6	1.6	221	Pregnancy wk 10
78	F	2	0.4		1.0	0.6	0.7	NA	CMV mononucleosis with heterotypic anti-EBV IgM
79/A	F	7		1.9	0.0	NA	0.1	>400	Recent EBV infection
79/B				2.3	0.8	0.2	0.1	383	15-day interval
80	F	17		3.3	0.7	0.3	0.2	62	Mononucleosis, multiple IgM reactivities (CMV, EBV, toxoplasma)
81/A	F	35		1.4	2.9	0.2	2.0	1,040	Pregnancy wk 11, multiple IgM reactivities (CMV, EBV, toxoplasma)
81/B				1.0	2.7	0.3	1.3	>400	Pregnancy wk 17, multiple IgM reactivities (CMV, EBV, toxoplasma)
82	F	18	0.9		0.6	0.7	0.8	>400	EBV mononucleosis, heterotypic IgM (toxoplasma)

[a] Groups: 1, proven recent rubella virus infection; 2, likely recent rubella virus infection; 3, recent rubella virus infection unlikely.
[b] M, male; F, female.
[c] Equivocal range.
[d] NA, not available for further testing because of insufficient amount of serum.
[e] CMV, cytomegalovirus.
[f] EBV, Epstein-Barr virus.

TABLE 2. Characteristics of four anti-rubella virus IgM tests[a]

Test	% Sensitivity	% Specificity	Predictive value of negative result (%)	Predictive value of positive result (%)
A	94.6	88.7	97.1	80.5
B	97.3	92.7	98.6	86.8
C	83.8	96.7	92.4	92.5
D	94.6	90.1	97.1	82.4

[a] Samples from groups 1 and 2 were considered true positives. Those from groups 3 and 4 were counted as true negatives. Equivocal results were counted as positives.

samples from the same patient were tested with the same technique.

Virus detection. Rubella virus was detected by inoculation of urine or throat swabs onto monolayers of Vero cells (ATCC cc181) and staining of the cells with an indirect fluorescent-antibody technique using a monoclonal antibody to rubella virus hemagglutinin (clone 379-810; Biosoft Clonatec, Paris, France) after 10 days of incubation at 35°C (19). Samples were considered negative if no cytoplasmic fluorescence was observed after two blind passages.

Statistical analysis. Box plots, two-tailed Wilcoxon signed-rank tests for differences between tests, and linear regression calculations were done by using the StatView II program, version 1.01, and in accordance with reference 11.

RESULTS

Clinical and laboratory information on 82 patients is presented in Table 1. Of 103 serum samples, 97 were available for testing with all four assays. The results of the IgM assays are presented as sample/cutoff ratios. In group 1, early acute-phase sera were frequently negative for IgG and IgM by all of the tests (Table 1). Test C failed to detect antibodies in three sera which were positive by the other assays (samples 8A, 10B, and 14B). Two of these (10B and 14B) were obtained about 1 month after the first signs of rubella. In group 2, antibodies were similarly not detected by test C in patients 20, 22, 50, 52, and 64. Whereas test C was less sensitive, tests A, B, and D proved to be comparable in groups 1 and 2 (Table 2 and Fig. 1a and b). When the first samples of seroconversion pairs in group 1 were excluded from the calculations because of the expected delayed appearance of anti-rubella virus IgM, the sensitivities were 100% (65 of 65) for tests A, B, and D and 89% (58 of 65) for test C.

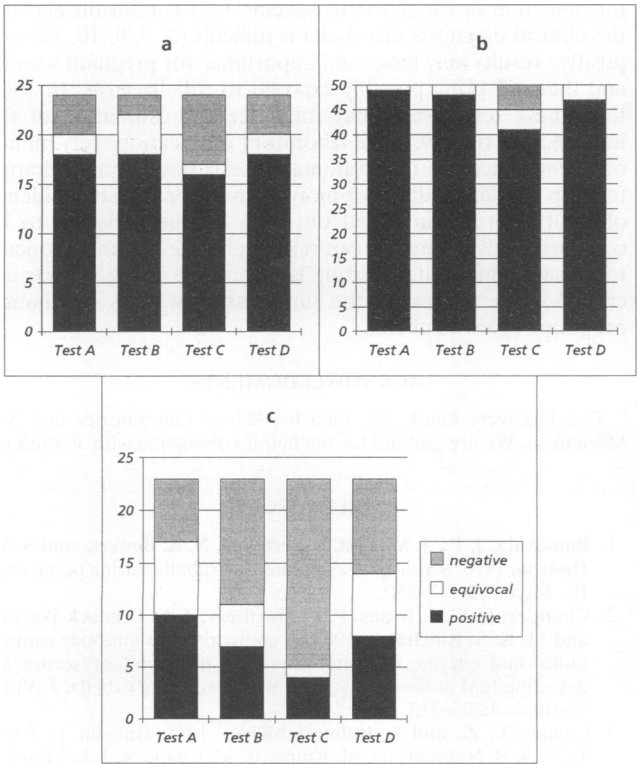

FIG. 1. Qualitative results of anti-rubella virus IgM measurement with four different assays. Panels: a, group 1; b, group 2; c, group 3.

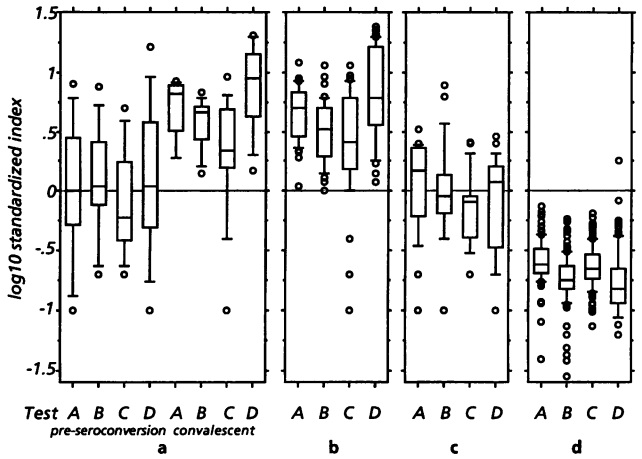

FIG. 2. Box plots of $\log_{10}$-transformed, standardized index values for four different anti-rubella virus IgM tests of four patient groups, showing the median and 50% of the values within the boxes. The line at $\log(x) = 0$ marks the cutoff value. Panels: a, group 1; b, group 2; c, group 3; d, group 4.

Group 3 contained samples which should be rubella virus IgM negative, thus providing information on the specificity of the tests in a panel of "difficult" negative controls (Table 1 and Fig. 1c). Each of the four tests had outlying results (Fig. 2c), most conspicuously the test B results for sera from patients 74 and 76; these sera contained antibodies to uninfected cells which did not, however, affect other assays lacking uninfected-cell controls. Although in this group, test C had three results in the range encountered in the positive panels of groups 1 and 2, this test had the highest specificity (Table 2). Test D was the only one to give a weak positive reaction (ratio, 1.4) with the negative controls of group 4 (Fig. 2d).

To attain comparable values for the four tests, the sample/cutoff ratios were converted to standardized index values by dividing the ratios by the lower limit of the equivocal range of each test (4). While the index values of tests A, B, and C levelled at around 8, test D frequently reached values above 10 without predilution. Thus, in the convalescent-phase samples of group 1 and in group 2, the values of test D were higher than those of the three other tests ($P < 0.01$) (Fig. 2a and b). The results obtained with the preseroconversion samples of group 1 did not differ significantly from each other. In group 3, all four tests were similar except for a significant difference between A and C ($P < 0.05$) (Fig. 2c). On the other hand, in group 4, test D had lower values than tests A and C ($P < 0.01$)

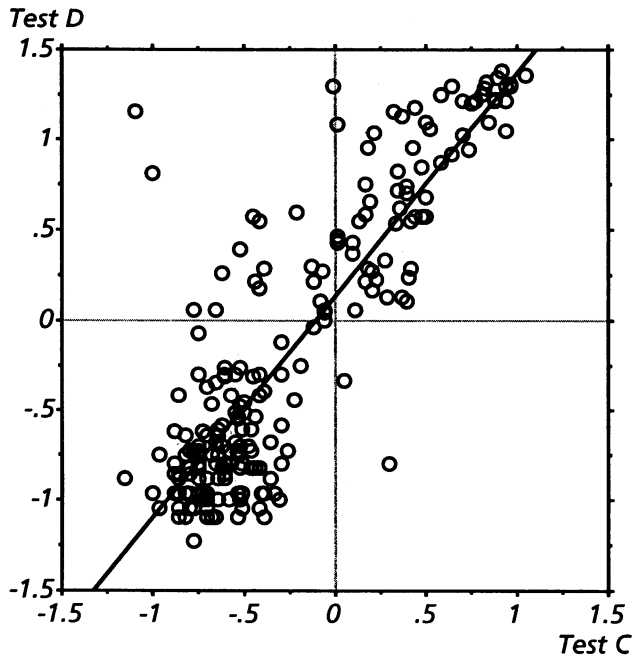

FIG. 3. Comparison of tests C and D for all samples. Log_{10}-transformed, standardized index values are plotted. The lines at log(x) = 0 indicate the cutoffs for tests C and D, respectively. The results are significantly correlated ($r = 0.85$; $r^2 = 0.73$; $P < 0.01$).

(Fig. 2d). Therefore, the range of measurements was largest with test D. A comparison of tests C and D is shown in Fig. 3.

The overall accuracies of the four tests were comparable, with percentages of correct results and 95% confidence intervals (7), respectively, of 91% and 87 to 95, 94% and 91 to 97, 92% and 89 to 96, and 92% and 88 to 95 for tests A, B, C, and D, respectively.

DISCUSSION

By using two different enzyme immunoassays for detection of rubella virus-specific IgM, we collected sera which were reactive by at least one of them in the course of the diagnostic evaluation of 82 patients. This provided panels of sera from patients with proven or likely recent infection or false-positive results. Consecutive IgM-negative sera in both tests were used as negative controls. The concomitant prospective use of two different assay formats mitigated the selection bias of samples used to evaluate two recently developed automated anti-rubella virus IgM tests in terms of their diagnostic performance. As cost considerations preclude the prospective use of multiple tests over periods long enough for collection of a sufficient number of reactive samples, this approach is a reasonable compromise. The sensitivity of the primary tests may, however, be slightly overestimated.

The overall accuracies of the four tests were comparable, with 91 to 94% correct results. All four tests missed some samples in the preseroconversion phase, when IgG was still negative or borderline. In addition, test C was clearly negative in two early convalescent-phase serum samples collected 4 to 5 weeks after seroconversion. The high anti-rubella virus IgG concentrations in some of the single serum samples from patients with likely recent infection (group 2) may indicate a late acute stage of the infection with declining IgM concentrations, which were not detected by this test in several cases. This is compatible with a short period of persistence for IgM, which is detectable by this test. Lowering the cutoff would avoid only a few of the false negatives and have an untoward effect on specificity. A consistently restricted persistence of detectable IgM may be helpful for estimation of the time of infection, especially in the context of prenatal diagnosis of rubella. However, further evaluation with appropriately spaced seroconversion panels should define more precisely the kinetics of the IgM response and the sensitivities of different tests. Until such data are available, we will use test C only in combination with a more sensitive test.

As expected, specificity was best for the test with the lowest sensitivity, but discrepant false-positive results were obtained with each of the tests. Therefore, to improve the specificity of this diagnostic procedure and the predictive value of positive results, the sequential or concomitant use of two or more different tests is preferable. In addition, the levels of specific IgM and IgG antibodies may help in the correct interpretation of IgM test results, especially if an evolving immune response can be demonstrated with follow-up samples. IgM tests which cover a wide range of semiquantitative values may facilitate estimation of the phase of the immune response in which a sample was obtained. Furthermore, consideration should be given to the possibility of heterotypic IgM responses due to other viral infections (particularly Epstein-Barr virus) or immune processes (18). The inclusion of appropriate control antigens in the test procedure may help in the detection or elimination of nonspecific reactivity.

The practical performance of the two automated tests posed no difficulties and required less hands-on time than conventional semiautomatic microplate ELISAs; appropriate maintenance of the machines was critical for test C. Tests A and B come in a microplate format which is amenable to various degrees of automation. The wide availability of reliable tests for detection of rubella virus-specific IgM is relevant because the clinical diagnosis of rubella is difficult (1, 3, 9, 10, 12) and positive results may have vital importance for pregnant women and their offspring possibly exposed to rubella virus. In addition, these test results may influence the estimation of the incidence of rubella, since laboratory notifications rely mainly on them. Since the new automated tests have overall characteristics similar to those of more conventional tests, epidemiological information based on them can be expected to be comparable over time, as can results obtained by their concurrent use by different reporting laboratories. These aspects are crucial in the context of the surveillance of mass vaccination programs against rubella.

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