EVALUATION OF CANDIDATE INTERNATIONAL REFERENCE METHODS FOR THE RUBELLA HEMAGGLUTINATION INHIBITION TEST: REPORT OF A COLLABORATIVE STUDY[1, 2]

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Various rubella hemagglutination-inhibition (HI) test procedures are used around the world to detect rubella antibodies in human sera. This article describes the work of a WHO-sponsored international research project designed to select one such procedure as an international reference method.

Introduction

Rubella virus infection in children or adults results in a self-limited, benign disease characterized by mild upper respiratory symptoms, an erythematous rash, and suboccipital lymphadenopathy. Complications of arthralgia and arthritis which may follow disappearance of the rash are most common in young women. Severe complications occur rarely. Infection of the fetus during the first trimester of pregnancy and, to a lesser degree, in the second and third trimesters may result in congenital rubella, a frequent cause of malformations and disabilities *(1, 2)*.

Of the various serologic methods for detecting or measuring rubella antibodies, the hemagglutination-inhibition (HI) test has had the widest use and has been evaluated the most frequently. When the test is performed properly, the indicated presence of antibodies correlates well with resistance to developing rubella. The test can be used to determine which individuals need vaccination and which pregnant women are at risk of giving birth to an infant with congenital rubella. However, the test does not distinguish between IgM and IgG antibody, and in order to determine whether a positive test reflects a recent infection, a test for IgM antibody is necessary.

The basic principles of all the modifications of the HI test are as originally described *(3)*. In addition, attempts have been made to standardize the HI procedure. In the United States, the Centers for Disease Control (CDC) and National Committee for Clinical Laboratory Standards (NCCLS) have published standardized HI procedures *(4, 5)*.

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[1]From WHO Document LAB/82.1 with the same title, World Health Organization, Geneva, 1982. Also appearing in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*.

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However, the need for an international rubella HI reference method was recognized by the World Health Organization and WHO advisory groups in 1976. A collaborative study was therefore designed in which nine participants tested a preselected panel[7] of coded human serum specimens with the HI techniques normally employed in their laboratories. Protocols for the nine techniques used were submitted by the participants and are on file at the WHO Virus Diseases Unit and Health Laboratory Technology Unit.[8] Basic differences between these nine techniques are shown in Table 1.

Objectives

The purpose of the study described here was to select a rubella HI test that could be designated as a WHO reference method. It was decided that the method selected should have the best score for the following measurements used in the study: (1) overall precision (overall reproducibility), (2) same-day precision (same-day reproducibility), (3) specificity, and (4) sensitivity. For purposes of the study these terms and the term "reactivity" were defined as follows:

1) The *overall precision* of the method is proportional to one standard deviation (S.D.) from the mean that includes all variations in the results except variations due to the specimen component.

2) The *same-day precision* of the method is proportional to one standard deviation (S.D.) that includes all variations in the results except variations due to the specimen component and factors that changed from one testing day to another.

3) The *overall reproducibility* of the method is indicated by the proportion of distinct specimens yielding a pair of titers, obtained on two different days, that differ by no more than a factor of two. This reproducibility reflects both the between-day and same-day components of variation.

4) The *same-day reproducibility* of the method is indicated by the proportion of distinct specimens yielding a pair of titers, obtained in the same daily titration run, that differ by no more than a factor of two. This reproducibility reflects only the same-day component of variation.

5) The *specificity* of the method is shown by the

[7] A set of sera with varying degrees of reactivity or different titers.

[8] Copies of these protocols are available upon request. A detailed description of the technique ultimately selected as the WHO reference method is contained in WHO mimeographed document LAB/82.1, which may be obtained from the World Health Organization, 1211 Geneva 27, Switzerland.

Table 1. The nine rubella HI techniques tested, showing the serum treatments, serum volumes, cell types, and starting dilutions employed.

Participant's code	Serum treatment	Serum volume treated	Cell type	Starting dilution
A	Kaolin (treatment x); heparin-$MnCl_2$ (treatment y)	0.2 ml	Pigeon	1:4
B	Heparin-$MnCl_2$	0.1 ml	Chick	1:8
C	Heparin-$MnCl_2$	0.2 ml	Chick	1:8
D	Kaolin	0.2 ml	Chick (freshly hatched)	1:4
E	Kaolin (treatment x); heparin-$MnCl_2$ (treatment y)	0.1	Pigeon	1:10
F	Kaolin	0.2 ml	Goose	1:8
G	Kaolin	0.2 ml	Chick (day old-unfed)	1:10
H	Kaolin	0.2 ml	Chick	1:10
J	Kaolin	0.025 ml	Chick (day old)	1:8

proportion of negative specimens correctly read as negative by the method (correct negative rate).

6) The *sensitivity* of the method is shown by the proportion of positive specimens correctly read as positive by the method (correct positive rate).

7) The *reactivity* of the method is indicated by the dilution factor of the antigen. That is, a method requiring an antigen dilution of 1:8 (i.e., a dilution factor of eight) is less reactive than a method requiring an antigen dilution of 1:32 (i.e., a dilution factor of 32).

Study Planning and Design

Phase One

Each of the nine participants used the rubella HI microtitration procedure normally employed in his or her laboratory to test 80 to 100 (40 to 50 duplicate) randomly coded human serum specimens. The testing was to be done on three different days, at least one week apart, with different sets of randomly coded serum samples being supplied by the U.S. Centers for Disease Control for each day of testing. In addition, each participant received three control sera—one negatively-reacting serum (<8), one low-titered positive-reacting serum (16-32), and one high-titered positive-reacting serum (≥ 128). All the participants were asked to include these sera along with their normal control sera in each run.

A portion of the same lot of CDC antigen was provided to each participant. The 40 to 50 pairs of blind-coded human serum specimens included the following: (1) eight to 10 different pairs of sera yielding negative (<8) results at the CDC when tested for rubella by HI and radioimmunoassay (RIA), and (2) 30 to 40 different positive sera, including eight to 10 low-titered sera (HI 8-16) and two to four high-titered sera (≥ 256), with the remaining sera having HI titers ranging from 32 to 128.

The negative and low-titered sera were tested by both the CDC standardized HI technique and the RIA technique of Dr. Olli Meurman at the Department of Virology, University of Turku, Turku, Finland.

Phase Two

The two procedures that ranked first and second for reproducibility, specificity, and sensitivity in phase one (those of laboratories B and J) were used by all the participants in phase two. The specimens were the same as those used in phase one, but in addition six new randomly coded sets (three for each test procedure) were prepared and used in phase two. The initial testing was done on three separate days, each at least one week apart, using the laboratory J procedure (serum adsorption with kaolin). After this work was completed, the testing was repeated in the same manner using the laboratory B procedure (serum adsorption with heparin-manganous chloride).

Except for the fact that items 5 and 9 below were provided to only one participant because of procurement problems, the following materials were provided to all the participants by the sources indicated:

1) Rubella antigen; CDC.

2) Rubella reference control sera; CDC. The set of reference reagents included one negative (<8), one low-titered (16-32), and one high-titered (>128) human serum.

3) Kaolin, lot 771130; Fisher Scientific Company.

4) Sodium heparin, 5000 USP heparin units per ml, lot 787FX; Upjohn Company.

5) Gelatin, lot J 1 DIOK; Baltimore Biological Laboratories.

6) Bovine albumin powder, fraction V, control number S 12506, stock number 2293-01; Reheis Chemical Company.

7) HEPES,[9] lot 0697, ICN.

8) $MnCl_2.4H_20$, lot 775323; Fisher Scientific Company.

9) Cyanmethemoglobin, Hycel Reagents No. 116, lot 6349A 1, standard No. 117, lot 6819A 1.

Phase Three

In phase three the nine participants performed the procedure B (heparin-manganous

[9] HEPES = N-2-hydroxyethylpiparazine-N-2-ethanesulphonic acid.

chloride serum treatment) HI test that was used in phase two on three sets of 49 duplicate coded serum specimens at least one week apart. After completing this procedure, the participants followed the same testing schedule with the procedure J (kaolin serum treatment) HI technique that was used in phase two. During this phase the only materials provided to the participants were the six sets of coded serum specimens and the three CDC reference control sera.

Serum Panels

The blind-coded human serum specimens used to make each serum panel were obtained from single donors and were not pooled. The donors were adult males and females between the ages of 19 and 55. Sodium azide (0.1 per cent) was added to each serum as a preservative, and the sera were stored at 4°C.

Sterility testing was done on the first and last vials dispensed by placing 0.5 ml of the serum on two blood agar plates and into one tube of thioglycollate broth. One plate was incubated at 23°C, and the second plate and the broth culture were incubated at 37°C, all for 72 hours. All of the results were negative. In addition, all of the specimens were tested by the RIA technique for HB_sAg in the CDC Hepatitis Laboratory at Phoenix, Arizona. The only specimen found to be positive was withdrawn from the study. Preliminary testing of all the serum specimens used in the three phases of the study was performed in two CDC laboratories that used the CDC Standardized HI Method in which heparin-$MnCl_2$ is used to treat the serum *(4)*.

The serum specimens used only in phases two and three of the study were also tested in two CDC laboratories by applying procedures J and B.

After the expected titer values were established, all of the negative (<8) and low positive (16-32) specimens were further tested with the RIA method by Dr. Meurman. All the specimens were dispensed at 0.5 ml per 2 ml sterile glass bottle, stoppered with white rubber stoppers, and capped with aluminum tear-away caps.

The random coding of the sera and decoding of the results were done by the Statistical Office of the CDC. The bottles were stored at 4°C after the coded labels were affixed to the sets of vials during each phase of the study.

The same serum panel, but coded differently, was used in phases one and two. A second panel was used in phase three.

Results

Phase One

Table 2 shows a summary of the results obtained in phase one. The procedures were then ranked according to their overall precision (Table 3) and same-day precision (Table 4), and the F-test was used to decide if observed differences between the various precision and reproducibility values for the procedures were significant at the 5 per cent level. As indicated in the tables, procedures F, H, C, J, and B had similar overall precision (and thus overall reproducibility) values. The same was true of procedures D and E (treatment x), and also of procedures G, A, and E (treatment y). However, G, A, and E (treatment y) had significantly less overall precision than procedures F, H, C, J, and B.

Procedures F, H, E, B, J, C, and G had similar same-day precision (and thus same-day reproducibility) values, and the same was true of procedures H, E, B, J, C, G, and D. However, procedure D had significantly less same-day precision than procedure F.

The results shown in Tables 2, 3, and 4 were provided to the nine participants, and each of them was asked to rank the coded procedures. In each case, the procedure ranked in the best position was assigned a numerical grade of 1, and so on, in order of position. If a participant stated that several procedures were equivalent, those procedures were assigned the same rank. When the rank numbers were

Table 2. A summary of phase one results from the WHO rubella HI study.

Procedure code	Estimated inverse measure of overall precision (1 S.D.)	Estimated overall reproducibility (%)	Estimated inverse measure of same-day precision (1 S.D.)	Estimated same-day reproducibility (%)	Estimated specificity (%)[b]	Estimated sensitivity (%)[c]	Dilution factor of antigen dilution used			
							Serum set 1	Serum set 2	Serum set 3	Mean
A (treatment x)	1.07[a]	68	.85	79	100	94 (33)	ND[d]	8	8	8
A (treatment y)	1.11[a]	66	.96	73	100	92 (36)	ND	8	8	8
B	.52	96	.49	97	100	100 (35)	100	64	64	76
C	.51	97	.51	97	100	97 (36)	40	40	40	40
D	.75	85	.57	94	100	100 (36)	80	88	80	83
E (treatment x)	.87	78	.46	98	91	91 (30)	ND	ND	12	12
E (treatment y)	1.08	68	.50	97	82	91 (31)	8	8	16	11
F	.48	97	.39	99	100	91 (34)	8	8	8	8
G	1.05	69	.53	96	100	93 (27)[e]	32	16	32	27
H	.50[f]	97	.44	98	100	100 (35)	256	ND	ND	256
J	.51	97	.50	97	100	100 (36)	32	32	32	32

[a]Set one was not tested. Thus, the day-to-day variation in this precision is from only two days of testing rather than from the three days called for by the protocol.

[b]Radioimmunoassay testing by an independent laboratory showed that there were 11 negative specimens.

[c]The number of positive specimens is shown in parentheses.

[d]ND = Not Done.

[e]Despite provision of over 27 sera initially yielding positive results, laboratory G reported only 27 positive sera.

[f]Serum sets 1, 2, and 3 were not tested on three different days, each separated by at least one week, as specified by the protocol.

added, the procedure with the lowest numerical total was taken to be the best, and so on, up to the highest numerical total.

As Table 5 shows, the top four rubella HI procedures selected by the participants in this manner were B, H, J, and C. When the procedures were ranked according to statistical analyses, as shown in Table 6, the same four procedures occupied the top four positions. Two of the four techniques used kaolin serum treatment, and the other two used heparin-$MnCl_2$. On the basis of these initial findings, the participants selected procedures B and J to be used in phases two and three.

Table 3. The test procedures ranked by order of overall precision and overall reproducibility. The letters "NS" in the right-hand column indicate that the differences between the bracketed results were not statistically significant.

Procedure code	Estimated inverse measure of overall precision (1 S.D.)	Estimated overall reproducibility (%)	
F	.48	97	NS
H	.50	97	
C	.51	97	
J	.51	97	
B	.52	96	
D	.75	85	NS
E (treatment x)	.87	78	
G	1.05	69	NS
A (treatment x)	1.07	68	
E (treatment y)	1.08	68	
A (treatment y)	1.11	66	

Table 4. The test procedures ranked by order of same-day precision and same-day reproducibility. The letters "NS" in the right-hand column indicate that the differences between the bracketed results were not statistically significant.

Procedure code	Estimated inverse measure of same-day precision (1 S.D)	Estimated same-day reproducibility (%)	
F	.39	99	NS
H	.44	98	
E (treatment x)	.46	98	
B	.49	97	
E (treatment y)	.50	97	
J	.50	97	
C	.51	97	
G	.53	96	
D	.57	94	
A (treatment x)	.85	79	NS
A (treatment y)	.96	73	

Table 5. Scores assigned to the various test procedures by participants on the basis of data presented in Tables 2, 3, and 4 and the resulting ranks of the five top-ranked procedures.

Procedure code	Scores assigned by participating laboratories of indicated countries										
	Japan	USA (California)	France	USA (CDC)	Czechoslovakia	Malaysia	Hong Kong	England	Australia	Total score	Ranking
B	1	2	2	2	2	4	1	1	3	18	1
H	1	1	1	3	1	5	5[a]	1	1	19	2
J	1	4	4	1	3	3	2	1	2	21	3
C	1	3	5	4	4	2	3	1	4	27	4
F	1	6	3	7	5	1	4	1	5	33	5
D		5	6	5	6	7			6		
E (x)		10	7		7	6			7		
G		7	8	6	8	8			9		
A (x)		8	9		10	10			8		
A (y)		9	10		11	11			10		
E (y)		11	11		9	9			11		

[a]Procedure H was not ranked by Hong Kong; a score of 5 was assumed to be appropriate.

Table 6. Numerical scores and rankings assigned to the various test procedures on the basis of statistical analyses of the data presented in Tables 2, 3, and 4.

Procedure code	Overall precision	Same-day precision	Specificity	Sensitivity	Antigen dilution	Total score	Ranking
A (x)	9	10	5	6	10	40	9
A (y)	11	11	5	8	10	45	11
B	5	4	5	2.5	3	19.5	2
C	3.5	7	5	5	4	24.5	4.5
D	6	9	5	2.5	2	24.5	4.5
E (x)	7	3	10	10	7	37	8
E (y)	10	5.5	11	10	8	44.5	10
F	1	1	5	10	10	27	6
G	8	8	5	7	6	34	7
H	2	2	5	2.5	1	12.5	1[a]
J	3.5	5.5	5	2.5	5	21.5	3

[a]Protocol for phase one not followed.

Phase Two

As previously noted, during phase two each of the nine laboratories tested six blind-coded sets of the same sera, three by procedure B (heparin-$MnCl_2$) and three by procedure J (kaolin). The six serum sets had different random codes. All testing with procedure B was completed before testing with procedure J was begun. The purpose was to select the better method with respect to precision (reproducibility), specificity, sensitivity, and antigen dilution.

Table 7 shows the precision and reproducibility results each laboratory obtained with the two procedures. These results, as assessed by the Wilcoxon Matched-Pairs Signed-Ranks Test, showed that the two procedures did not differ significantly ($p>0.05$) with regard to their overall or same-day precision and reproducibility values.

Table 8 shows the sensitivity and specificity results each laboratory obtained with the two procedures. These results, likewise assessed by the Wilcoxon Matched-Pairs Signed-Ranks Test, indicated that procedure B (hepa-

Table 7. Precision and reproducibility results obtained by the nine participating laboratories using HI test procedures B and J (phase two).

	Overall precision and reproducibility				Same-day precision and reproducibility			
	Procedure B (heparin-$MnCl_2$)		Procedure J (kaolin)		Procedure B (heparin-$MnCl_2$)		Procedure J (kaolin)	
Laboratory code	Estimated precision (1 S.D.)	Estimated reproducibility (%)	Estimated precision (1 S.D.)	Estimated reproducibility (%)	Estimated precision (1 S.D.)	Estimated reproducibility (%)	Estimated precision (1 S.D.)	Estimated reproducibility (%)
C	.44	98	.37	100	.43	99	.35	100
F	.46	98	.48	97	.39	99	.45	98
D	.52	96	.58	93	.46	98	.54	95
J	.56	94	.49	97	.52	96	.41	99
E	.62	91	.39	99	.56	94	.38	100
H	.63	91	.98	72	.59	93	.98	72
G	.65	90	.66	89	.60	92	.64	90
B	.69	88	.72	86	.61	92	.64	90
A	.69	88	–	–	.59	93	–	–

Table 8. Specificity and sensitivity results obtained by the nine participating laboratories using HI test procedures B and J (phase two).

Laboratory code	Specificity (%)[a]		Sensitivity (%)[b]	
	Procedure B (heparin-$MnCl_2$)	Procedure J (kaolin)	Procedure B (heparin-$MnCl_2$)	Procedure J (kaolin)
C	100	98	100	100
F	100	98	100	99
D	100	36	100	100
J	100	98	100	100
E	100	98	100	100
H	77	77	100	98
G	100	89	99	95
B	–[c]		–[c]	
A	–[c]		–[c]	

[a]Based on the existence of 11 negative specimens, as verified by an independent laboratory.

[b]Based on the existence of 37 positive specimens.

[c]Could not be estimated because "16" appeared in data (reciprocal of serum dilution too low to be of value in the statistical analysis).

Table 9. Dilution factors of the antigen used by the nine participating laboratories during phase two testing of procedures B and J.

Laboratory code	Procedure B (heparin-$MnCl_2$)	Procedure J (kaolin)
C	40	64
F	32	32
D	64, 64, 128	64
J	128	256
E	64	16
H	64	64
G	32	32
B	256	256
A	128	–

rin-$MnCl_2$) was significantly more specific than procedure B using kaolin ($p < 0.05$). No significant difference in the two procedures' sensitivity was detected.

Table 9 shows the dilution factors of the antigen dilutions used by each laboratory. No significant difference was observed between the two procedures' overall reactivities as measured in terms of these dilution factors.

Phase Three

The selection of a reference rubella HI procedure should not depend upon all laboratories having the same reagents. The purpose of phase three of the study was to determine which procedure, B or J, produced the most consistent results among and within laboratories when each laboratory provided its own reagents.

The study design used a collection of 12 negative and 37 positive serum specimens. Each of the nine participating laboratories tested six blinded-coded sets of the same sera—three by procedure B (one set per day for three spaced days) and three by procedure J (one set per day for three spaced days). The six sets of sera had different random codes, and all of the testing with procedure B was completed and reported before testing was begun with procedure J.

The precision and reproducibility results for the nine laboratories obtained with each procedure are shown in Table 10. The Wilcoxon Matched-Pairs Signed-Ranks Test indicated there was no significant difference between the results regarding either overall or same-day precision and reproducibility.

Table 11 shows the sensitivity and specificity results obtained by the nine laboratories with each procedure. The observed differences between the two procedures were not statistically significant.

Table 10. Precision and reproducibility results obtained using procedures B and J (phase three).

Laboratory code	Overall precision and reproducibility				Same-day precision and reproducibility			
	Procedure B		Procedure J		Procedure B		Procedure J	
	Estimated precision (1 S.D.)	Estimated reproducibility (%)	Estimated precision (1 S.D.)	Estimated reproducibility (%)	Estimated precision (1 S.D.)	Estimated reproducibility (%)	Estimated precision (1 S.D.)	Estimated reproducibility (%)
A	.71	87	.44	98	.67	89	.38	100
B	.38	100	.33	100	.38	100	.32	100
C	.34	100	.57	94	.34	100	.50	97
D	.70	87	.52	96	.69	88	.51	97
E	.62	91	.63	91	.59	93	.36	100
F	.91	75	1.05	69	.90	76	1.04	70
G	.63	91	.56	94	.54	95	.44	98
H	.50	97	.69	88	.49	97	.58	93
J	.54	95	.48	97	.53	95	.47	98

Table 11. Specificity and sensitivity results obtained using procedures B and J (phase three).

Laboratory code	Specificity (%)[a]		Sensitivity (%)[b]	
	Procedure B	Procedure J	Procedure B	Procedure J
A	98.6	98.6	99.5	100.0
B	100.0	100.0	100.0	100.0
C	100.0	98.6	100.0	100.0
D	90.3	100.0	100.0	100.0
E	98.6	100.0	99.5	100.0
F	41.8	45.8	99.5	100.0
G	98.6	100.0	96.3	100.0
H	–[c]	98.6	–[c]	100.0
J	98.6	98.6	100.0	99.5

[a]Based on the existence of 12 negative specimens, as verified by an independent laboratory.

[b]Based on the existence of 37 positive specimens.

[c]Could not be estimated because "<16" appeared in data (reciprocal of serum dilution too low to be of value in the statistical analysis).

Table 12. Dilution factors of the antigen dilutions used during phase three testing of procedures B and J.

Laboratory code	Dilution factors of antigen dilutions used with:	
	Procedure B	Procedure J
A	64	64
B	32	32
C	64	64
D	16	16
E	16, 25, 32	32, 16, 32
F	32	64, 32, 45
G	8	64, 64, 128
H	80	80
J	32, 32, 64	64

Table 12 shows the dilution factors of the antigen dilutions used by each laboratory. These factors differed in only four of the nine laboratories; however, these differences all showed the kaolin procedure (procedure J) to be more reactive than the heparin-$MnCl_2$ procedure (procedure B).

Conclusions

Four measures of test performance were used in this study to identify a recommended reference HI procedure, these being sensitivi-

ty, specificity, reactivity, and reproducibility.

The test sensitivity observed in phases two and three is shown in Tables 8 and 11. For procedure B (the heparin-$MnCl_2$ procedure) these sensitivity values were quite high. That is, among the 15 sets of results the lowest sensitivity percentage registered was 96.3 per cent, and 10 of the 15 sets of results indicated 100 per cent sensitivity. This shows that procedure B yielded very few false negative readings. Likewise, the lowest degree of sensitivity attained with procedure J (using kaolin) was 95.0 per cent, and 12 of the 16 sets of results attained 100 per cent sensitivity, leading to the same conclusion.

Tables 8 and 11 also show the observed specificity of the two procedures. Twelve of the 15 sets of results shown indicated a very high specificity for procedure B (in excess of 98 per cent). However, the two lowest procedure B specificity scores were 77 and 42 per cent, which shows that certain laboratories had a problem with false positive readings. The same was true of procedure H. That is, eight of the 16 sets of results shown indicated a specificity of 98 per cent or more, but the lowest specificity scores were 77, 46, and 36 per cent, which again indicated a problem with false positive readings.

Tables 7 and 10 indicate the overall reproducibility of the phase two and three results. Regarding procedure B, even though seven of the 18 sets of results indicated 95 per cent reproducibility or better, five others indicated less than 90 per cent reproducibility. This shows that the results obtained with procedure B varied substantially in certain laboratories. Likewise, eight of the 17 sets of procedure J results indicated 95 per cent reproducibility or better; but five indicated less than 90 per cent reproducibility, again revealing substantial variation within certain laboratories. It should be noted, however, that two of the laboratories reporting low procedure J reproducibility were different from those reporting low reproducibility with procedure B.

Overall, the observed differences between the two procedures were not significant; but since it was necessary to designate only one reference procedure, a letter was sent from WHO asking the participants in this study to select one of these two procedures to be recommended as the WHO rubella reference HI procedure. Five of the nine participants chose procedure B (heparin-$MnCl_2$), which has therefore been designated as the recommended procedure.

SUMMARY

Various rubella hemagglutination-inhibition (HI) test procedures are used around the world to detect rubella antibodies in human sera—in order to determine which individuals need vaccination and which pregnant women are at risk of giving birth to an infant with congenital rubella. In view of the need to have a single international rubella HI reference method, the World Health Organization sponsored a collaborative study involving the testing of various procedures by nine laboratories in eight countries.

The initial tests singled out four procedures as being superior in terms of their sensitivity, specificity, reactivity, and reproducibility, and the chief participants at the nine laboratories selected two of these for further testing. This subsequent testing indicated that both methods were very sensitive, yielding few false negative results; that both showed a high degree of specificity (few false positive results), though some of the participating laboratories did have problems with false positive readings; and that high levels of precision and reproducibility were attained in some laboratories but not in others.

Overall, the observed differences between the two procedures were not significant. The nine study participants were therefore asked to select which of the two methods they preferred, and they accordingly selected the one employing heparin-manganous chloride to treat the sera. A detailed description of this reference method is available from the World Health Organization upon request.

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JAMAICA LAUNCHES PUBLIC EDUCATION PROGRAM ON IMMUNIZATION

The Ministry of Health of Jamaica has launched an extensive public education program designed to focus attention on a national plan for immunizing children up to 12 years old. Due to be completed by the end of 1983, the plan represents further development of the ministry's existing Expanded Program of Immunization (EPI); its goal is to achieve a satisfactory immunization status for 80 per cent of the children in this age group against tetanus, polio, diphtheria, whooping cough, and measles.

The education program, which was preceded by a recently concluded survey of knowledge, attitudes, and practices in relation to immunization, is being funded by UNICEF and PAHO with grants of J$50,000 and J$ 40,000, respectively. On the local scene, a well-known musician has allowed his hit song "Treat the Youth Right" to be used as a jingle in the immunization program, and the Graduate Theatre Company of the Jamaica School of Drama has used its talents to highlight the importance of immunization.

Source: Jamaica Information Service as reported in *CAJANUS* 16(2):99-100, 1983.