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Multicenter Evaluation of Five Commercial Rubella Virus Immunoglobulin G Kits Which Report in International Units per Milliliter

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In a multicenter study, the consistency of international units expressed by five commercially available rubella virus immunoglobulin G kits was evaluated. The linearity and within-run and between-run precision were determined for each kit. All kits demonstrated good linearity and had within-run and between-run precision coefficients of variation ranging from 5.1 to 21.7% and from 9.5 to 51.0%, respectively. To compare the international units expressed, the results from 40 samples tested in duplicate were compared with the results of a reference enzyme immunoassay calibrated with World Health Organization international standard serum and a hemagglutination inhibition test. The results of the kits were plotted against those of the reference tests, and linear regression analysis was applied. The Pearson correlation coefficient ranged from 0.64 to 0.75 when the commercial kit results were compared with those of the reference enzyme immunoassay, indicating only a moderate degree of correlation. Therefore, the international units expressed by the commercial kits are insufficiently consistent to be of practical use in diagnostic clinical microbiology.

Before the late 1970s, the serological investigation of rubella virus infection in humans was achieved by hemagglutination inhibition (HAI). This quantitative method was relatively well standardized worldwide (19, 20). Several new techniques to detect rubella virus-specific immunoglobulin G (IgG) then became available; these techniques include single radial diffusion, indirect immunofluorescence, latex agglutination (13, 18), and enzyme immunoassays (EIAs) (4, 9, 19, 20, 23, 25). In many countries the EIA has become the most popular method of testing for rubella immunity because of technical ease, automation, sensitivity, and specificity and the low cost of reagents. However, most commercial kits reported results in absorbances or indices that did not relate to results reported by other kits. This produced problems in (i) the interpretation of results by clinicians who use more than one pathology laboratory, (ii) the comparison of results from different commercial kits (13), and (iii) the introduction of standardized reporting of results.

Recently many manufacturers have used a World Health Organization international standard for anti-Rubella serum (World Health Organization, Copenhagen, Denmark) to calibrate the kits and therefore report in international units (IU) per milliliter. A multicenter study was established to determine the degree of correlation of these units between different manufacturers. An in-house EIA, which was calibrated by using the World Health Organization international standard for anti-Rubella virus serum (second international standard preparation), and an HAI test were used as reference methods. Linearity and within-run and between-run precision were calculated for each kit by using methods commonly used in clinical chemistry laboratories.

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MATERIALS AND METHODS

Specimens. All but three serum samples were from patients requiring routine rubella virus serology to determine immune status; the other three specimens were obtained from blood packs to ensure large volumes. The specimens were aliquoted into appropriate volumes and stored at 4°C. Specimens with visible bacterial contamination were discarded and replaced with fresh aliquots. All sera were randomized and coded. A detailed protocol was distributed to each institution.

HAI. The HAI test was performed as previously described (14). Briefly, 0.2 ml of serum was pretreated with 0.6 ml of 25% kaolin in borate saline (Flow Laboratories, Irvine, Scotland) for 20 min at room temperature. The tubes were centrifuged at 800 × *g* at 4°C for 20 min. Then 50 μl of 50% pigeon erythrocytes in Alsevers solution (Innes Scientific Co., Sydney, Australia) was added to each tube, and the tubes were incubated overnight at 4°C. The tubes were then centrifuged at 800 × *g* at 4°C for 10 min. All dilutions were performed in duplicate.

Pretreated serum diluted 1:4 was then double-diluted in 0.2% bovine albumin fraction V (Commonwealth Serum Laboratories, Melbourne, Australia) in dextrose-gelatin-Veronal buffer (pH 7.0 to 7.2). Then 25 μl of rubella hemagglutinating antigen (Behring Diagnostics, Marburg, Germany), pretitrated to give four agglutinating doses, was added. Plates were incubated at 37°C for 40 min and then at 4°C for 2 to 3 min. Then 25 μl of 0.16% pigeon erythrocytes was added to the test and control wells. The plates were shaken on a plate shaker and incubated at 4°C for 90 min. The results were read after incubation at room temperature for a further 5 min. The endpoint of the titration was taken as the last well showing 50% or less agglutination. Patient

serum, pigeon erythrocytes, and agglutinating antigen were controlled with each run.

Reference EIA. An in-house EIA was standardized by using the World Health Organization international standard for anti-rubella virus serum (second international standard preparation). This method was described previously (21) and was modified as follows: (i) by substituting poly-L-lysine (Sigma Chemical Co., St. Louis, Mo.) for albumin as a coating solution, (ii) by substitutions *o*-phenylenediamine (Sigma) for 4 amino-antipyrine as the substrate, and (iii) by freeze-drying prepared plates instead of storing them at −70°C.

Briefly, sera were tested at dilutions of 1/50 and 1/1,000. Diluted serum (125 μl) was added to the appropriate well, and the wells were incubated at room temperature overnight. The plates were washed in phosphate-buffered saline, and 100 μl of rabbit anti-human IgG conjugated with horseradish peroxidase (Dako Immunoglobulins, Copenhagen, Denmark) was added. After incubation at 37°C for 30 min, the plates were washed and 100 μl of *o*-phenylenediamine was added. After incubation for 5 min at room temperature, the reaction was stopped by adding 100 μl of 1 N H_2SO_4. The results were read in a Microlyser-Auto reader (MR-600; Dynatech Laboratories Inc., Alexandria, Va.) at a wavelength of 490 nm.

The *E* value for each result was calculated, where *E* is the optical density which would be obtained with a 1-cm light path. Since the actual light path used was 0.33 cm, the *E* value was calculated by multiplying the optical density readings obtained from the test and controls by three. When the *E* value of the 1/50 serum dilution was less than 0.200, the results were converted to IU per milliliter by using a linear regression line. With this equation we were able to calculate results that were ≤20 IU/ml. When the *E* value of the 1/50 dilution was greater than 0.200, a second curve with the results of the 1/1,000 dilution was used to calculate the IU per milliliter. To construct this calibration curve, the World Health Organization standard serum (1,000 IU/ml) was diluted from 1/80 to 1/2,560 and tested. The absorbance was plotted against the known unit value, and the resulting curve can be described by the following equation as determined by a computer program (Fig P, version 5; Biosoft, Ferguson, Mo.): $y = -665.22x/(384.08 + x) + -316.40x/(1.43 + x) + 261.02x$, where x is the *E* value of the 1/1,000 dilution and y is in IU per milliliter.

Commercial kits. All commercial enzyme immunoassay systems were performed as specified by the manufacturer. To limit technical error, laboratories that routinely use the methods tested were chosen to participate. All reagents and equipment were supplied by the manufacturer unless otherwise stated. The runs were only accepted if all of the control guidelines of the manufacturers were fulfilled.

(i) IMx. The fully automated IMx kit (Abbott Laboratories, North Chicago, Ill.) was previously described (1, 5, 16). Briefly, microparticles that were sensitized with the Gilchrist strain of rubella virus were reacted with patient serum. A glass fiber matrix separated the particles from the reaction mixture because of the high affinity of the matrix for the protein-coated microparticles. Goat anti-human IgG conjugated with alkaline phosphatase was added and was bound to the captured patient anti-rubella virus antibodies. The substrate methylumbelliferyl phosphate was used as an indicator of this reaction. The results were converted to IU per milliliter by using the manufacturer's software with a stored, six-point calibration curve. The calibration curve was determined by analyzing calibrators standardized with the World Health Organization international standard for anti-rubella virus serum.

(ii) Sorin. The E.T.I.-Rubek-G kit (Sorin Biomedical, Saluggia, Italy) is a standard microtiter EIA. All samples were diluted 1/500 with phosphate buffer. Standards calibrated against the World Health Organization international standard for anti-rubella virus serum (second standard preparation) and negative controls were diluted 1/10 with phosphate buffer. Then 100-μl samples of prediluted standards, negative controls, and patient sera were dispensed into their respective wells, which were coated with the Putnam strain of rubella virus. After 1 h at 37°C, the plates were washed in phosphate-buffered saline with Tween 20. Then 100 μl of goat anti-human IgG conjugated with horseradish peroxidase was added, and the plates were incubated for 1 h at 37°C. After a wash, 100 μl of tetramethylbenzidine diluted 1/50 with 0.005% hydrogen peroxide in citrate buffer was added. After the reaction was incubated for 30 min at room temperature, 200 μl of 1 N sulfuric acid was added to stop the reaction. The resulting color change was read by using a Sorin 311 Turbo spectrophotometer at a wavelength of 490 nm. The absorbance was converted to IU per milliliter by using a four-point calibration curve generated from the manufacturer's software.

(iii) Rubazyme quantitation panel. The Rubazyme quantitation panel (Abbott), a bead EIA, was described previously (13). All standards and specimens were diluted 1/300 in specimen dilution buffer. Then 200-μl samples of diluted standards were pipetted into duplicate reaction wells, and 200-μl samples of the specimens were pipetted into single reaction wells. A rubella virus-coated bead was added to each well, and the trays were incubated for 60 min at 37°C. After the wells were washed with distilled water, 200 μl of goat anti-human IgG conjugated with horseradish peroxidase was added. After 120 min of incubation at 37°C, the beads were washed and transferred to EIA reaction tubes. Then 300 μl of *o*-phenylenediamine substrate was added; after 30 min of incubation at room temperature, the reaction was stopped with 2.5 ml of 1 N sulfuric acid. The results were read with a Quantum II dual wave spectrophotometer (Abbott). All results were converted to IU per milliliter by using the Quantum II software and five standards that had been calibrated with the World Health Organization international standard rubella virus serum (second standard preparation).

(iv) Clark. Serum samples and controls were diluted 1/20 in test tubes before analysis. A 100-μl sample of diluted specimen was added to the appropriate microtiter well coated with viral antigen. After 20 min of incubation at room temperature, the plates were washed with Tris-buffered saline and 100 μl of anti-human IgG conjugated with horseradish peroxidase was added. After 20 min of incubation at room temperature, the plates were washed and 100 μl of freshly prepared *o*-phenylenediamine was added. The reaction was stopped with 100 μl of 1 N sulfuric acid after 10 min of incubation at room temperature. The plates were read on a MR 5000 spectrophotometer (Dynatech Laboratories Inc., Alexandria, Va.), at 490 nm.

Each lot number of Clark rubella virus IgG (Clark Laboratories, Jamestown, N.Y.) has a numerical factor designated on the packaging. The average absorbance of the low positive calibrator is multiplied by this factor to obtain the cutoff value. Each test absorbance is divided by the cutoff value to determine a ratio value. This ratio is converted to IU per milliliter by using a linear calibration curve described by $y = 0.853x - 0.97$, where x is the ratio value. This calibration curve was calculated at Clark Laboratories by

TABLE 1. Linearity study: calculated results (CR) verses test results (TR)

Serum dilution	EIA kit results (IU/ml)											
	Rubazyme		IMx		Clark		Sorin		Organon		Reference EIA	
	CR	TR	CR	TR	CR	TR	CR	TR	CR	TR	CR	TR
Neat	240	267	168.3	149.4	111	134	84.9	80.6	94.7	106	131	118.3
		216		183.5		94		79.1		86		127.1
		238		172.1		106		94.9		92		148.6
1/5	48	19	33.7	23.6	22	16	17	9.3	18.9	7	26	18.7
		20		24.2		16		8.9		10		18.7
		16		25.8		16		8.8		7		20
1/10	24	3	16.8	10.4	11	9	8.5	4.2	9.4	7	13	8
		2		9.9		9		4.4		7		10.1
		1		10.5		10		3.7		7		10.5
1/20	12	0	8.4	5.0	5.6	6	4.2	2.4	4.6	6	6.6	5.5
		0		4.8		6		2.7		6		6.1
		0		5.1		6		2.6		6		5.5
1/50	4.8	0	3.4	0	2.2	0	1.7	0.4	2	5	2.6	2.1
		0		0		0		0.4		5		2.4
		0		0		0		0.3		5		1.7

plotting the mean ratio value of eight dilutions against the natural logarithim of the value (IU per milliliter) of the World Health Organization international standard rubella virus serum for several different lots of Clark rubella virus IgG.

(v) Rubenostika IgG. The Rubenostika IgG kit (Organon Teknika, N.V. Turnhout, Belgium) required serum samples to be diluted 1/25 in serum diluent before testing. Then 100 μl of diluted sample, controls, and calibrators were added to virus-coated microdilution tray wells. After incubation for 60 min at 37°C, the wells were washed in phosphate buffer and 100 μl of sheep anti-human IgG conjugated with horseradish peroxidase was added. After incubation for 60 min at 37°C, the plates were washed and 100 μl of freshly prepared tetramethylbenzidine dissolved in dimethyl sulfoxide was added. After incubation for 30 min at room temperature, 100 μl of 2-mol/liter sulfuric acid was added. The results were read with a Titertek M.C. reader (Flow Laboratories, Irvine, Scotland) at 450 nm. The results were converted to IU per milliliter from a calibration curve constructed by plotting the absorbance of the two calibrators supplied with the kit against the result. The potencies of the calibrators were determined at Organon Teknika by using World Health Organization international standard for anti-rubella virus serum.

Evaluation protocol. The evaluation protocol was based on previously published methods for kit evaluations (2, 3, 7, 8, 10–12, 15, 24, 26).

(i) Linearity study. A human serum with a high level of anti-rubella virus IgG was diluted 1/5, 1/10, 1/20, and 1/50. Each dilution and the neat sample were analyzed in triplicate in the same run. Predilutions, when required, were also performed in triplicate. The mean result of the neat serum for each kit was divided by 5, 10, 20, and 50 to obtain the expected values of the dilutions. The expected results were plotted against the test results, and the slope, *y* intercept, and correlation coefficient were calculated.

(ii) Within run precision. Three serum samples with negative, equivocal, and high levels of anti-rubella virus IgG as determined by HAI were labeled B1, B2, and B3, respectively, and tested 20 times in the same run. The mean, mode, standard deviation, and coefficient of variation (CV) were calculated for each serum sample.

(iii) Between-run precision. The same sera used to determine the within-run precision were tested 20 times in consecutive runs. The means, modes, standard deviations, and CV were calculated and compared with those of the within-run precision results.

(iv) Comparison test. Forty sera with anti-rubella virus IgG levels ranging from undetectable to high were split into duplicate samples, randomized, and tested blindly. The Pearson coefficient of correlation of the results for each kit were calculated and compared. The results of each kit were plotted against those obtained with the reference EIA. The slope, *y* intercept, and standard error of the estimate were determined for the line of best fit as determined by linear regression analysis. The Deming correction of the linear regression slope was used to correct for the imprecision of the reference EIA (17, 22). The results from each kit were also plotted against the HAI titers.

RESULTS

Linearity. The mean value of the neat serum ranged from 84.9 to 240 IU/ml (Table 1). Anti-rubella virus IgG was not detected with the Rubazyme, IMx, or Clark system at the 1/50 dilution or with the Rubazyme system at the 1/20 dilution. When the expected results were plotted against the test results, the slope (a measure of standard error) ranged from 1.00 to 1.12 and the *y* intercept (a measure of constant error) ranged from −1.84 to −29.97 (Table 2). The coefficient of correlation (*r*) was close to 1.00 for all kits.

Within-run precision. The results of the within-run precision study are summarized in Table 3. The CV results for all sera (B1, B2, and B3) with all kits ranged from 5.08 to

TABLE 2. Least-squares regression analysis of linearity study

Test kit	Slope	*y* intercept	*r*
Rubazyme	1.12	−29.97	0.99
IMx	1.04	−6.93	0.99
Clark	1.02	−2.49	0.98
Sorin	1.04	−3.90	0.99
Organon	1.00	−1.84	0.98
Reference EIA	1.02	−2.9	0.99

TABLE 3. Results of the within-run precision test

Serum sample and statistical analysis	EIA kit results					
	Rubazyme	IMx	Clark	Sorin	Organon	Reference EIA
Serum B1						
Mean (IU/ml)	4.10	5.54	7.95	ND[a]	6.10	ND
Mode (IU/ml)	4	5.2	8		6	
SD (IU/ml)	0.79	0.60	0.69		0.31	
CV	19.3	10.8	8.7		5.08	
No. of samples outside ± 2 SD	0	1	0		0	
Serum B2						
Mean (IU/ml)	35.95	51.01	10.45	10.44	14.80	19.54
Mode (IU/ml)	38	58.4	11	11.1	15	20.2
SD (IU/ml)	2.28	4.48	0.76	0.99	2.14	2.36
CV	6.3	8.8	7.3	9.5	14.5	12.1
No. of samples outside ± 2 SD	1	0	0	0	1	1
Serum B3						
Mean (IU/ml)	236.65	197.85	57.85	90.26	55.85	144.31
Mode (IU/ml)	250	189.8	53	>100	42	155.3
SD (IU/ml)	26.89	13.56	8.52	10.9	12.1	14.8
CV	11.4	6.8	14.7	12.1	21.7	10.2
No. of samples outside ± 2 SD	0	1	0	0	1	1

[a] ND, no rubella virus-specific IgG detected.

21.67%. When the F test ($P = 0.05$) was applied, the Rubazyme, IMx, and Clark tests had standard deviations for serum B1 that were statistically larger than those of the Organon system. The reference EIA and the Sorin system failed to detect anti-rubella virus IgG in this sample. IMx had a standard deviation that was significantly greater than those of the reference EIA and the Rubazyme and Organon systems, all of which had standard deviations greater than those of the Sorin and Clark systems for serum B2. The Rubazyme assay had a standard deviation for serum B3 that was significantly greater than those of the other kits.

All kits had fewer than 2 of 20 replicates outside ±2 standard deviations. Six of 20 replicates of B3 were reported as greater than 100 IU/ml when tested with the Sorin kit. This would have decreased the CV, because all "greater-than" results were recorded as the highest detectable value.

There was poor correlation among the means of the results obtained by each kit for sera B1, B2, and B3. The differences between the means were much greater for B3, which contained the highest amount of rubella virus-specific IgG.

Between-run precision. The result of the between-run precision studies are summarized in Table 4. The Rubazyme, Clark, and Sorin kits had only 7, 12, and 10 replicates tested, respectively, because of kit failures and inadequate reagents. The Sorin kit and the reference EIA failed to detect anti-rubella virus IgG in serum B1. The Sorin kit reported 6 of 10 replicates of serum B3 as greater than 100 IU/ml. The CVs for the between-run precision tests were uniformly higher than those of the within-run results; however, the mean results varied marginally.

When an F test ($P = 0.05$) was used, the standard deviations from the Rubazyme and Clark kits for serum B1 were significantly greater than those of the other tests. There was no significant difference between the standard deviations for serum B2 of the IMx and Clark kits. There was, however, a significant difference between the standard deviations of the IMx kit and those of the other kits tested. The Clark and Organon kits had standard deviations for serum B3 that were significantly greater than those of the other kits.

Comparison test. The results of the 40 paired sera were analyzed by using the Pearson correlation coefficient (r) (Table 5). The r values ranged from 0.63 for the IMx kit versus the Clark kit to 0.93 for the two Abbott kits and the IMx and Rubazyme kits. The Sorin, Organon, and Rubazyme kits had the best correlation with the reference EIA.

The standard deviation of the difference between the paired sera, which was not reported as a greater-than value, was calculated for each kit. The results were as follows (in international units per milliliter): Rubazyme, 171.1; reference EIA, 124.20; IMx, 87.6; Organon, 59.1; Clark, 54.1; and Sorin, 19.7.

The results for each commercial kit were plotted against the reference EIA results (Fig. 1) and the HAI titers (Fig. 2). The slope, y intercept, correlation coefficient, and standard error of estimation for the least-squares line are summarized in Table 6. Because the reference EIA had a relatively high degree of imprecision, the Deming correction of linear regression was calculated (Table 6).

The results from the IMx kit had a slope of 1.00 when plotted against the reference EIA, indicating a low level of proportional error. The results from the Clark and Sorin kits, however, had slopes of 0.19 and 0.23, respectively, indicating high degrees of proportional error. The Organon kit results had the lowest y intercept. Deviation from a y intercept of 0.00 indicates the degree of constant error relative to the reference method. The results with the IMx kit showed the highest y intercept (82.4); however, after correction with the Deming method, the IMx y intercept value was −2.0. The standard error of estimation provides an indicator of the amount of scatter around the line of best fit. The low $Sx.y$ values with the Clark and Sorin kits are shown in Fig. 1. The two Abbott Diagnostic tests and the IMx and Rubazyme kits had a markedly larger degree of scatter around the linear regression line.

The results of each test were plotted against the HAI titers (Fig. 2). All the sera with an HAI titer of 1/256 or greater were found to contain >100 IU/ml with all of the kits except the Clark kit. The IMx kit had four serum replicates with

TABLE 4. Results of the between-run precision test

Serum sample and statistical analysis	EIA kit results					
	Rubazyme (7)[a]	IMx (20)	Clark (12)	Sorin (10)	Organon (20)	Reference EIA (20)
Serum B1						
Mean (IU/ml)	6.0	5.49	8.83	ND[b]	5.15	ND
Mode (IU/ml)	6.0	5.9	9.0		5.0	
SD (IU/ml)	2.58	0.93	1.75		0.67	
CV	43.0	16.9	19.8		13.0	
No. of samples outside ± 2 SD	0	1	1		0	
Serum B2						
Mean (IU/ml)	37.0	55.31	14.75	11.34	13.95	21.36
Mode (IU/ml)	38.0	61.9	18.0	9.9	15.0	21.7
SD (IU/ml)	3.51	6.67	4.14	2.48	1.57	2.60
CV	9.5	12.1	28.1	21.9	11.3	12.2
No. of samples outside ± 2 SD	0	0	0	0	2	0
Serum B3						
Mean (IU/ml)	205.29	183.64	100.25	92.65	114.65	161.72
Mode (IU/ml)	191.0	175	65	>100[c]	105	115
SD (IU/ml)	25.12	25.48	51.16	12.90	34.3	30.75
CV	12.2	13.9	51.0	13.9	29.91	19.0
No. of samples outside ± 2 SD	0	1	0	1	1	0

[a] Numbers within parentheses indicate the numbers of replicates.
[b] ND, no rubella virus-specific IgG detected.
[c] Six of 10 replicate results were >100 IU/ml.

HAI titers of 1/64 and one replicate with a 1/32 titer reported as >500 IU/ml. Rubazyme had four samples with titers of 1/64 reported as >500 IU/ml.

Six sera had HAI titers of <1:8; the IMx and Rubazyme kits both reported two of these six sera as containing >40 IU/ml. All other commercial kits reported six of the six sera as containing <10 IU/ml. The reference EIA reported four of the six sera as containing <10 IU/ml and two sera as containing between 10 and 20 IU/ml. The mean values and the ranges of results of sera with HAI titers of 1/16 and 1/32, when tested with the EIA kits, are shown in Table 7. Sera with HAI titers of 1/64 or greater had EIA results expressed as greater-than values.

TABLE 5. Comparison test: Pearson correlation coefficients

Commercial kit	Pearson coefficient of correlation with the following kits:					
	Rubazyme	IMx	Clark	Sorin	Organon	Reference EIA
Rubazyme	1	0.93	0.65	0.82	0.83	0.75
IMx	0.93	1	0.63	0.79	0.73	0.64
Clark	0.65	0.63	1	0.79	0.63	0.65
Sorin	0.82	0.79	0.79	1	0.74	0.74
Organon	0.83	0.73	0.63	0.74	1	0.75
Reference EIA	0.75	0.64	0.65	0.74	0.75	1

TABLE 6. Least square regression analysis comparing commercial kit results with reference EIA results

Test kit	Slope	y intercept	SE of estimate	Deming correction of linear regression	
				Slope	y intercept
Rubazyme	1.76	56.1	172.7	2.35	−171.9
IMx	1.00	82.4	134.7	1.56	−2.0
Clark	0.19	22.9	24.0	0.29	−3.7
Sorin	0.23	34.2	23.1	0.31	−16.7
Organon	0.56	6.0	55.2	0.75	−57.1

DISCUSSION

Recently, many commercial rubella virus antibody kits have used the World Health Organization international standard for anti-rubella virus serum to calibrate their tests, thereby enabling the results to be expressed in IU per milliliter. Our study compared the IU values from these kits to determine the degree of correlation. We also examined the linearity and precision of these commercial kits.

All commercial kits and the reference EIA demonstrated good linearity throughout the range of rubella virus levels commonly found in human sera. There was a low level of standard error as determined by the slope of the linear regression curve when the test results were plotted against the expected results.

TABLE 7. Range and mean value of EIA kits for sera with HAI titers of 1/16 and 1/32

Test kit	EIA results (IU/ml) at HAI titer of:			
	1/16		1/32	
	Mean	Range	Mean	Range
Rubazyme	58.4	44.0–130.0	135.9	33.0–199.0
IMx	81.5	29.0–190.6	143.9	23.0–497.3
Clark	19.6	14.0–24.0	32.7	16.0–52.0
Sorin	26.9	18.3–43.2	42.6	16.4–78.0
Organon	20.9	15.0–27.0	27.4	14.0–67.0
Reference EIA	66.9	13.0–290.0	73.8	43.0–300.0

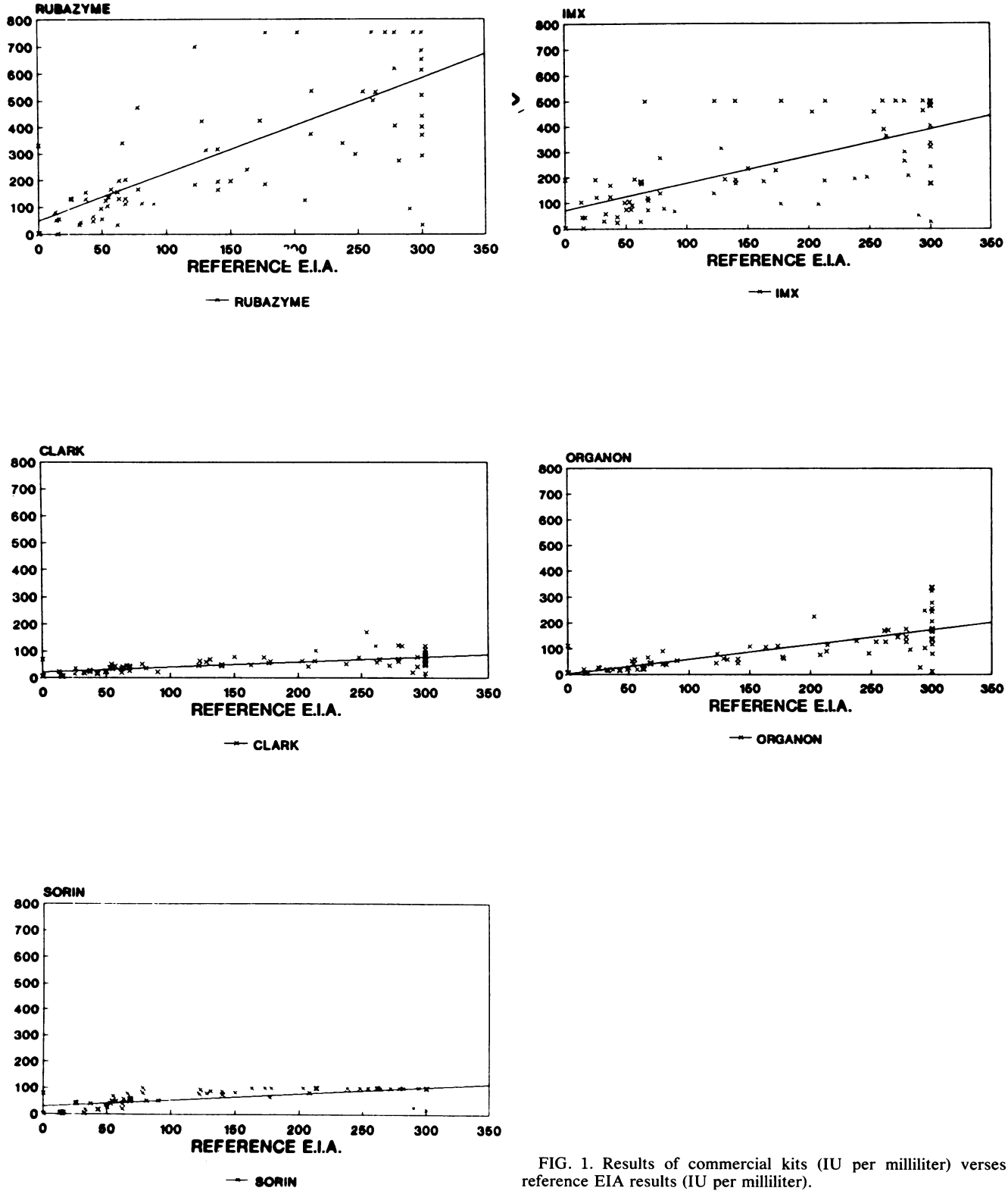

FIG. 1. Results of commercial kits (IU per milliliter) verses reference EIA results (IU per milliliter).

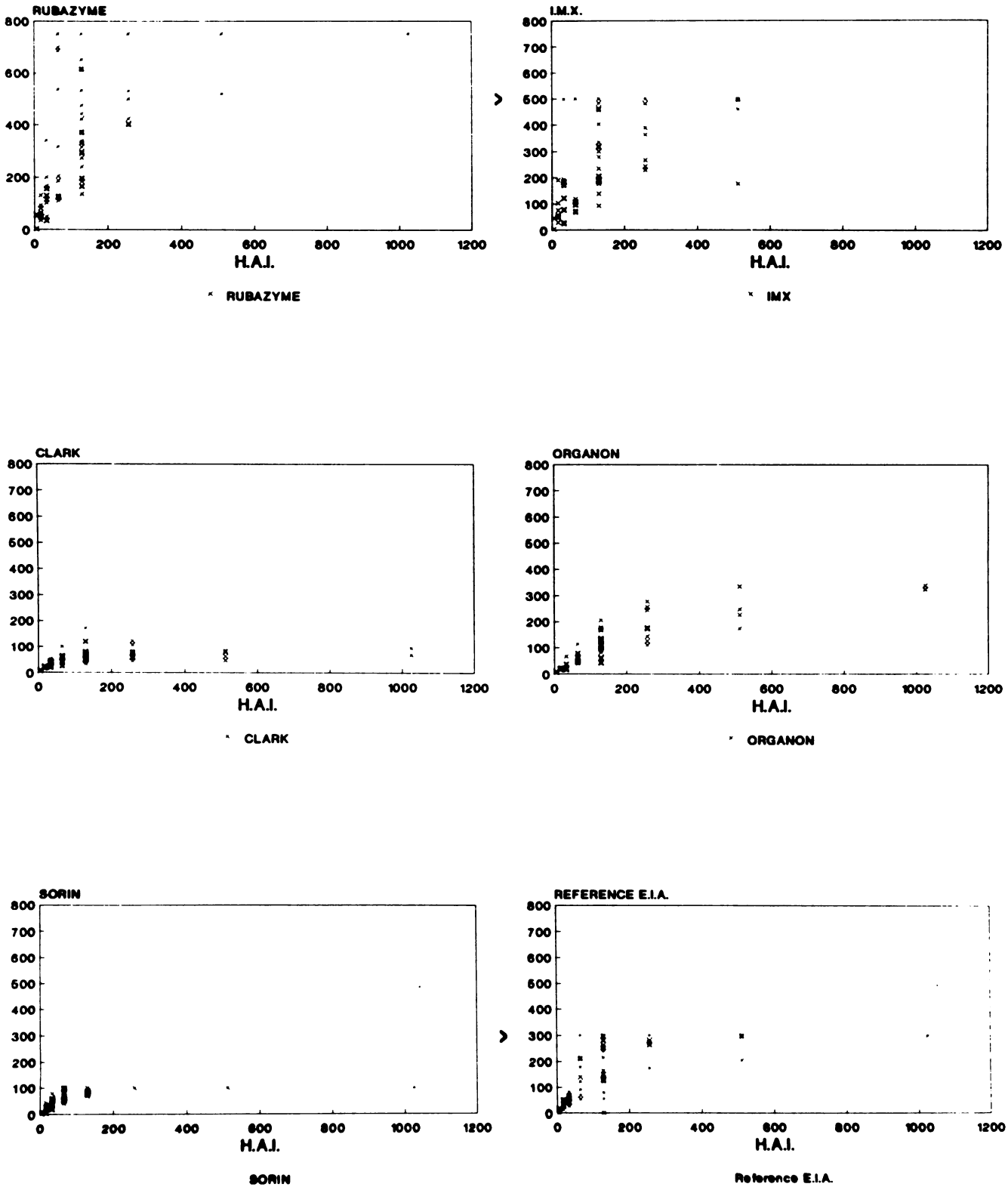

FIG. 2. Results of EIA kits (IU per milliliter) verses HAI results (1/titer).

The within-run precision test demonstrated a CV for the rubella virus kits ranging from about 5 to 20%. Acute rubella virus infection can be diagnosed by the detection of a significant increase in rubella virus-specific IgG antibodies when acute and convalescent sera are tested in parallel. The within-run CV must be small enough to avoid an increase due to experimental error, which could contribute to a significant rise in titer. Only the Organon and Rubazyme kits specify the percent increase required between the results of the acute and convalescent sera. With the Organon kit a 2-fold increase, and with the Rubazyme kit a 1.65-fold increase, is indicative of an acute infection. Therefore, even a 20% CV may be an acceptable error. All kits encourage the detection of rubella virus-specific IgM to confirm the diagnosis of acute rubella virus infection.

Rubella virus serology is also used to determine the immune status of an individual. A level of >10 IU/ml in serum or an HAI titer greater than 1/10 have been used as indicative of previous exposure to rubella virus (6). Therefore it is preferable to have a high degree of precision when testing sera with low levels of rubella virus IgG antibody. The Organon kit demonstrated the lowest CV for serum B1, which contained rubella virus antibody at a concentration close to 10 IU/ml.

The CV obtained in the between-run precision test was uniformly greater than those obtained in the within-run precision test. This is due to the introduction of more experimental variables, such as changes in temperature, incubation time, and substrate and conjugate concentrations. However, even a CV of 50% would not change the clinical interpretation.

There was only a moderate correlation between the kits when the results of the 40 split samples were compared. This lack of correlation can be attributed to many variables, including differences in rubella virus antigen, substrate and conjugate affinities, incubation periods, temperatures, predilutions of the specimens, and technical errors. Many of these variables may be overcome by using an international standard serum to standardize the kits. However, the methods of calibrating the kits also vary in the number and frequency of calibrators used and in the methods of calculating the result. Therefore, the units expressed with the commercial kits do not correlate sufficiently well to enable laboratories to change kits without changing their reference ranges.

In the comparison test with six sera with a HAI titer of less than 1/8, both the Rubazyme and IMx kits indicated that two samples contained >40 IU/ml. Therefore, these sera would have been interpreted as having a protective level of rubella virus-specific IgG, whereas the reference HAI method indicated a lack of immunity. These EIA kits may have a lower detection limit and may detect low levels of rubella virus IgG; however, these levels may not confer immunity to the virus (9).

All of the commercial kits tested demonstrated good linearity and comparable precision. Many factors examined when selecting a method, such as cost, capital equipment requirements, supply, shelf life, and technical ease, were not addressed in this study. Although there was a moderate degree of correlation of the IU, it is insufficient to be of practical use. It is suggested that the results of rubella virus antibody testing be confined to a statement concerning immunity rather than a numerical value. Furthermore, when selecting a commercial kit, a study to ascertain the immune-nonimmune cut-off value should be undertaken by studying the results in IU per milliliter obtained with sera with HAI titers of 1/8 to 1/16.

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