Asian Pacific Journal of Allergy and Immunology (1992) 10 : 47-54

Re-Evaluation of ELISA and Latex Agglutination Test for Rheumatoid Factor Detection in the Diagnosis of Rheumatoid Arthritis

Napatawn Banchuin[1], Kritsana Janyapoon[2], Suphannee Sarntivijai[4] and Lek Parivisutt[3]

SUMMARY **An indirect ELISA for the determination of each isotype (IgM, IgG, IgA, IgD, IgE) of rheumatoid factors (RF) was performed with sera obtained from 77 patients with either classical or definite rheumatoid arthritis (RA) and 319 controls, using rabbit IgG as the antigen. The results were compared with those of a commercial latex agglutination test, using denatured human gamma globulin as the antigen for rheumatoid factor determination. At the cut-off level at which positive results were found in less than 5% of normal controls, ELISA for IgM RF determination had sensitivity, specificity, efficiency, positive predictive value and negative predictive value of 46.75%, 98.12%, 88.13%, 85.71%, 88.41%, while those for IgA RF were 46.75%, 93.42%, 84.34%, 63.16%, 87.91% and for IgG RF were 59.74%, 92.16%, 85.86%, 64.78%, 90.46%, respectively. These indices by latex agglutination test were 83.11%, 93.73%, 91.67%, 76.19% and 95.83%, respectively. IgD RF titre $\geq$ 1:5 was detected in 19/77 RA patients and 4/200 normal controls while IgE RF titre $\geq$ 1:5 was detected only in 7/77 RA patients. Thus, ELISA did not appear to have any advantage over latex agglutination test for diagnosis of RA.**

One of the criteria used for the diagnosis of rheumatoid arthritis (RA), as revised by the American Rheumatism Association in 1987, is the presence of rheumatoid factor (RF) in sera of the patients.[1] One of the tests usually performed in routine laboratories for the detection of RF is latex agglutination, which is claimed to detect mainly the IgM class of RF.[2] This test has been reported to be positive in approximately 60-70% of the patients with rheumatoid arthritis.[3,4] However, the existence of other immunoglobulin classes of RF have been reported by many investigators.[2,3,5-8] One of the methods used for the detection of various RF classes is enzyme immunoassay.[2,5,6,9-13] It has been reported that the percentages of positive RF in RA patients increase with the use of enzyme-linked immunosorbent assay (ELISA) for the detection, in comparison with the use of latex agglutination. The RF found by ELISA in the proportion of RA patients who had negative latex agglutination tests were IgM, IgG and IgE.[2] However, the value of ELISA has usually been assessed only in terms of its sensitivity in the detection of RF in RA patients and the correlation of each RF isotype with clinical activity of the disease. The specificity of the assay has rarely been considered. Moreover, the control group used in some previous reports did not include a wide range of other diseases that could have positive RF.

In this study, we compared the results of ELISA for the determination of each isotype of RF (IgM, IgG, IgA, IgD, IgE) with those of a commercial latex agglutination test in the differential diagnosis of rheumatoid arthritis from other rheumatic diseases as well as from other conditions and normal controls.

MATERIALS AND METHODS

Sera

Seventy-seven sera obtained from patients with classical or defi-

From the Department of Microbiology[1], Preventive Medicine[2], and Department of Medicine[3], Faculty of Medicine Siriraj Hospital, Department of Clinical Microbiology[4], Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand.

Correspondence : Dr. Napatawn Banchuin Division of Immunology, Department of Microbiology, Faculty of Medicine Siriraj Hospital Mahidol University, Bangkok 10700, Thailand.

nite rheumatoid arthritis (RA), based on the criteria established by American Rheumatism Association[1] (ARA), were used in this study. Thirty-three sera were obtained from patients with other rheumatic diseases which consisted of 14 patients with systemic lupus erythematosus (SLE), 2 with Reiter's syndrome, 3 with reactive arthritis, 1 with psoriasis, 3 with polymyositis, 1 with osteoarthritis, 7 with ankylosing spondylitis and 2 with scleroderma.

Sera were also obtained from 30 patients with cancer, 56 patients with various viral infections including rubella, dengue, measles, mumps, cytomegalovirus, and 200 normal healthy individuals.

Twenty-seven sera of RA patients with positive RF by latex agglutination test were pooled and used as the reference positive control serum.

All the sera were aliquot and kept at -20°C until used.

ELISA for the determination of rheumatoid factors

The indirect ELISA was used for the determination of each RF isotype. The solid phase used in ELISA was Microelisa Immulon 1® plate (Dynatech, Verginia, USA). Normal rabbit IgG (NRIgG) (Sigma Chemical Company, St. Louis, MO, USA) was used as the antigen for coating the plate at the concentration of 5 μg/ml in 0.01 M phosphate buffered saline, pH 7.1, (PBS) containing 0.02% sodium azide. The plate was incubated at 37°C for 3 hours. After 3 time washes with 0.85% sodium chloride solution containing 0.05% Tween 20 (NSST), the plate was dried. Serial 2-fold diluted serum samples in PBS containing 0.05% Tween 20 (PBST) were then incubated in the NRIgG coated plate, at 4°C overnight. After the washing step, the conjugate was added. For the determination of IgM RF and IgA RF, the conjugate used was F(ab)$_2$ fragment of goat Ig specific to human IgM or IgA, which was labelled with alkaline phosphatase (Sigma Chemical Company). For the determination of IgG RF, the conjugate used was the F(ab)$_2$ fragment of rabbit Ig specific to human IgG which was labelled with peroxidase (Dakopatts, Glostrup, Denmark). The conjugate used for the determination of IgD RF and IgE RF was goat Ig specific to human IgD or IgE, which was labelled with alkaline phosphatase (Sigma Chemical Company). The diluent used for the conjugates was PBST containing 0.5% BSA (PBST-BSA). The incubation of this step was carried out at 37°C for 3 hours. Then, the excess conjugate was washed out and the substrate was added. The substrate for alkaline phosphatase was 1 mg/ml of p-nitrophenyl phosphate (Sigma Chemical Company) in 0.05 M carbonate buffer containing 0.005 M $MgCl_2$. The substrate solution for peroxidase was 0.0125% H_2O_2 in 0.1 M citric acid phosphate buffer, pH 5.0, containing 0.67 mg/ml of 1,2-phenylene diamine (OPD) (Dakopatt). The reaction between enzyme and substrate was allowed to take place at 37°C for 1 hour in the case of IgMRF, IgARF, IgDRF and IgERF and 45 minutes for IgGRF determination. Then the reaction was stopped by the addition of 25 μl 3 N NaOH for alkaline phosphatase reaction and 50 μl of 2 M H_2SO_4 for peroxidase reaction.

In each plate, a direct conjugate control (DCC) and reference positive control serum were also included. The PBST was used instead of a serum sample for the well of DCC. Thus, the DCC provided a control for the extent of non-specific conjugate binding to the plate. Each sample was assayed in duplicate.

To evaluate the results, the mean absorbance value of the DCC was always subtracted from the mean absorbance value of each tested sample. Such a corrected absorbance value of tested samples was then used for the calculation of results. The level of RF present in each specimen was determined by reading the dilution of that specimen which gave the corrected absorbance value 0.2 unit above base line level of semilogarithmic curve, obtained by plotting the corrected absorbance value against corresponding reciprocal dilutions of that specimen. Such a dilution was then expressed as the titre of RF in each specimen tested.

Latex agglutination test for the determination of rheumatoid factors

The determination of rheumatoid factors by latex agglutination test was carried out with a rheumatoid arthritis test kit (Rheumafac®) (ICL Scientific, CA, USA). The Rheumafac latex reagent provided in the kit, for the detection of RF, was stabilized polystyrene latex particles coated with human albumin which was chemically bonded with denatured human gamma globulin. The RF reference and serum samples were diluted in serial 2 fold dilution with the glycine buffer provided, in test tubes. The starting dilution of the former was 1:5 while that of the latter was 1:20. Each dilution of the sample was tested for agglutination reaction with Rheuma-fac latex reagent, on a black glass slide. The agglutination reaction was read 2 minutes after the sample was mixed with the reagent, using a direct light source. The titre of the RF in the specimen was determined as the highest dilution that still gave a positive reaction in the test.

Statistical analysis

Kruskal Wallis test was used for comparison of more than 2 groups of data and Bonfessoni pairwise comparison was used for comparison of each pair of data when the Kruskal Wallis test was significant. These statistical analyses were computed by using the Statview 512+ software (Brain Power, Inc, Cababasas, CA, USA) run on a McIntosh microcomputer.

The sensitivity, specificity, efficiency, positive predictive value and negative predictive value were calculated as follows:

$$\text{sensitivity} = [a/(a+c)] \times 100$$
$$\text{specificity} = [d/(b+d)] \times 100$$
$$\text{efficiency} = [(a+d)/(a+b+c+d)] \times 100$$
$$\text{positive predictive value} = [a/(a+b)] \times 100$$
$$\text{negative predictive value} = [d/(c+d)] \times 100$$

where a, b, c, d are the numbers of true positive samples, false positive samples, false negative samples and true negative samples, respectively.

RESULTS

ELISA for the determination of rheumatoid factors

The results of ELISA for the determination of IgMRF, IgARF, IgGRF, IgDRF and IgERF in sera of patients with rheumatoid arthritis, other rheumatic diseases, cancer, viral infections and normal healthy individuals are shown in Figs 1, 2, 3 and Table 1 respectively.

The geometrical mean of IgM RF titre in the group of patients with RA was significantly higher than those of the other groups (Kruskal Wallis test, $p < 0.001$, followed by Bonfessoni pairwise comparison, $\alpha = 0.05$). Such a difference was also found in the case of IgA RF and IgG RF. However, detectable levels of IgDRF ($\geqslant 1:5$) and IgERF ($\geqslant 1:5$) were found in very small number of sera tested. (Table 1)

The sensitivity and specificity of ELISA for IgMRF, IgARF and IgGRF determination, for the diagnosis of rheumatoid arthritis, were then calculated with the use of various cut-off levels and the results are shown in Table 2. It can be seen that different degrees of sensitivity and specificity were obtained with the use of different cut-off levels.

When the criteria set up by the American Rheumatism Association (that the test for RF which can be included as one criterion for the diagnosis of RA must be positive in less than 5% of normal individuals) was used for the selection of the optimal cut-off level, this level of IgMRF, IgARF and IgGRF was found to be 1:2,500, 1:150, and 1:5,500, respectively. Thus, with such a cut-off level, ELISA for IgMRF determination had sensitivity, specificity, efficiency, positive predictive value

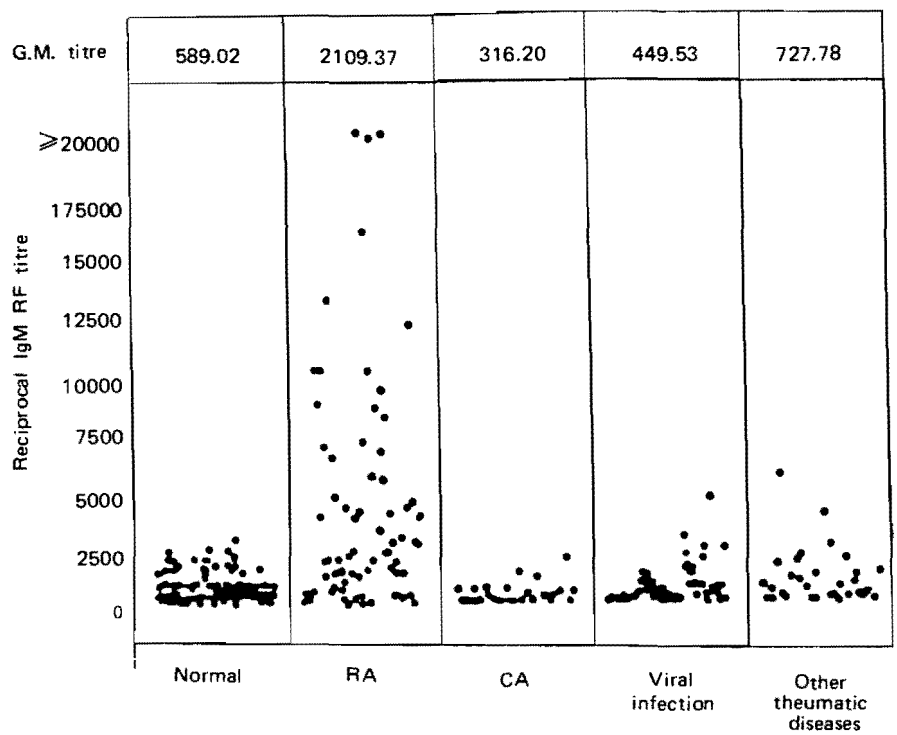

Fig. 1 Titres of IgM rheumatoid factor (RF) in sera obtained from normal healthy individuals, patients with rheumatoid arthritis (RA), cancer patients (CA), patients with viral infections and patients with other rheumatic diseases, as determined by ELISA.

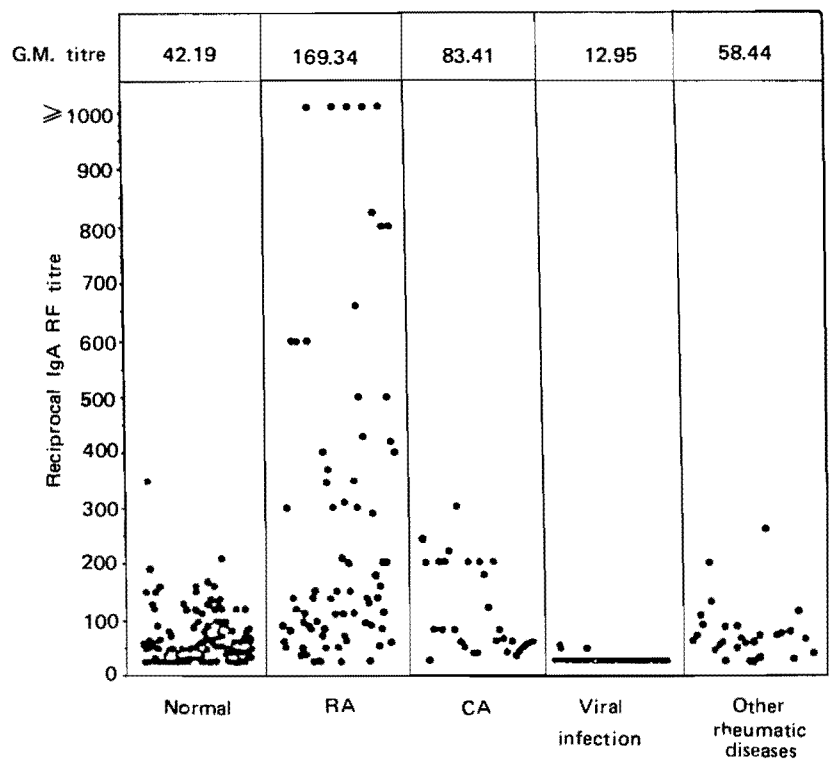

Fig. 2 Titres of IgA rheumatoid factor (RF) in sera obtained from normal healthy individuals, patients with rheumatoid arthritis (RA), cancer patients (CA), patients with viral infections and patients with other rheumatic diseases, as determined by ELISA.

Table 1. Number of subject with serum IgD and IgE rheumatoid factors titres ⩾ 1:5, as determined by ELISA

Subjects	Numbers		
	Total	IgD RF titre ⩾ 1:5	IgE RF titre ⩾ 1:5
Normal individuals	200	4	0
Patients			
– rheumatoid arthritis	77	19	7
– other rheumatic diseases	33	3	0
– viral infections	56	0	0
– cancer	30	0	0

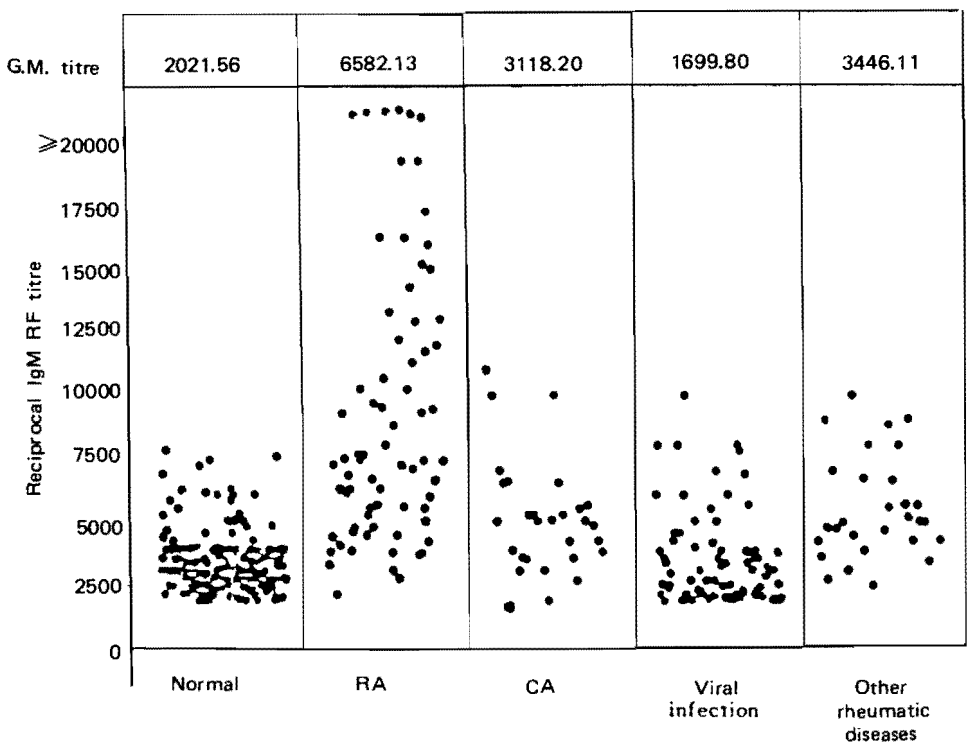

Fig. 3 Titres of IgG rheumatoid factor (RF) in sera obtained from normal healthy individuals, patients with rheumatoid arthritis (RA), cancer patients (CA), patients with viral infections and patients with other rheumatic diseases, as determined by ELISA.

Table 2. Sensitivity and specificity of ELISA for IgM RF, IgA RF and IgG RF determination in the diagnosis of rheumatoid arthritis, using various cut–off levels.

IgM RF			IgA RF			IgG RF		
cut–off titre	sensitivity %	specificity %	cut–off titre	sensitivity %	specificity %	cut–off titre	sensitivity %	specificity %
1:500	85.75	51.41	1:50	89.61	54.42	1:2500	93.50	54.55
1:600	81.81	52.66	1:70	81.81	73.98	1:3000	87.01	73.35
1:800	72.72	72.73	1:100	67.53	83.70	1:4000	77.02	81.19
1:1000	71:14	82.45	1:150	46.75	93.42	1:4500	67.75	86.83
1:1500	62.33	89.34	1:200	38.96	95.56	1:5000	64.93	89.34
1:2000	55.84	93.42	1:250	35.06	99.06	1:5500	59.74	92.16
1:2500	46.75	98.12	1:400	24.66	100.00	1:6000	55.84	94.04
1:3000	42.86	99.37				1:6500	42.86	96.24
1:9000	12.99	100.00				1:7000	41.56	96.87
						1:10000	29.87	100.00

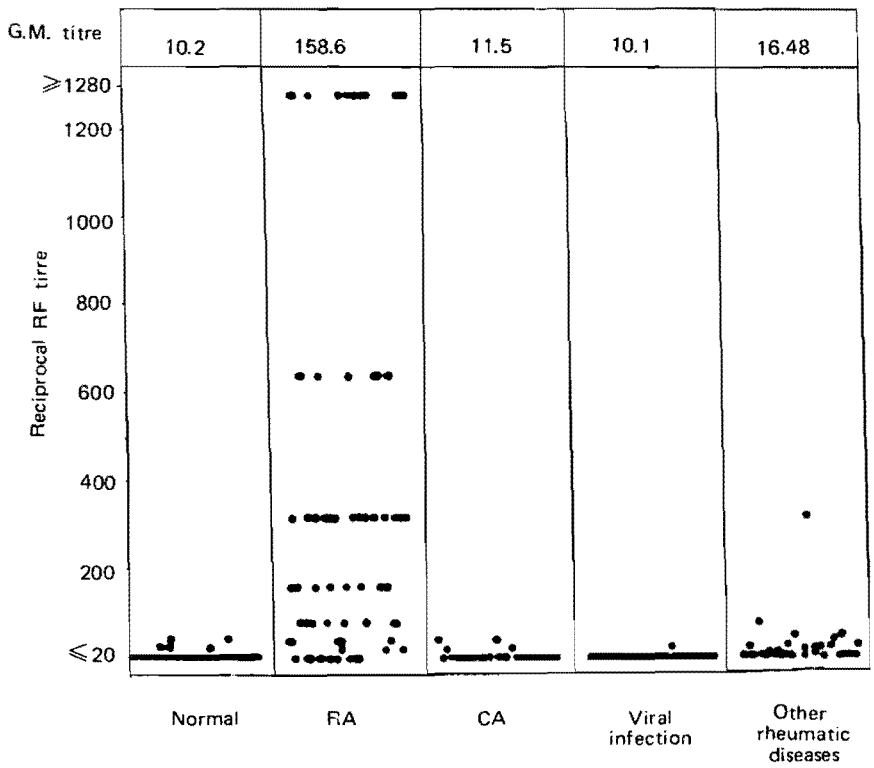

Fig. 4 Titres of rheumatoid factors (RF) in sera obtained from normal healthy individuals, patients with rheumatoid arthritis (RA), cancer patients (CA), patients with viral infections and patients with other rheumatic diseases, as determined by latex agglutination test.

and negative predictive values of 46.75%, 98.12%, 88.13%, 85.71%, 88.41%, while those for IgARF were 46.75%, 93.42%, 84.34%, 63.16%, 87.91%, and for IgGRF were 59.74%, 92.16%, 85.86%, 64.78%, 90.46%, respectively.

False positive IgM RF determinations were found in 2/200 normal individuals, 2/33 patients with other rheumatic diseases and 2/56 patients with viral infections.

False positive IgARF readings were found in 9/200 normal subjects, 2/33 patients with other rheumatic diseases and 10/30 cancer patients while false-positive IgGRF readings were found in 5/200 normal controls, 9/33 patients with other rheumatic diseases, 4/56 patients with viral infections and 7/30 cancer patients.

When the results of IgMRF, IgARF and IgGRF were considered together with positives for anyone of the 3 classes of RF being considered as a positive RF assay, then positive RF assays were found in 16/200 normal individuals, 57/77 RA patients, 14/33 patients with other rheumatic diseases, 8/56 patients with viral infections and 12/30 cancer patients. Thus, the sensitivity, specificity, efficiency, positive and negative predictive values of the ELISA were 74.03%, 84.33%, 82.32%, 58.27% and 84.33%, respectively.

Latex agglutination for the determination of rheumatoid factors

The RF titres in sera of RA patients and controls, as determined by latex agglutination test, are shown in Fig 4. By Kruskal Wallis test and multiple comparison with Bonfessoni pairwise comparison, the geometrical mean titre of RF in RA patients was found to be significantly different from those in other groups ($p < 0.001$, $\alpha = 0.05$).

When a positive result was defined by macroagglutination observed with serum diluted at 1:20 or greater, according to the manufacturer's recommendation, positive results were observed in 4/200 (2%) normal healthy individuals. This cut-off level then fulfilled the criteria set up by the ARA for diagnosis of RF. At this cut-off level, the sensitivity, specificity, efficiency, positive and negative predictive values of latex agglutination test for the diagnosis of RA were 83.11%, 93.73%,

91.6%, 76.19%, 95.83%, respectively.

False positive results were observed in 4/200 normal healthy individuals, 11/33 patients with other rheumatic diseases, 1/56 patients with viral infection and 4/30 cancer patients.

DISCUSSION

The presence of RF in a serum sample is one criterion used for the diagnosis of rheumatoid arthritis.[1] A laboratory test which is routinely used for RF detection is latex agglutination, which is claimed to detect mainly the IgM class of RF.[2] This test is usually positive in approximately 60-70% of RA patients.[3,4] The use of ELISA for the detection of other isotypes of RF has been found to be positive in RA patients at higher percentages than the latex agglutination test.[2]

However, the value of ELISA for RF detection has rarely been reported in terms of its specificity for the diagnosis of RA. As high levels of both sensitivity and specificity are essential for a valuable diagnostic test, we therefore have evaluated the ELISA for RF isotype determination and also the latex agglutination test for RF determination for their validity, including sensitivity and specificity, in the diagnosis of RA. A wide range of other rheumatic diseases and other conditions that could have positive RF were included as the control groups in this study as well as normal individuals, in order to obtain the more accurate evaluation of these tests.

In this study, we found that the geometric mean titre of IgMRF, IgARF and IgGRF, as determined by ELISA in the RA patient group was significantly higher than those in various control groups, including patients with SLE, Reiter's syndrome, reactive arthritis, osteoarthritis, ankylosing spondylitis, scleroderma, viral infection, cancer and normal controls. This was also true for RF titre determined by the latex agglutination test. However, only very small numbers of all the subjects studied had IgDRF and IgERF detectable by ELISA. When ELISA and latex agglutination test were evaluated in terms of sensitivity, specificity, efficiency, positive and negative predictive values, several points were raised. The use of different cut-off levels for positive results gave rise to different sensitivity and specificity. This also affected other parameters of the tests, ie efficiency, positive and negative predictive values. At lower cut-off levels, the sensitivity of the test increased while the specificity decreased. At the cut-off level at which positive results were observed in less than 5% of normal individuals (the criterion set up by ARA for acceptable RF test) the latex agglutination test was found to have higher sensitivity than ELISA for IgMRF, IgARF or IgGRF detection in the diagnosis of rheumatoid arthritis. (83.11% versus 46.75%, 46.75%, 59.74% respectively).

Even though the results of ELISA for IgMRF, IgARF, IgGRF were considered together, the sensitivity of the ELISA for RF detection in the diagnosis of RA was 74.03% and the specificity was 84.33%. The use of different antigens may contribute to different results being observed between ELISA and latex agglutination test. While rabbit IgG was used as the antigen in ELISA, aggregated human IgG was used in the latex agglutination test. However, the use of human IgG as the antigen can result in false positive results of ELISA for RF detection due to the direct binding of conjugate to the human IgG antigen, especially the detection of IgGRF. Thus, the rabbit IgG was then selected for use as the antigen in ELISA in this study.

The sensitivity of the latex agglutination test found in this study was comparable to that reported by the ARA (83.11% versus 80.4%).[1] However, the specificity found in this study was higher (93.73% versus 87.0%). In one study, which was carried out with RA patients in Thailand, the latex agglutination test was found to have only 69% sensitivity, which was similar to those reported from other countries.[4] The discrepancy found between the previous study and this study could be due to the different groups of RA patients studied. In this study, only patients with either classical or definite RA were included in the RA patient group, which enable more accurate evaluation of the tests for the diagnosis of this disease. Thus, it would appear that the accuracy of subject group classification has an effect on the results of diagnostic test evaluation. The sensitivity of the test may appear to be low if that test is very specific for a particular disease but patients with other diseases may also be included as subjects.

In comparison with other studies, our ELISA appeared to have comparable or higher sensitivity when the assays were considered at the same level of specificity. Karsh *et al,*[10] found positive IgMRF by ELISA in 53/60 RA patients and 45/131 controls. Thus, the sensitivity and specificity of their assay would be 88.33% and 65.65%, respectively. At a level of specificity between 52.66-72.73% our ELISA for IgMRF determination had 81.81-72.72% sensitivity (Table 2).

Gioud-Paquet *et al* found that their ELISA for IgGRF were positive in 66/100 RA patients and 58/202 controls. This would result in 66% sensitivity and 71.28% specificity.[2] We have found that, at the level of 73.35% specificity, ELISA for IgGRF determination had 87.01% sensitivity for the diagnosis of RA. However, for IgARF determination, our ELISA had lower sensitivity than that reported by Gioud-Paquet *et al,* when it was considered at the same level of speci-

ficity (between 46.75-67.53% versus 65%) (Table 2).

Although the ELISA for IgMRF, IgARF or IgGRF had rather low sensitivity in the diagnosis of RA patients, they had rather high negative predictive values (>87%).

In conclusion, the results from this study have indicated that the latex agglutination test is suitable for use as a RF test, since apart from being convenient for use, it also has both high sensitivity and specificity, provided that the reagents used have the same quality as the one evaluated in this study. ELISA performed with rabbit IgG did not have an advantage as RF test in the diagnosis of RA.

ACKNOWLEDGMENTS

This work was supported by a research grant from Mahidol University, Thailand.

REFERENCES

1. Arnett FC, Edworthy SM, Bloch DA, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31 : 315-24.
2. Gioud-Paquet M, Auvinet M, Raffin T, *et al.* IgM rheumatoid factor (RF), IgARF, IgERF and IgGRF detected by ELISA in rheumatoid arthritis. Ann Rheum Dis 1987; 46 : 65-71.
3. Carson DA, Lawrence S, Catalano MA, Vaughan JH, Abraham G. Radioimmunoassay of IgG and IgM rheumatoid factors reacting with human IgG. J Immunol 1977; 119 : 285-300.
4. Parivisutt L, Chandanayingyong D, Nilganuwong S. Chapter 3.7 Rheumatoid Arthritis in Thais, Immunogenetics in Rheumatology. Exerpta Medica Int Congress Series 1982; 602 : 98-100.
5. Florin-Christensen A, Arana RM, Garcia Morteo O, Rouse MEB, Hubscher O. IgG, IgA, IgM and IgD antiglobulins in juvenile rheumatoid arthritis. Ann Rheum Dis 1974; 33 : 32-4.
6. Koopman WJ, Schrohenloher RE, Salomon A. A quantitative assay for IgA rheumatoid factor. J Immunol Methods 1982; 50 : 89-98.
7. Walker DJ, Pound JD, Griffiths ID, Powell RJ. Rheumatoid factor tests in the diagnosis and prediction of rheumatoid arthritis. Ann Rheum Dis 1986; 45 : 684-90.
8. Wernick R, Lo Spalluto JJ, Fink CW, Ziff M. Serum IgG and IgM rheumatoid factors by solid phase radioimmunoassay. Arthritis Rheum 1981; 24 : 1501-11.
9. Halbert SP, Karsh J, Auken M. A quantitative enzyme immunoassay for IgM rheumatoid factor using immunoglobulin G as substrate. Am J Clin Pathol 1980; 74 : 776-84.
10. Karsh J, Halbert SP, Klima E, Steinberg AD. Quantitative determination of rheumatoid factor by an enzyme-labelled immunoassay. J Immunol Methods 1980; 32 : 115-26.
11. Maiolini R, Ferrua B, Quaranta JF, Pineteau A, Euller L, Ziegler G, Masseyeff R. A sandwich method of enzyme immunoassay. II Quantification of rheumatoid factor. J Immunol Methods 1978; 20 : 25-34.
12. Vejtorp M, Horer-Madsen M, Halbery P. Enzyme linked immunosorbent assay for determination of IgM rheumatoid factor. Scand J Rheumatol 1979; 8 : 65-70.
13. Zrein M, De Marcillac G, Van Regenmortel MHV. Quantitation of rheumatoid factors by enzyme immunoassay using biotinylated human IgG. J Immunol Methods 1986; 87 : 229-37.