Technical method

A modified latex-fixation test for the detection of rheumatoid factors

F. KLEIN[1], W. BRONSVELD[2], W. NORDE[3], L. K. J. VAN ROMUNDE[2], AND J. M. SINGER[4] [1]*Institute of Epidemiology, Medical Faculty, Erasmus University, P.O. Box 1738, Rotterdam, The Netherlands,* [2]*Department of Rheumatology, University Hospital, Leiden, The Netherlands,* [3]*Laboratory for Physical and Colloid Chemistry, Agricultural University, Wageningen, The Netherlands, and* [4]*Montefiore Hospital, Bronx (New York), USA*

The latex fixation test, as described by Singer and Plotz (1956), has become a widely used laboratory tool for the diagnosis of rheumatoid arthritis. Modifications were suggested by Valkenburg (1963), while Klein *et al.* (1966) investigated the biochemical background of the test. Correlations between titre value, prozone phenomena, and clinical data were investigated by Cats and Klein (1970) for the latex-fixation test and for the Rose-Waaler test.

Although Singer (1974) has proposed his test as an international standard procedure, some disadvantages are still attached to it (Klein *et al.*, 1976). One of these is the difficulty of reading a clearcut titration endpoint. The widely used Difco Bacto latex particles (0·8 μm diameter) do not produce very clear endpoints apart from varying noticeably from one batch to another and sometimes even within one batch.

In this communication a test with a new latex preparation is described, and its correlation with clinical parameters is given. The new procedure does not use essentially new principles but comprises technical improvements, which result in a sharper and more reproducible endpoint. The latex can be easily prepared in any laboratory with relatively simple equipment.

Methods

PREPARATION OF LATEX (Norde, 1976)
In a bottle of 250 ml, a mixture of 20 ml styrene (freshly distilled) and 175 ml of a $KHCO_3$ solution in water is saturated with nitrogen at 70°C, after which 5 ml of a nitrogen saturated $K_2S_2O_8$ solution

in water is added. In this mixture the final concentration of $KHCO_3$ should be $0·5 \times 10^{-2}$ M and that of $K_2S_2O_8$ $37·4 \times 10^{-4}$ M. The bottles are then glass-stoppered immediately and attached to a wheel rotating vertically with a speed of 40 rpm in a waterbath thermostatted at 70°C. The polymerisation is allowed to proceed over a period of at least 30 h. After this, agglomerates formed during the polymerisation are removed by filtering through Pyrex glass wool. The latex is then transferred to well-boiled Visking dialysis tubing and dialysed against water at a volume ratio of about 1:25 in order to remove low molecular weight substances. The dialysis is carried out at 40°C over a period of three weeks, with a change of water daily. The latex thus obtained has a surface charge density of about -15 μC/cm² caused by covalently bound sulphate groups.

The average diameter as determined by electron microscopy was $0·6$ μm. Some heterogeneity was noted but this does not interfere with the results of the latex-fixation test. Stock solutions prepared this way contained approximately 8% solids and were used for the latex fixation test in the following way:

PERFORMANCE OF LATEX-FIXATION TEST
1 Glycine buffer 0·1 mol/l containing 0·1 M NaCl is adjusted to pH 8·2. Part of the buffer contains 0·4% bovine serum albumin. This part should be used within one day after preparation.
2 Serum dilution series ranging from 1/20 to 1/10240 are made in tubes with the buffer containing albumin.
3 To 1-2 ml latex stock solution is added 25 ml 1% human gammaglobulin (made from the Dutch Red Cross 16% solution by dilution with buffer *without* albumin). This is made up to 1 litre with buffer containing albumin. Reagents should always be added in this sequence. The final mixture should have a transmission of 3% at 650 nm.
4 One millilitre of latex-gammaglobulin mixture is added to 1 ml of each serum dilution and mixed. The reaction mixture is then incubated for 1½ h at 56° in a waterbath.
5 The tubes are left for 20 h at room temperature and are then centrifuged for 4 min at 1200 g, not including acceleration time. Using the brake while running down should be avoided.
6 When reading the assay, the last tube containing

an agglutinate in the form of at least a complete ring with or without a clear supernatant should be considered the last positive reading of the series.

The addition of bovine serum albumin is necessary to stabilise the latex against false agglutination in negative sera or even in the absence of serum. Standing overnight improves the clarity of reading. Incubation at $\leqslant 37°C$ instead of $56°C$ produces agglutination in almost every serum, due to Clq, just as it does in the system with Difco latex (Klein *et al.*, 1966). Such agglutinations disappear after previous inactivation of the sera for 30 min at $56°C$, demonstrating the thermolabile nature of this type of agglutinator. H. Clasener (private communication) has carried out the test on microtitre plates using a Compu-pet 100 dilution apparatus (General Diagnostics). Clear readings were obtained with the new latex preparation but not with Difco latex.

Material

For two months the new method was tested parallel with the old one (Valkenburg's modification). During this time American Rheumatism Association criteria were recorded for 230 patients (with and without rheumatoid arthritis). This number was found to be sufficient in view of the close parallelism between the titre values of both methods, which appeared already in preliminary investigations. Table 1 gives the composition of the test sample.

Table 1 *Composition of the test sample*

	Female	Male	Total
All patients	149	81	230
Rheumatoid arthritis	85	44	129

Results

Correlations between the old and the new methods, with and without previous inactivation, are given in Table 2. The correlations between titre values and number of American Rheumatism Association (ARA) criteria at the time of investigation (8 criteria

Table 2 *Regression and correlation of titres in the old and new methods*

y	x	All patients	r	RA patients	r
O	N	y = 1·11x + 0·37	0·956	1·11x + 0·39	0·951
O	N56	y = 1·19x − 0·41	0·920	1·20x − 0·34	0·902
N	N56	y = 1·04x − 0·61	0·939	1·07x − 0·63	0·944

x and y = numbers of positive tubes
O   = old method
N   = new method
N56   = new method (with previously inactivated sera)

Table 3 (A) *Regression of ARA criteria on titre values for all patients (n = 230). Eight criteria recorded, serology not included*

Regression equations	
O	y = 0·24x + 2·61
N	y = 0·27x + 2·54
N56	y = 0·30x + 2·33

y = number of ARA criteria
x = number of positive tubes

Table 3 (B) *Correlation of tests with clinical data expressed as r^{2}* (coefficient of determination)*

Test	ARA criteria	Radiological abnormalities	Severity of symptoms
O	0·261	0·263	0·304
N	0·272	0·269	0·324
N56	0·277	0·282	0·342

*r^{2} measures the fraction of the total variance that can be explained by the regression.

recorded), the presence or absence of radiological abnormalities, and an estimation of the activity of the disease, graded from 0 to 4, are given in Table 3. This table also gives the regression equations of the number of ARA criteria (serology not included) on number of positive tubes for the three test modifications. The expected number of criteria for a given titre value, as calculated from these equations, is given in Table 4. Mean titre values are $6·0 (= 1/640)$ for the old method, while the new method gives $5·0 (= 1/320)$ without and $5·2$ with previous inactivation.

Table 4 *Mean prediction of number of ARA criteria from titre values*

Tube no.	O	N	N56
0	2·6	2·5	2·3
1	2·8*	2·8	2·6
2	3·1*	3·1	2·9
3	3·3*	3·4	3·2
4	3·6	3·6	3·5
5	3·8	3·9	3·8
6	4·1	4·2	4·1
7	4·3	4·4	4·4
8	4·5	4·7	4·7
9	4·8	5·0	5·0
10	5·0	5·2	5·3

*Figures obtained by extrapolation of the regression line.

Reproducibility was tested on 125 sera in duplicate on different days and with different batches of latex or gammaglobulin. For 21 sera the titres differed by more than one dilution. When latex and gammaglobulin were the same, 13 out of 97 sera differed by more than one dilution when repeated on another day.

It is known (Klein *et al.*, 1966) that previous heating of sera for 30 min at 56°C destroys an inhibitor of the latex fixation test. This results in disappearance of the so-called prozone phenomenon observed in this test (Cats and Klein, 1970). It appears that in the new test 4·8% prozone phenomena were read in the test sample without previous inactivation, compared to 27·4% in the old method. After inactivation of sera, prozones are abolished and some low titres appear in otherwise negative sera. This phenomenon, which can also be observed in the old method, is due to traces of rheumatoid factor which in native sera are masked by an inhibitor (Klein *et al.*, 1966). In the new method such low titres are weaker and less frequent, and therefore this method seems to be less suitable for detection of this phenomenon, which is of no importance for clinical diagnosis but of some interest for the epidemiology of rheumatoid factor (Greenwood *et al.*, 1971).

Discussion

The results show clearly that the new test correlates at least as well with clinical parameters as the old one. The main advantage of the procedure described here is its better readability. Reproducibility also appears to be satisfactory. The suspension of latex particles can be relatively easily prepared by any laboratory that wants to have the test system under complete control, since this does not require more than a shaking machine and a waterbath. The possibility of preparing the latex oneself may become of importance in future standardisation efforts.

Although in our hands the new test system seemed to be less sensitive to small variations in gammaglobulin preparations, it remains advisable to try out different gammaglobulin batches in a comparative test with a battery of sera with known titres. Because of agglutination by C1q the new method cannot be used as a slide test without previous inactivation (Cheng and Persellin, 1971).

The question arises whether sera should be inactivated before being tested. Correlations with clinical parameters are slightly better after inactivation but hardly sufficiently so to warrant an extra step in the laboratory procedure. Cats and Klein (1970) attached some clinical importance to the prozone phenomenon. Since this phenomenon is greatly reduced in the new test, even without

previous inactivation, it should be disregarded when a test without inactivation is used.

The use of borderline titres may be questioned, since the relation between clinical parameters and titre value is a continuous one, making any division arbitrary. Therefore it is preferable not to establish a new borderline titre but to give the mean number of ARA criteria corresponding to a chosen titre value.

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