COMPARATIVE STUDY OF THE HAEMAGGLUTINATION
TEST AND A SIMPLE LATEX-FIXATION TEST FOR THE
DETECTION OF THE RHEUMATOID FACTOR

BY

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The exact nature of the “rheumatoid factor” is
unknown, but it appears to be an antibody-like
substance composed of a gamma globulin complex
of high molecular weight. Two groups of tests
have been used to detect it: the haemagglutination
test and the latex-fixation or Bentonite flocculation
test. In the first group the indicator consists of
sheep red cells with rabbit anti-sheep haemolyhin as
the reactant. In the second group the indicators are
either polystyrene latex particles or bentonite
particles with gamma globulin as the reactant. In
both groups of tests the indicator becomes sensitized
or coated with reactant which then reacts with sera
containing the rheumatoid factor, causing agglutina-
tion of the indicator.

In the following series of 176 cases, serum from
each was examined for the rheumatoid factor,
using a standard haemagglutination test and a simple
slide latex-fixation test.

Methods

(A) Haemagglutination Test

Reagents

(1) Sheep red cells.
(2) Rabbit anti-sheep haemolyhin serum.

Doubling dilutions of rabbit anti-sheep haemolyhin
serum were incubated at 37° C. for 1 hour with equal
quantities of a 1 per cent. suspension in saline of sheep
red cells. After a further hour at room temperature the
agglutination end-point was read. Then a 1 per cent.
solution of sheep red cells was sensitized with haemolyhin
serum (agglutination titre previously determined). After
30 min. equal quantities of doubling dilutions of a
patient’s serum (previously decomplemented at 56° C.
for 30 min.) were incubated for 1 hour at 37° C. with
equal quantities of the sensitized sheep cells. As a
control, the patient’s serum was also incubated at the
same time with unsensitized sheep cells.

After a further hour at room temperature, if the
agglutination titre of the sensitized cells to unsensitized
cells exceeded 16, the test was considered positive.

(B) Latex-Fixation Test

Reagents

(1) Latex-globulin reagent (indicator already coated
with reactant).
(2) Positive control serum.
(3) Negative control serum.
(4) Glycine-saline buffer diluent (adjusted to simplify
preparation of diluted serum).

A 1:20 dilution of serum in glycine-saline buffer
diluent was made. One drop of this was placed on a
slide and to it was added one drop of latex globulin
reagent. These were mixed with a stick and the slide
was gently tilted from side to side for 1 min. Sera con-
taining the rheumatoid factor produced visible floccula-
tion of the reagent, and a smooth suspension indicated
a negative reaction. Positive and negative controls were
carried out. The results of the test were read as follows:

Negative
Weakly Positive (+)
Strongly Positive (++)

Results

The results in the 176 persons tested are shown in
Table I (opposite). The 100 cases classed as rheuma-
toid arthritis satisfied the accepted diagnostic criteria.
The fifteen “suspected” cases were under obser-
vation as ones which might develop into rheumatoid
arthritis, but it has not been possible to carry out a
follow-up on this group.

Table I shows that 89 per cent. of the rheumatoid
arthritis group gave positive results in the latex-
fixation test, while the haemagglutination test carried
out in parallel was positive in 74 per cent. Two out of fifteen “suspected” cases gave positive results in the latex-fixation test, and one in the
haemagglutination test. Three out of six cases of
disseminated lupus erythematosus gave positive
TESTS FOR THE DETECTION OF THE RHEUMATOID FACTOR

TABLE I
RESULTS IN 176 SERA

<table>
<thead>
<tr>
<th>Test</th>
<th>Haemagglutination</th>
<th>Latex-Fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Rheumatoid Arthritis (100)</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>Suspected Rheumatoid Arthritis (15)</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Disseminated Lupus Erythematosus (6)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Other Diseases* (45)</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Normal Controls (10)</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid Arthritis</td>
<td>150</td>
<td>107</td>
</tr>
<tr>
<td>Osteo-arthritis and Other Arthritis</td>
<td>250</td>
<td>5</td>
</tr>
<tr>
<td>Rheumatic Fever and Rheumatic Heart Disease</td>
<td>250</td>
<td>4</td>
</tr>
<tr>
<td>Hyperglobulinaemia</td>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td>Disseminated Lupus Erythematosus</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Other Cases (Non-Articular)</td>
<td>560</td>
<td>17</td>
</tr>
<tr>
<td>Normal Subjects</td>
<td>200</td>
<td>2</td>
</tr>
</tbody>
</table>

* Osteo-arthritis (13), Gout (4), Rheumatic fever (8), Scleroderma (1), Erythema multiforme (1), Other diseases (18, including Pyelitis, Hypochromic anaemia, Pernicious anaemia, Gastric ulcer, and Bronchitis).

results in the haemagglutination test, but all the latex-fixation results were negative. It is apparent that the latex-fixation test is more sensitive and more specific for rheumatoid arthritis. No false positive results were given in this series.

Singer and Plotz (1956) described the latex-fixation test performed in tubes. This involved maintaining the constancy of the latex concentration, the make and source of the gamma globulin used, the time of incubation and temperature, and a borate buffer at pH 8.2 for optimal conditions for the test in rheumatoid arthritis. A series of tests gave the results shown in Table II.

A comparison of the latex test and the sheep cell agglutination test gave the results shown in Table III.

It may be concluded that in the present series of cases the slide latex test was superior in that it gave 89 per cent. positive results in rheumatoid arthritis as compared with the 71.3 per cent. obtained by Singer and Plotz. The probable reason for this is that the present reagents are superior to and more stable than those used by Singer and Plotz.

Bozicevich, Bunim, Freund, and Ward (1958) have described a Bentonite flocculation test for rheumatoid arthritis. The test consists of coating Bentonite particles with human gamma globulin. When mixed with the serum of patients with rheumatoid arthritis, flocculation occurs. In a series of 41 cases, 78 per cent. gave positive results. In a control series, including cases of rheumatic disease...
other than rheumatoid arthritis (Rheumatic Heart Disease, Gout, Systemic Lupus Erythematosus, Rheumatic Fever), carcinoma, and other conditions, 2 per cent. gave positive results, i.e. one case of systemic lupus erythematosus, one case of brain tumour with macroglobulinaemia, and one case of acute leukaemia. A series of tests on normal people all gave negative results. In the above series of cases sera tested by the sheep cell agglutination test and the Bentonite flocculation test showed agreement in 98 per cent. From these results it can be concluded that the latex test is superior to the Bentonite test in rheumatoid arthritis since only 78 per cent. were positive as compared with 89 per cent. in the present series.

Summary

176 persons (115 with rheumatoid disease and 6 with disseminated lupus erythematosus) were examined for the rheumatoid factor, using a standard haemagglutination test and a simple latex-fixation test. It is concluded that the latex-fixation test is simple and time-saving, and superior to the former test.

I should like to thank Dr. W. I. Leslie for his advice, and Dr. J. H. Glyn for his clinical help.

The reagents for the latex-fixation test were supplied by the Hyland Laboratories, California, U.S.A.

REFERENCES


Etude comparée de la réaction d'hémagglutination et d'une simple réaction de fixation sur latex pour déceler le facteur rhumatismal

RéSUMÉ

On rechercha le facteur rhumatismal chez 176 personnes (dont 115 atteintes de maladie rhumatismale y 6 de lupus erythèmeux diséméine) au moyen de la réaction d'hémagglutination habituelle et d'une simple réaction de fixation sur latex. On conclut que la réaction de fixation sur latex est simple, rapide et supérieure à la première réaction.

Estudio comparativo de la reacción de hemaglutinación y un simple test de fijación sobre latex para descubrir el factor reumatoide

SUMARIO

Se buscó el factor reumatoide en 176 personas (115 con artritis reumatoide y 6 con lupus eritematoso diseminado) usando la reacción de hemaglutinación clásica y un simple test de fijación sobre latex. Se concluye que el test de fijación sobre latex es sencillo, rápido y superior a la reacción mencionada.