Rate nephelometric determination of rheumatoid factor: comparison between Kallestad QM-300 and Beckman ICS-II (RF) methods

R J Collins, J C Neil, R J Wilson

Abstract
The Kallestad Corporation recently suggested that their new buffer system for the nephelometric detection of rheumatoid factor conferred advantages over existing systems. Two rate nephelometric procedures, the Kallestad QM-300 and the Beckman ICS-II (RF), were therefore compared. Sera (n = 157) were selected on the basis of a previous ICS-II value. The results on the QM-300 of sera with an initial rheumatoid factor value of <400 IU identified two groups. Group 1 (n = 109) showed a good correlation with the ICS-II method while group 2 (n = 13) was highly discordant with the QM-300, producing significantly higher values. The values of 35 sera with an initial rheumatoid factor of >400 IU were likewise highly discordant, with the QM-300 producing significantly lower values. Dilution recovery experiments implied that the Beckman buffer was likely to be contributory. As the formulae of the buffers remain proprietary, the reasons for the differences are speculative. The findings could be taken to indicate that the Kallestad value is a more accurate indicator of the quantity of rheumatoid factor than the Beckman value.

Since the original description of rheumatoid factor by Waaler and its documented clinical association with rheumatoid arthritis, the detection and quantitation of rheumatoid factor have been hallmarks in the differential diagnosis of autoimmune disease. Its demonstration is a major laboratory contribution to the American Rheumatism Association's diagnostic criteria for rheumatoid arthritis. Because of the clinical importance of rheumatoid factor, considerable attention has been directed to its accurate quantitation. Numerous methods have been used, including the principles of haemagglutination (Rose-Waaler test), latex agglutination, complement fixation, radioimmunoassay, enzyme linked immunosorbent assay (ELISA) and endpoint and rate nephelometry. Recent quality assurance programmes show considerable interlaboratory variability in the detection of rheumatoid factor using manual procedures, and it has been suggested that automated rate nephelometry has the precision and reproducibility to warrant its routine use in clinical practice.

Since nephelometry was first described several improvements in methodology have occurred, including the use of various polymers to enhance the antigen-antibody formation, and automation to monitor the rate of complex formation. As the Kallestad Diagnostic Corporation has recently suggested that its improved buffer system is advantageous in the detection of rheumatoid factor we compared their QM-300 Protein Analysis System (Kallestad, Austin, Texas) with the Beckman Auto Immunochemistry System ICS-II (RF) (Beckman Instruments Inc, Brea, California).

Methods
Whole blood was collected by standard venepuncture technique using evacuated tubes (Beckton-Dickinson, Sunnyvale, California). After centrifugation (1300 × g for 10 minutes), serum was aliquoted (2 ml) and stored at −20°C. All sera were heat inactivated at 56°C for 30 minutes and centrifuged at 8000 × g for 10 minutes.

Rheumatoid factor was quantitated in all sera strictly according to the manufacturers' instructions. Two groups of rheumatoid factor positive sera, selected on the basis of a previous ICS-II value, were studied. They consisted of 122 sera with rheumatoid factor values between 60 and <400 IU and 35 sera with rheumatoid factor values of >400 IU.

Within-run precision was determined by analysing up to 12 replicates of six sera. Between-run precision was determined by analysing four sera on both instruments on up to eight occasions. The instruments were...
recalibrated at the beginning of each determination.

The rheumatoid factor binding characteristics and linear response range of each method were determined using 11 sera with rheumatoid factor values of < 400 IU determined by both methods. The sera were manually diluted using precision pipettes from 1:2 to 1:10 with either Kallestad QM-300 diluent, Beckman buffer, or normal heat-inactivated serum (rheumatoid factor of < 60 IU).

All statistical analyses of results from the two methods including means, standard deviations, Student's t test for matched pairs, and linear regression were performed using the CSS statistical analysis software (Statsoft Inc, Tulsa, Oklahoma).

**Results**

**Precision**

The rheumatoid factor values of the six sera examined for within-run precision ranged from 80 IU to 1900 IU. The precision of both methods was high with the coefficient of variation (CV) being less than 5% over the entire range tested (table 1).

The rheumatoid factor values of the four sera examined for between-run precision ranged from 94 to 1951 IU. The CV for the QM-300 ranged from between 2.5% and 8.3% (mean = 5.5%), the CV for the ICS-II ranged from between 4.1% and 11.6% (mean = 8.7%) (table 2).

**Sera**

Although the precision of both methods was high, significant differences in the rheumatoid factor values were shown by the two methods. With the QM-300, the results of 122 sera with initial ICS-II values of < 400 IU identified two groups (fig 1). While the results of group 1 (109 sera) showed a good correlation (r = 0.906), the QM-300 values were significantly higher (p = 0.01). Thirteen sera, however (group 2), gave highly discordant results. In group 2 there was no correlation between the values obtained by each method (r = −0.15), and the QM-300 values were significantly higher than the ICS-II results (p = 0.000) (table 2).

While the results of the 35 sera, chosen with an initial ICS-II value of > 400 IU, showed good correlation (r = 0.74), significant differences were again recorded, with the QM-300 producing lower values (p = 0.000) (table 3).

**Dilution recovery experiment**

All 11 sera examined produced similar dilution recovery curves (fig 2). The curves obtained for sera diluted with Beckman buffer and run on the ICS-II were significantly different from the curves of sera diluted with Kallestad buffer and run on the QM-300 and from the curves of sera diluted with normal human serum and run on both instruments (p = 0.0003 at 1:6 dilution).

**Discussion**

The results of this study indicate that significantly different rheumatoid factor values can be obtained depending on which nephelometric system is used. There were several important findings.

Firstly, while the rheumatoid factor results for group 1, obtained using the Kallestad QM-300 method, were significantly higher than those obtained using the Beckman ICS-II, the difference was of questionable clinical relevance.

Secondly, and of potential clinical relevance, are the findings associated with group 2 sera and all sera with rheumatoid factor values of > 400 IU by both methods. Two apparently conflicting phenomena occurred. Some sera, whose reaction products near the peak rate of light scatter, had significantly higher values (p = 0.000) determined by the QM-300 compared with the ICS-II system. All sera whose rheumatoid factor values were greater than 400 IU with the Beckman ICS-II system produced significantly lower values (p = 0.000) determined by the Kallestad QM-300.

These discrepancies are unlikely to be caused by the instrumentation itself as both instruments' precision and reproducibility are of a high standard. Our results implicate the Beckman buffer as the likely cause. Both instruments' precision and reproducibility are of a high standard. Our results implicate the Beckman buffer as the likely cause.

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**Table 2** Reproducibility of between-run determinations of four sera on QM-300 and ICS-II

<table>
<thead>
<tr>
<th>Instrument</th>
<th>QM-300</th>
<th>ICS-II</th>
<th>QM-300</th>
<th>ICS-II</th>
<th>QM-300</th>
<th>ICS-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>1 SD</td>
<td>101</td>
<td>94</td>
<td>101</td>
<td>94</td>
<td>156</td>
<td>117</td>
</tr>
<tr>
<td>% CV</td>
<td>8.3</td>
<td>5.2</td>
<td>11.6</td>
<td>5.5</td>
<td>11.6</td>
<td>5.5</td>
</tr>
</tbody>
</table>

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**Table 3** Quantitation of rheumatoid factor in sera by ICS-II and QM-300

<table>
<thead>
<tr>
<th>ICS-II &lt; 400 IU</th>
<th>QM-300 &lt; 400 IU</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>(n =)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>178 (97)</td>
<td>189 (103)</td>
<td>*</td>
<td>109</td>
</tr>
<tr>
<td>Group 2</td>
<td>395 (40)</td>
<td>1205 (227)</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>ICS-II &gt; 400 IU</td>
<td>2387 (266)</td>
<td>1380 (703)</td>
<td></td>
<td>35</td>
</tr>
</tbody>
</table>

*Values obtained by the QM-300 significantly higher than those obtained by the ICS-II (p = 0.01) and p = 0.000, respectively.

Values obtained by the QM-300 significantly lower than those obtained by the ICS-II (p = 0.000).
Rate nephelometric determination of rheumatoid factor

Figure 2. Dilution recovery experiments of a representative serum diluted with Beckman buffer ( — — ) or normal human serum ( — — — ) and run on the ICS-II, or diluted with Kallestad Diluent ( — — — ) or normal human serum ( — — — ) and run on the QM-300. At 1:6 dilution the rheumatoid factor values for sera ( n = 11 ) diluted with Beckman buffer and run on the ICS-II are significantly higher ( p = 0.0003 ) than the other combinations.

Instruments are automated and use a 1:6 dilution of sera to determine the rheumatoid factor value for sera whose peak rate light scatter is above a predetermined level. Our dilution recovery experiments show that the Beckman ICS-II system produces a higher value only when diluted in the Beckman buffer ( p = 0.0003 at 1:6 dilution). Results concordant with the Kallestad QM-300 value are obtained when sera are diluted with normal human serum. As the formulae of the buffers are proprietary, the reasons for the differences remain speculative. We interpret the findings, however, to indicate that the Kallestad method produces values which are a more accurate indication of the quantity of rheumatoid factor than the Beckman value. The clinical usefulness of a procedure which has the discriminatory power to identify patients of the group 2 category is yet to be determined. Furthermore, the findings have obvious implications for quality assurance programmes.

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