method), and this result was still within the reference range.

We repeated tests for three of the patients on the following day. Two (J. E. and R. C.) showed little change in total CK, apparent CK-MB, and CK-MB (Hybritech). However, one (M. J.) showed a significant decrease in apparent CK-MB activity which, without the total CK or electrophoresis results, could have been indicative of a myocardial infarction.

The Hybritech kit involves use of specific monoclonal antibodies for the detection of CK-MB. Our results with it show an absence of false-positive macro CK or increased CK-BB.

Improved 125I Radioimmunoassay

To the Editor:

The report of Knight et al. (1) concerning improvements in the 125I immunofluorescence for cotinine prompts us to report our observations on the same problem and an alternative approach to its solution.

Antibodies to a trans-4'-carboxycotinine-bovine serum albumin (BSA) conjugate (coupled in an 8:1 molar ratio) were prepared in sheep by injecting intramuscularly 25 µg of the conjugate emulsified with complete Freund's adjuvant, followed by a booster immunization at four weeks and a subsequent bleed sampling a week later. The serum obtained was absorbed against a formaldehyde/glutaraldehyde-BSA polymer to remove the anti-BSA antibody fraction.

N-(p-Hydroxyphenethyl)-trans-cotinine-carboxamide (HPTCC) was prepared by a simplified procedure of the method of Langone et al. (2), and the iodinated tracer was prepared in a way similar to that reported by Knight et al. Using this tracer, we observed reasonable antibody titers, a 4000-fold dilution of antiserum sufficient for 50% tracer binding, so we used this dilution to study the displacement reactions shown in Figure 1.

Displacement of this tracer by cotinine was disappointing (curve 2), yielding an assay of poor sensitivity, while displacement by the precursor of the tracer gave a much more sensitive assay system (curve 5). This suggested that bridge recognition was a problem, a well-known phenomenon in immunoassays of steroids. Delayed addition of the tracer after a preincubation of cotinine and antibody did not improve the sensitivity of the assay, because equilibrium was reached rapidly. At the short incubation times needed for good sensitivity, assay drift was a major problem.

References


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Fig. 1. Displacement curves of the tracers 125I-labeled HPTCC (—) and VI (—) with various analytes

To change the nature of the bridging group, we prepared a tracer based on the tyramine adduct of succinylhydroxymethylcotinine (V) by the following sequence of reactions. trans-4'-Carboxycotinine (I) was methylated in methanol/sulfuric acid in the presence of 4A molecular sieves to yield the methyl ester of trans-4'-carboxycotinine (II). Reduction of this compound with excess sodium borohydride in methanol (16 h, room temperature), acetylation with 50% aqueous acetic acid, and adjustment of the pH to greater than 10, followed by continuous dichloromethane extraction, gave virtually quantitative yields of the hydroxymethylcotinine derivative (III). Nuclear magnetic resonance and ultraviolet and infrared spectroscopy confirmed the identity of this compound. This material was transformed to the succinate by refluxing with succinic anhydride and an equivalent amount of triethylamine in dry benzene to give compound IV. This can be linked to tyramine (producing V) and iodinated to tracer VI by methods similar to those for the trans-carboxycotinine derivative (2).

Using the tracer VI in similar molar and radioactive concentrations as the HPTCC tracer requires more antibody (1000-fold dilution for 50% tracer binding). Displacement of the tracer by cotinine, however, leads to a much more sensitive assay system. Comparison of curves 2 and 3 in the figure.
demonstrates that, with use of this tracer, assay sensitivity improves 100-fold, being similar to the sensitivity of assays with tritiated ligands, but with better antibody titers (unpublished).

It would be interesting to see whether using this tracer with the absorbed antiserum of Knight et al. might lead to even more sensitivity. Comparing the displacement of the tracer with cotinine and with unlabeled tracer (curves 3 and 4) suggests that there still appears to be some bridge recognition by the antibodies we have been using.

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References

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Rheumatoid Factors Measured in Serum with a Fully Automated Laser Nephelometer, and Correlation with Agglutination Tube Titers

To the Editor:

We have recently evaluated the immunonephelometric test (LN-Latex-RF test; Hoechst Canada Inc., Montréal, Québec, Canada H4R 1R6) for the quantitative determination of rheumatoid factors (RF) in serum with the fully automated Behring laser nephelometer (Hoechst). Serum RF react with polystyrene particles coated with modified human gamma-globulins and agglutinate the latex particles. The increase in light-scattering is measured by the nephelometer (I). Thermal inactivation is not required, because complement factors do not interfere with the test. The assay is calibrated against the WHO Reference Serum, and RF values are expressed in int. units/mL. Behring’s protocol was precisely followed.

We have compared the nephelometric procedure with our routine procedure, the “Rapitex RF” (Hoechst), a latex-agglutination test. The results of this semiquantitative test are reported in terms of dilution titers, the titer at which agglutination can still be observed in this tube.

Because the clinical course of rheumatoid arthritis appears to be related to RF concentrations (2), we had to establish an equivalence table between results expressed in titers and in international units per milliliter, to assist clinicians in their therapy, before we discontinued the agglutination test.

The sera of 113 patients with a titer of at least 1:40 were assayed by the two procedures. Two different technicians performed the two tests at two different periods of the year: winter (54 patients) and fall (59 patients). The results (Figure 1) show that the samples obtained during autumn had much lower titer values vs int. units/mL than did those collected in winter. A long-term precision study demonstrated the stability of the nephelometer over that period (LN Rheumatoid Factor Control Serum, Hoechst, n = 30, = 77 int. units/mL, CV = 7.3%). Thus the observed discrepancy was ascribable to the inaccuracy and subjectivity of the latex test, one technician seeing agglutination where the other did not. It would be very surprising indeed, considering published evidence (3–6), if all sera analyzed during the fall reported to have a titer of 1:640 in fact had a RF concentration lower than 150 int. units/mL (Figure 1).

Therefore we established the following relation between RF tube titer and concentration (x), using only the 54 data points obtained in the winter study: 1:40 = 47 int. units/mL; 1:80 = 64; 1:160 = 85; 1:320 = 143; 1:640 = 289; 1:1280 = 513; and 1:2560 = 946.

Our results agree with those of Finley et al. (3), who compared a rate-nephelometric reaction (Beckman Instruments Inc., Fullerton, CA 92634) with a latex precipitation test ("RA slide and test tube"; Hyland Diagnostics, Bannockburn, IL 60015), except for the 1:80 and 1:160 tube titers, where we found 64 int. units/mL instead of 50, and 85 instead of 62.5, respectively. Such a close agreement between results by two different assays is surprising indeed, considering the subjectivity of the visual tests and the heterogeneity of RF.

Measurement of RF in serum appears to have been greatly improved with the introduction of nephelometry. Our precision data for the fully automated Behring laser nephelometer confirm this, especially when one considers that tube titer precision reportedly (4) is at best ± 1 tube titer:

<table>
<thead>
<tr>
<th>RF, int. units/mL</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within run (n = 10)</td>
<td>76</td>
<td>1.32</td>
<td>1.7</td>
</tr>
<tr>
<td>427</td>
<td>4.90</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Day-to-day (n = 9)</td>
<td>69</td>
<td>4.56</td>
<td>5.1</td>
</tr>
<tr>
<td>408</td>
<td>13.95</td>
<td>3.4</td>
<td></td>
</tr>
</tbody>
</table>

These data are from four pools of patients’ sera. In the day-to-day study, aliquots of two pools were frozen and analyzed on nine consecutive working days.

Measuring patients’ RF concentrations in int. units/mL during the clinical follow-up of these patients will eventually allow us to verify whether RF concentrations are effectively correlated with the clinical course of rheumatoid arthritis.

Fig. 1. Equivalence between RF values expressed in titers and concentration for results obtained during winter (8) and autumn (x).

Two results were outside the concentration scale: 2464 and 3840 int. units/mL.