A rapid micro-agglutination technique for the detection of antibody to *Legionella pneumophila* serogroup 5

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Summary. A rapid micro-agglutination test (RMAT) for the detection of antibody to *Legionella pneumophila* serogroup 5 is described. It was found to be both sensitive and specific when compared with the indirect immunofluorescence test. Evaluation of 89 paired sera from patients with respiratory symptoms showed that the incidence of *L. pneumophila* serogroup 5 respiratory infection in East Anglia is low: only one case was found in this study. The RMAT would be easy to perform as a screening test in a routine serological laboratory.

Introduction

Legionella species are a significant cause of infection in man and give rise to 2% of community-acquired pneumonias.¹ Although more than 20 species of *Legionella* have been described, and 14 serogroups of *L. pneumophila*, most laboratories test only for *L. pneumophila* serogroup 1. Studies² have suggested that pneumonia is rarely caused by other serogroups but investigation has been hampered by the lack of rapid serological screening techniques. Currently, serological diagnosis of infection by strains other than serogroup 1 depends either on the time-consuming indirect immunofluorescence antibody test (IFAT), requiring skilled personnel, or on culture of the organism.

We have been particularly interested in serogroup 5 infections since 1979, when we reported the first case of fatal pneumonia caused by this serogroup.³ At that time we used the IFAT to screen sera from 470 patients with respiratory symptoms in Cambridgeshire,⁴ and we found antibody to serogroup 5 in 5.5%.

In a recent local hospital survey, serogroup 5 was a common, and indeed the predominant, serotype isolated from the hot water calorifiers (personal communication, Dr R. E. Warren). Therefore, the potential for infection with serogroup 5 appeared to be substantial. However, the commonly used rapid micro-agglutination test (RMAT) or IFAT for serogroup 1 does not detect specific antibody to serogroup 5; therefore, outbreaks or individual cases of infection might have been missed. The aim of this study was to develop a rapid RMAT screening technique to assess the incidence of disease due to serogroup 5 in Cambridgeshire.

Materials and methods

Human and rabbit sera

Several groups of human sera were used: six positive sera⁴ with antibody to *L. pneumophila* serogroups 1–6; 30 sera⁴ collected between January and December 1979 and stored at –20°C, with antibody to serogroup 5 by IFAT; single samples from 96 blood donors, collected in January 1987; single samples from 96 patients with respiratory symptoms, collected in November or December 1987; 89 pairs of sera from in-patients and out-patients with respiratory symptoms, July to September 1987.

Six high-titre rabbit sera, specific for serogroups 1–6, were obtained from the Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5DF.

Rapid micro-agglutination test (RMAT)

The method was based on that described by Harrison and Taylor⁵ for *L. pneumophila* serogroup 1.

Bacterial suspensions. Six strains of *L. pneumophila* serogroup 5 (634, 214, 638, 357, 40, CAMB 2) from Birmingham, Leeds or Cambridge were stored in glycerol on beads at –70°C. Five of them were isolates from the environment and one from a patient. Formolised suspensions were prepared as described by Harrison and Taylor.⁵ Briefly, strains were incubated on Buffered Charcoal Yeast-Extract (BCYE) agar at 37°C for 72 h, harvested into formol saline, incubated at 37°C overnight, stained with safranin 0.005%, washed, and reconstituted to a final optical density (OD₆₀₅) of 1.5 in 0.1 M phosphate-buffered saline (PBS), pH 6.4, with sodium azide 0.08%. These suspensions were used in the RMAT; they were stable at 4°C for at least 6 months.

Agglutination. Twofold dilutions of serum in PBS were made in 25- μ l volumes in the wells of a V-bottomed microtitration plate (Sterilin, Feltham, Middlesex), and 25 μ l of bacterial suspension was added to each well, commencing at the 1 in 8 dilution. The plates were sealed with clear adhesive plastic tape and shaken on a Micro-Shaker (Dynatech) for 10 s. They were incubated at room temperature for 10 min and then centrifuged at 1000 rpm for 4 min. After removal of the plastic seals, the plates were left at an angle of 70° to the horizontal on a plate reader for 10 min before recording the results. A negative result was read as one in which the tight button of stained bacteria had streaked down the well to produce a tear-drop pattern. A positive result showed as a tight button in the bottom of the V, with no streaking.

Suitability of bacterial strains for RMAT

The six strains were evaluated in the RMAT with human reference sera selected from the above groups by the IFAT results (see below). Some were IFAT-positive with serogroup 5; the others, IFAT-negative with serogroup 5, were either positive with other serogroups or without detectable antibody to *Legionella* spp.

Indirect immunofluorescence antibody test (IFAT)

The test was performed, as described by Harrison and Taylor,⁶ with formalised suspensions of *L. pneumophila* serogroups 1–6 grown on BCYE agar.

Results

Specificity of bacterial suspensions and selection for use in the RMAT

All six strains of *L. pneumophila* serogroup 5 showed good specificity by IFAT. Rabbit antiserum to serogroup 5 reacted with them at high titre (≥ 125), but rabbit sera with high titres against serogroups 1–4 and 6 did not react.

Two of the six strains (40 and 357) gave appropriate buttons in the RMAT with positive and negative

Figure. Detection of antibody to serogroup 5 of *L. pneumophila* in 30 human sera: comparison of agglutinin titre against strain 357 in the rapid micro-agglutination test (RMAT) with IgM titre in the indirect immunofluorescence antibody test (IFAT). There was good correlation between the two methods except for two sera with RMAT titres of <16.

human reference sera; these were used for all further studies. The other four strains gave non-specific results with these sera and failed to form buttons in the RMAT.

Human serum samples, which were shown by IFAT to have IgM antibody to serogroup 5, were tested by RMAT against strain 40 and strain 357 (figure) and gave similar results—correlation coefficient 0.699 and 0.7 respectively. Two sera gave discrepant results; they had titres of <16 by the RMAT, but had IFAT IgM titres of 32 and 256. Sera without antibody by IFAT or with a positive IFAT for serogroups 1–3 were negative in the RMAT. Several batches of strains 40 and 357, prepared at intervals over 6 months, gave similar results.

RMAT results with sera of patients and blood donors

Most serum samples from 96 blood donors and 96 patients with respiratory symptoms had RMAT titres of <32, but a few in each group had titres of ≥ 32 (table). Three (3%) of 96 blood donors and four (4%) of 96 patients with acute respiratory symptoms had

Table. Agglutinin titres of sera tested by RMAT against two strains of *L. pneumophila* serogroup 5

Source of sera (number)	Strain no. of agglutinable suspension	Number of sera with RMAT titre					
		<16	16	32	64	128	>128
Blood donors (96)	357	81	6	6	0	3	0
	40	80	10	3	2	1	0
Patients with respira- tory symptoms (96)	357	84	6	2	2	1	1
	40	83	7	1	3	1	1

titres of ≥ 64 . There was no significant difference in the distribution of titres between the two groups.

Paired serum samples from a further 89 patients with acute respiratory symptoms were tested; only one patient showed a rise in titre, from 16 to 64 with both strains, and this increase was confirmed by IFAT. The patient, aged 40, had had a cough with pleuritic chest pain and diarrhoea beginning 2 days before the first sample was collected. The second sample was taken 13 days later. On admission to hospital he had been pyrexial with a leucocyte count of $26 \times 10^9/L$ and pulmonary consolidation. He had been treated empirically with erythromycin and had made an uneventful recovery. The general distribution of titres in these 89 patients was similar to that shown in the table.

Discussion

The RMAT for antibodies to *L. pneumophila* serogroup 5 was straightforward to develop and perform, and would be within the scope of most routine diagnostic laboratories. Good correlation was found between RMAT and IFAT titres with serogroup 5 reference sera. It was unfortunate that only two of six strains proved suitable for use in this assay: although four failed to form buttons in the RMAT, all six reacted with serogroup 5 antibody by IFAT, and they showed no cross-reaction with other groups. Thus, it would be necessary to test and select strains suitable for the RMAT.

The small study of 96 blood donors (presumed to be from the normal population), and 96 patients with

respiratory infection, confirms the low background incidence of serogroup 5 antibodies in the population. A larger study would be needed to evaluate this adequately. Single high titres of 64 or 128 were found in sera from three blood donors; no clinical information was available to indicate recent respiratory symptoms, but this does not preclude recent infection. Further work will be needed to define significant RMAT titres in patients with single serum samples. Existing RMAT guidelines relate to serogroup 1; they give diagnostic criteria as a four-fold rise in titre of paired sera, or a single titre of ≥ 32 in serum from a patient with a relevant clinical history.⁶ In the present study, a four-fold rise from 16 to 64, in the patient with clinical symptoms and signs of Legionnaires' disease, is consistent with recent infection. We feel that these are valid criteria for our serogroup 5 RMAT: the maximum variation in titre on repeated assay of our samples was two-fold; but, with the sera from the patient with a four-fold rise in titre, this difference in titre was reproducible when they were tested in parallel on several occasions.

The RMAT is a simple test; a single serum dilution of 1 in 32 would enable rapid screening of cases, though a negative result would not exclude the diagnosis. Positive sera would need to be titrated fully and confirmed by the IFAT. The RMAT could be used to investigate the extent of an outbreak of Legionnaires' disease in patients exposed to the organism.

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