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Technical methods

Serogrouping of *Legionella pneumophila* by double diffusion and counter immuno-electrophoresis

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Ten serogroups of *Legionella pneumophila* have now been described.[1] Although the direct fluorescent antibody technique is widely used to identify and serologically differentiate *L pneumophila* isolates,[2] some cross reactivity between serogroups[3] and some staining of non-legionella organisms with legionella antisera[4] have been reported. Several simple alternatives to the direct fluorescent antibody technique have been described which do not require fluorescence microscopes and competent microscopists. These include slide agglutination,[2] staphylococcal coagglutination,[5] latex agglutination,[6] and antiserum-agar plates.[7]

Counter immunoelectrophoresis and double diffusion have been used to detect human antibodies to *L pneumophila*.[8 9] The studies reported here suggest that both double diffusion and counter immunoelectrophoresis can be used as alternatives to direct fluorescent antibody when serogrouping isolates of *L pneumophila*.

Material and methods

Antisera to *L pneumophila* serogroups 1 to 6 were obtained from the Division of Microbiological

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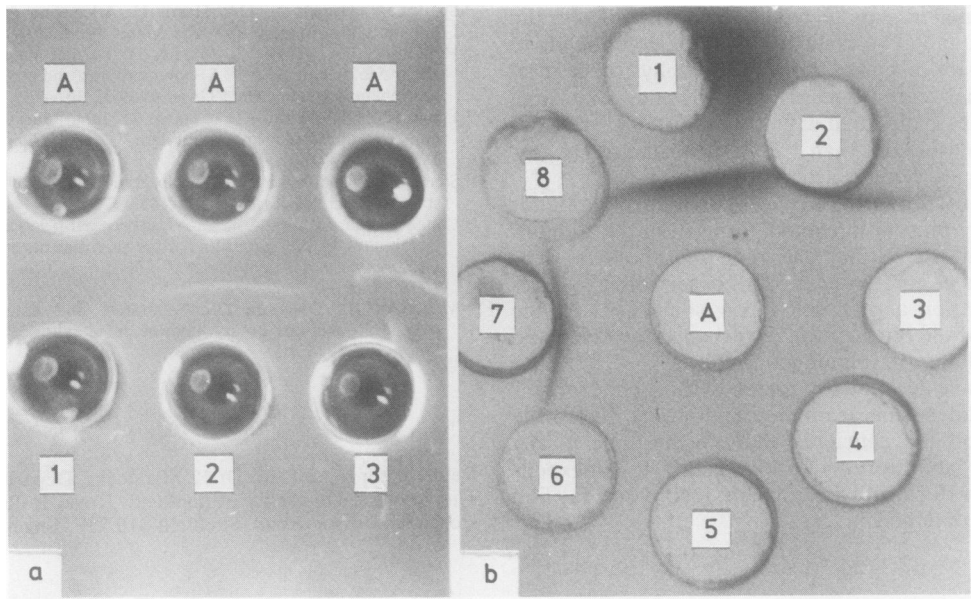

(a) Counter immunoelectrophoresis slide (unstained) and (b) double diffusion slide (stained) showing urea extracts of L pneumophila strains tested against DMRQC serogroup 1 antiserum.
Counter immunoelectrophoresis wells: (A) antiserum, (1) serogroup 6 strain (Oxford-1) (2) serogroup 1 strain (Philadelphia-1), (3) serogroup 1 strain (Pontiac-1).
Double diffusion wells: (A) antiserum, (1) serogroup 1 strain (Philadelphia-1), (2) serogroup 1 strain (East Birmingham EB-1), (3) serogroup 2 strain (EB-2), (4) serogroup 3 strain (EB-3), (5) serogroup 4 strain (EB-4), (6) serogroup 5 strain (Cambridge-2), (7) serogroup 1 strain (Pontiac-1), (8) serogroup 6 strain (Oxford-1).

Results of testing 35 Legionella pneumophila strains, 132 non-Legionella strains, and calorifier and lung samples containing L pneumophila by counter immunoelectrophoresis and double diffusion

L pneumophila antisera	*L pneumophila strains*						*Other bacterial species*	*Calorifier* deposit*	*Lung samples†*
	Serogroup								
	1	*2*	*3*	*4*	*5*	*6*			
Serogroup 1	+	−	−	−	−	−	−	−	−
Serogroup 2	−	+	−	−	−	−	NT	−	−
Serogroup 3	−	−	+	−	−	−	NT	−	−
Serogroup 4	−	−	−	+	−	−	NT	−	−
Serogroup 5	−	−	−	−	+	−	NT	−	−
Serogroup 6	−	−	−	−	−	+	NT	−	−
Pooled 1–6	−	−	−	−	−	−	NT	−	−
Normal rabbit serum	−	−	−	−	−	−	NT	−	−
Total	12	5	3	1	3	11	132	1	4

**L pneumophila* serogroup 1 isolated.
†All from positive cases of Legionnaires' disease.
NT = not tested.
+ = positive reaction.
− = negative reaction.

Reagents and Quality Control (DMRQC), Colindale, London. Fluorescent antibody titres were between 4000 and 16 000.

An antiserum to *L pneumophila* serogroup 1 was also prepared in rabbits immunised with an ultrasonically disrupted antigen.[8] This was designated Phil-1 antiserum. Fluorescent antibody titre was 5000.

Antigens for counter immunoelectrophoresis and double diffusion were extracted from 24 or 48 h old cultures on buffered charcoal yeast extract agar (Oxoid Ltd, Basingstoke, England). Organisms under test were suspended in 35 μl of phosphate buffered saline (PBS) in a glass bijou bottle to about 3×10^{10} colony forming units (CFU) /ml (roughly equivalent to two 3 cm sweeps through confluent growth). Diethyl ether (100 μl) was added, and the contents were shaken. The bottle was incubated at room temperature for 10 min and then 200 μl of 9 M urea was added, mixed, and the bottle left for a further 10 min. The mixture was centrifuged for 5 min at 2000 *g* and the supernatant was used as antigen.

Counter immunoelectrophoresis was performed as described previously[8] except that the antigen and antibody wells were 3 mm in diameter and 3 mm apart and 10 μl volumes were employed in each (Fig (a)).

Double diffusion was carried out on glass microscope slides with 3 ml of 1% agarose (BDH) in PBS, pH 7·2. Nine wells (3 mm diameter) were made: one central antiserum well and eight others at 3 mm from it for antigen extracts (Figure (b)). The slides were incubated at room temperature overnight in a moist chamber.

Results

Serogroups determined by counter immunoelectrophoresis and double diffusion showed 100% correlation with previously determined fluorescent antibody serogroups when 35 strains of *L pneumophila* were tested (Table). No cross reactions between serogroups were seen. No reactions were noted when these 35 strains were tested against pooled serogroup 1 to 6 antiserum, or when 132 non-legionella strains were tested against serogroup 1 antiserum. A calorifier deposit known to contain *L pneumophila* and four lung samples from confirmed cases of Legionnaires' disease were repeatedly negative with all antisera by both counter immunoelectrophoresis and double diffusion.

Discussion

A positive counter immunoelectrophoresis or double diffusion result was denoted by the appearance of a single slightly diffuse, almost central, precipitin line (Figure), although one serogroup 3 strain and four serogroup 1 strains occasionally produced negative results when tested. These strains were all positive when repeated. No morphological differences were seen between these strains and others apart from slightly less luxuriant growth and a tendency for the urea extract to be slightly sticky after centrifugation; however, these occasional false negative results mean that all organisms examined by these techniques should be extracted and tested in duplicate in an attempt to eliminate this problem. Strains which persist in giving a negative reaction should be examined by conventional means or sent to a reference laboratory. *L pneumophila* strains cultured on legionella blood agar (Oxoid) gave comparable results to strains grown on charcoal yeast extract agar when tested by counter immunoelectrophoresis and double diffusion. Occasional non-legionella isolates such as *Pseudomonas*

fluorescens and *Bacteroides fragilis* have been shown to give positive reactions with *L pneumophila* antisera by double diffusion and direct fluorescent antibody,[4] owing to shared antigens, but none of the 132 non-legionella strains tested here (which included 13 *P fluorescens* and 33 *B fragilis*) produced any reaction with serogroup 1 antisera (DMRQC or Phil-1) by counter immunoelectrophoresis or double diffusion.

The results indicate that counter immunoelectrophoresis and double diffusion are not sufficiently sensitive to detect the relatively small amount of antigen presumably present in clinical or environmental samples such as calorifier deposits and lung tissues. The lack of sensitivity of counter immunoelectrophoresis in detecting legionella antigens has been noted previously.[10]

Both counter immunoelectrophoresis and double diffusion can be used to group and identify strains of *L pneumophila* accurately. Double diffusion is a much simpler and more economic test than counter immunoelectrophoresis when testing multiple strains, as up to eight extracts can be tested simultaneously against 10 μl of each antiserum, whereas each extract tested requires 10 μl of antiserum by counter immunoelectrophoresis. Thus although both techniques could be used in laboratories where fluorescent antibody techniques are not available, double diffusion should be the method of choice.

The fact that none of the *L pneumophila* strains reacted with the pooled serogroup 1–6 antiserum is probably due to the consequent dilution of each individual antiserum, and it is suggested that only neat, high titre antisera such as those obtained from DMRQC should be used for counter immunoelectrophoresis and double diffusion.

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New medium for enhancing pigment production of group B streptococci

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Group B streptococci are important pathogens in neonates[1 2] and in adults.[3 4] It is important to identify group B streptococci either by serogrouping or by demonstrating one of their unique physiological characteristics. The sensitive and specific physiological method is pigment production.[5 6] Specific media[6–8] for enhancing pigment production of group B streptococci need anaerobic conditions and contain serum. The purpose of this study was to develop a new medium which does not require serum and anaerobic conditions.