

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 media⁶⁻⁸ 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.

Material and methods

One hundred and fifty five isolates of group B streptococci were obtained from clinical specimens: 127 were from vaginal or cervical specimens and 28 were from perianal regions. Another 210 isolates of streptococci from human sources were tested along with group B streptococci.

MEDIUM FOR ENHANCING PIGMENT FORMATION

The new starch medium, PPR medium, for enhancing pigment production of group B streptococci contains 3% Proteose Peptone no 3 (Difco), 5% rice powder, 0.5% NaCl, and 1% agar (pH 7.2). The medium was distributed into 13 × 75 mm test tubes and sterilised by autoclaving. The medium was allowed to cool and kept in the refrigerator overnight before further use.

Each isolate was heavily stabbed by the side of the tube down to the bottom of the PPR tube. The tubes were incubated at 35°C aerobically. The pigment producing ability was noted after 24, 48, and 72 h incubation.

All isolates were serologically identified by coagulation (Phadebact Streptococcal test; Pharmacia Diagnostic, Uppsala, Sweden) and by capillary precipitation (Antistreptococcal antisera, Difco). Their haemolytic activity and their ability to grow on 6.5% NaCl, and bile aesculin reactions were recorded accordingly.

Results

The pigment producing ability of 152 β-haemolytic strains of group B streptococci on the new pigment enhancing medium (PPR) was 100%. The three non-haemolytic isolates of group B streptococci and 210 other isolates of streptococci did not produce any pigment at up to 72 h incubation. One hundred and forty nine isolates (98%) of pigment producing group B streptococci gave positive results in 24 h, two isolates produced pigment at 48 h, and one at 72 h.

Discussion

The development of PPR medium is based on the starch serum medium described by Islam. The buffer system was removed and replaced by sodium chloride. To make the medium more opaque and efficient the soluble starch was replaced by rice powder.

Heavy stabbing of the isolates by the side of the tube down to the bottom of the medium enhanced anaerobiosis, and pigmented colonies were easily seen. Serum free Proteose Peptone no 3 was able to

support the growth of 149 of 152 isolates of group B streptococci as orange colonies within 24 h. The other three required a longer period of incubation.

The specificity and sensitivity of PPR is 100% for haemolytic strains of group B streptococci, which compares well with other effective pigment enhancing media.⁶⁻⁸ There were no false positive results among 210 isolates of streptococcal types other than

Production of pigment in PPR medium of 365 isolates of streptococci

	Serogroup				Non-grouped	
	B	A	C	D	G	
Strain	155	29	38	45	53	45
β lysis	152	28	35	30	46	7
Pigment production	152	0	0	0	0	0

group B streptococci. Serum and buffer are not needed in this medium, nor does it require anaerobic conditions. Furthermore, PPR medium is easy to obtain and inexpensive.

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