

# A note on the use of immunofluorescent methods for the detection of *Pseudomonas aeruginosa* in bronchitic sputum

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**SYNOPSIS** By immunofluorescent microscopy of sputum from 67 cases of bronchitis *Pseudomonas aeruginosa* was detectable in 14 as compared with nine by cultural methods.

Recent studies have shown that immunological methods may be of value for the detection of infective agents in respiratory disease (Nicholls *et al*, 1975). Immunofluorescence has been employed for the diagnosis of *Haemophilus* in sputum (Sell *et al*, 1963).

Severe respiratory tract infections may be caused by *Ps. aeruginosa* during hospitalization and this organism may prove invasive. Thus its identification, in the early infective stages, is desirable (Perry and Sellors, 1963; Fraser and Paré, 1970).

## Material and Methods

### HOMOLOGOUS ANTISERUM

*Ps. aeruginosa* was obtained from a case of pneumonia in the Queen Elizabeth Hospital, Birmingham and found to be a non-typable polyagglutinating strain by the Cross-infection Laboratory, Colindale. Antiserum was raised in rabbits by the method of Yager *et al* (1960) using the inoculation schedule of Thomason and Cherry (1963). The agglutination titre of the antiserum against the homologous strain of *Ps. aeruginosa* was found by the Widal technique to be 1/250, and it was subsequently shown by immunofluorescence to react with this strain and 14 newly isolated strains but failed to react with one. No cross-reactions were observed by either method with *Haemophilus influenzae*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, group A streptococcus,  $\alpha$ -haemolytic streptococcus or *Neisseria catarrhalis*.

### ANTISPECIES IMMUNOFLUORESCENT CONJUGATE

Commercial reagents having proved unsatisfactory, Received for publication 15 July 1975

immunofluorescent conjugate was prepared; sheep anti-rabbit immunoglobulin G was obtained from the Department of Experimental Pathology, University of Birmingham, and conjugated to fluorescein isothiocyanate (FITC) isomer 1 using the method of Nairn (1969). Unconjugated fluorochrome and impurities present in the FITC were removed by passing *c* 80 mg of protein through a 2.5 × 42 cm chromatography column containing Sigma G-25 Sephadex in a gel sample ratio of 6:1 (v/v).

The purified conjugate was checked for the presence of unconjugated dye using polyacrylamide gel electrophoresis (PAGE) (Ornstein, 1964; Davis, 1964) and running the gels at 493 nm in a microdensitometer (Cartwright and Jaynes, 1972). The preparation was absorbed with ox liver homogenate supplied by the Department of Rheumatism Research, University of Birmingham by the technique of Nairn (1969).

The prepared conjugate was examined by PAGE, double gel diffusion (Ouchterlony, 1948), immunoelectrophoresis (Scheidegger, 1955), chessboard titration, incorporating normal rabbit serum against conjugate dilutions as a test for non-specific staining (Hale and Bergquist, 1971), and the fluores-

PAGE	No free dye detected
Double gel diffusion	Single arc of identity
Immuno-electrophoresis	Single arc of the IgG class
Chessboard titration	PEP 1/64
	PT 1/200 to 1/500
Molar fluorescein to protein ratio	2:3

PEP = plateau end-point  
PT = plateau titre (Hale and Bergquist, 1971)

Table I *Assessment of a fluorescent antibody conjugate*

cein to protein ratio (F:P) was calculated (Jobbágy and Király, 1966) (table I).

The conjugate was also tested for specificity against the previously listed bacteria, using the homologous antibacterial antiserum, prepared in this laboratory, for the indirect immunofluorescence test. Staining reactions were controlled for inhibition of fluorescent antibodies by pretreatment with unconjugated sheep anti-rabbit immunoglobulin G, and substitution of normal rabbit serum for the homologous antiserum.

#### INDIRECT STAINING TECHNIQUE

Heat-fixed bacterial smears were covered with homologous antiserum and incubated at room temperature for 1 hour in Petri dishes containing moist filter paper. Slides were washed in phosphate buffered saline (PBS) pH 7.2, 0.145M for 30 minutes and after the removal of excess moisture were incubated with homologous antispecies conjugate for 30 minutes. After the slides had been washed in PBS for 30 minutes the preparations were mounted in glycerol/PBS (9:1) and the coverslips were sealed with wax. Preparations were examined by dark-ground, blue fluorescence with the  $\times 55$  oil immersion lens of a Gillett and Siebert 'Research Conference' microscope.

#### BACTERIOLOGICAL METHODS

Sputum samples were homogenized after pancreatin digestion and, for the purposes of comparison, were examined by Gram's stain and inoculated on horse blood agar and McConkey agar, incubated at 37°C, and inspected after 24 and 48 hours. Colonies were picked, Gram-stained, and subcultured for further examination, in accordance with routine procedures.

#### Results

A specimen of sputum was taken from each of 67 subjects of various ages and both sexes, clinically diagnosed as bronchitic. Specimens were examined

	<i>Pseudomonas aeruginosa</i>
Immunofluorescence located	14
Gram stain and culture	9
Location by both immunofluorescence, Gram stain, and culture	8
Negative for immunofluorescence but positive for Gram stain and culture	1
Positive for immunofluorescence but negative for Gram stain and culture	6

Table II *A comparison of immunofluorescence and routine bacteriology*

Total number of patients investigated—67

by both cultural and immunofluorescence methods as soon as possible. The results are given in table II and show that more positive samples were obtained by immunofluorescence examination, while substantiating, in all but one case, the results of cultural methods. Control reactions were all negative.

#### Discussion

The results recorded show that immunofluorescence provides a rapid and sensitive method of detecting a potentially important pathogen in bronchitic sputum, where rapid diagnosis provides the possibility of early chemotherapy that might otherwise be delayed.

Those cases in which a positive immunofluorescent diagnosis of *Ps. aeruginosa* was made, in the absence of a positive culture, are explicable either by cultural failure or as false positives. The former might be caused by inadequate methods, transfer of antibiotics with the inoculum, low or non-viability of the bacteria. Improved selective cultural methods should eliminate the discrepancy, but the work, as presented, is intended to provide a comparison between this method and the cultural techniques currently applied in a large number of hospital laboratories.

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