Technical methods

Counterimmunoelectrophoresis in the diagnosis of whooping cough

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Bordetella pertussis is still a major cause of severe respiratory infection in childhood. The disease is prolonged and debilitating and may be responsible for bronchiectasis in later life. The laboratory diagnosis of whooping cough is difficult and unreliable. The initial catarrhal symptoms are often mild and mistaken for a "cold." It is during this period, however, that the isolation of the organism is most likely and the rate of isolation falls rapidly once the spasmodic stage has begun. Moreover, the organism *B. pertussis* is fastidious, grows poorly on artificial medium, and is sensitive to the delay in transport from patient to laboratory.¹

Attempts have been made to confirm the diagnosis using a number of serological techniques—namely, direct agglutination, haemagglutination, indirect haemagglutination, complement fixation, immunofluorescence, and enzyme linked immunosorbent assay. Results have been varied and have usually been unsatisfactory.²⁻⁷ As these methods require the detection of a fourfold rise in antibody titre, diagnosis is retrospective.

Counterimmunoelectrophoresis (CIE) is now a well established technique for the rapid diagnosis of bacterial meningitis and pneumonia.⁸ Its major advantage is that it detects bacterial antigen and is therefore positive early in the course of the illness.

The purpose of this feasibility study was to develop a CIE method which could detect *B. pertussis* antigen in clinical specimens.

Patients and methods

Serum and urine samples, and pernasal swabs were obtained from children (age range 5 weeks to 12 years) admitted to Belvoir Park Hospital, Belfast, in whom a clinical diagnosis of whooping cough had

been made. On the basis of their clinical histories the children were divided into early and late whooping cough. An age and sex matched control group was established from routine paediatric medical admissions to the Waveney Hospital, Ballymena, N Ireland, from whom serum and urine samples were also taken.

Pernasal swabs were transported to the laboratory with minimal delay, inoculated on to Lacey's modification of Bordet-Gengou agar, and incubated aerobically at 37°C for five days. Samples of serum and urine were stored at -20°C until examined by CIE.

Antiserum against pertussis vaccine (Wellcome) was prepared in rabbits by three intramuscular inoculations of 1 ml of vaccine at biweekly intervals. Rabbits were bled one week after the final inoculation and the potency of the antipertussis antiserum confirmed by titrating against the vaccine using CIE.

CIE was carried out in 0.75% agarose (Uniscience) in barbitone acetate buffer (pH 8.6, ionic strength 0.05 M) on glass slides 8 cm × 10 cm. Wells 2.5 mm in diameter were cut in parallel rows 2 mm apart; 6 µl of either serum or urine was placed in wells on the cathode side of the plate and antipertussis antiserum (6 µl) was added to the wells nearest the anode. Pertussis vaccine was used as a positive control.

After electrophoresis, at a constant current of 20 mA for 30 min, the plate was examined for the presence of precipitin lines using a ×8 hand lens and dark ground illumination. The plate was re-examined after storage at 4°C for 30 min and again after 24 h.

All negative specimens were concentrated, serum ×5 and urine ×50, using minicon concentrators (Amicon) and the CIE repeated.

Results and discussion

Of the 35 children studied a total of 17 were diagnosed as suffering from whooping cough: eight of these were classified as "early" and nine as "late" and *B. pertussis* was isolated from the pernasal swabs in eight of these. Seventeen serum specimens and nine urine specimens were obtained from these children and were tested against antipertussis antiserum using CIE. A single precipitin line was obtained with nine (53%) serum and three (33%) urine specimens (Table). In two cases both serum and urine from the same child were positive. All positive results were visible immediately after electrophoresis for 30 min.

None of the serum or urine specimens from the control group was positive by CIE (Table).

Although only a few patients were studied, our results indicate that CIE is a reliable method of

Comparison of counterimmunoelectrophoresis (CIE) using serum and urine specimens with culture of pernasal swabs

	No of patients	Number positive/Number tested (%)			
		CIE			Culture
		Serum	Urine	Total	Pernasal swab
Early pertussis	8	5/8 (63)	2/4 (50)	7/12 (58)	4/8 (50)
Late pertussis	9	4/9 (44)	1/5 (20)	5/14 (38)	4/9 (44)
Total	17	9/17 (53)	3/9 (33)	12/26 (46)	8/17 (47)
Control	18	0/18 (0)	0/18 (0)	0/36 (0)	ND

ND = Not done.

detecting *B pertussis* antigen and may be a useful addition in the diagnosis of whooping cough. There were no false positive reactions and no cross reactivity was found between the antipertussis antiserum and *Streptococcus pneumoniae* and *Haemophilus influenzae* antigens. The method was easy to perform and required only small quantities of specimens and laboratory reagents.

CIE was more sensitive than culture methods early in the course of the illness, when the importance of symptoms is more difficult to assess. Moreover, unlike pernasal swabs specimens of serum and urine are easy to obtain and the availability of the technique may encourage general practitioners to use this laboratory facility to complement clinical diagnosis of whooping cough.

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Sample collection for determination of plasma fibronectin concentration

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Convenient blood collection methods for human plasma fibronectin determination have not been fully explored. We have investigated whether glass tubes can be used as an alternative to the recommended plastic tubes for collection¹ and whether citrated blood samples can be stored at 4°C for 22-26 h before separation and testing.

Material and methods

INTRA-ASSAY COEFFICIENT OF VARIATION
Fibronectin concentrations were determined by the