Staphylococcus epidermidis to smooth surfaces. *Infect Immun* 1982;37:318-26.

² Peters G, Locci R, Pulverer G. Adherence and growth of coagulase negative staphylococci on surfaces of intravenous catheters. *J Infect Dis* 1982;146:479-82.

³ Kristinsson KG, Hastings JGM, Spencer RC. Coagulase negative staphylococcal infections. *Br Med J* 1985;290:1743.

⁴ Peters G, Pulverer G. Pathogenesis and management of *Staphylococcus epidermidis* "plastic" foreign body infections. *J Antimicrob Chemother* 1984 (Suppl D);14:67-71.

Commercial strip test for reduction of nitrate by bacteria

The ability of an organism to reduce nitrate is commonly tested by the Griess-Ilosvay method.¹ This entails two reagent mixtures, one of which contains naphthylamine, a potentially harmful chemical. An alternative is the Cook plate method, in which the organism is stab inoculated on a blood agar plate containing nitrate.² The reaction in the plate test is irreversible and is unaffected by any subsequent reduction of the nitrite. The zones of discolouration caused by the formation of methaemoglobin are, however, often indistinct, and the plate may require 48 hours of incubation. As an alternative to these methods we evaluated a commercial strip designed to screen for clinically important bacteriuria by detecting nitrite derived from dietary metabolites (Ames Division, Miles Laboratories). In this test the nitrite reacts with p-arsanilic acid to form a diazonium compound that is coupled with 1,2,3,4-tetrahydrobenzo(h)-quinoline-3-ol to produce a pink colour.

Material and methods

A nitrate broth was prepared containing potassium nitrate (nitrite free) 0.3% and tryptone (Oxoid L42) 0.5% w/v in distilled water. The broth was distributed in 3 ml volumes in bijoux bottles and autoclaved at 115°C for 10 minutes.

Clinical isolates from a representative range of nitrate positive and nitrate negative genera were collected. For the test a broth was inoculated from a fresh subculture to give a density of about 10⁸ colony forming units/ml. After incubation at 37°C the broth was tested for the presence of nitrite at 4 hours and again at 24 hours using the commercial strips. A strip was immersed in the broth, immediately removed, drained on the inside of the container, and read at 40 seconds, as recommended for urine testing by the manufacturer (Technical information, Miles Laboratories). Any degree of pink colour was regarded as a positive test. At 24 hours the broth was tested for nitrate by a modified Griess-Ilosvay method. Broth (200 µl) was pipetted into a microtitre well, and 50 µl of 0.8% sulphanilic acid and 0.5% Cleve's acid, dimethyl-α-naphthylamine, added. A pink colour indicated the reduction of nitrate to nitrite. If no colour change occurred the test was repeated in a well containing about 30 mg of zinc dust.³ Development of a pink colour at this stage indicated that the nitrate had not been reduced by the test organism, whereas absence of colour indicated total breakdown of nitrate and nitrite. All strains were tested by the Cook plate method using a paper strip impregnated with potassium nitrate and incubated for up to 48 hours.²

Results

Fifty eight of the 66 strains tested showed concordance of the commercial strip method, Cook plate, and modified Griess-Ilosvay method at 24 hours. The 58 strains included: Nitrate positive (1): *E. coli* (5), *Klebsiella* (4), *Haemophilus* (6), *Enterobacter* (2), *Proteus* (4), *S. aureus* (5), *Actinomyces* (3), and *Branhamella catarrhalis* (3); Nitrate negative (1): *Listeria* (5), *Bordetella* (2), *Flavobacterium* (2), *Lactobacillus* (5), and *A. nitratum* (12). The Table shows the results given by the other eight strains.

When tested by the strip method 63 of the 66 organisms gave the appropriate nitrate reaction¹ at four hours, and the other three

strains gave the appropriate reaction at 24 hours.

From the results so far obtained we concluded that the strip test is a reliable method for the detection of bacterial nitrate reduction, as most positive tests are readable at four hours. The test avoids the problems of chemical preparation, storage, and handling.

We thank Mr RG Newell of Ames Division, Miles Laboratories Limited, for supplying the nitrite reagent strips.

AP KEE

RG MITCHELL

Department of Microbiology

John Radcliffe Hospital

Headington

Oxford OX3 9DQ

References

- ¹ Cowan ST, Steel KG. *Manual for the identification of medical bacteria*. 2nd ed. London: Cambridge University Press, 1974.
- ² Cook GT. A plate test for nitrate reduction. *Clin Pathol* 1950;3:360-2.
- ³ Zobell CE. Factors influencing the reduction of nitrates and nitrites by bacteria in semi-solid media. *J Bacteriol* 1932;24:237.

Human parvovirus specific IgM in antigen positive serum

In 1984 a programme of virological studies on infection with the newly described human parvovirus,¹ now designated B19 virus,² was started in Florence. We report here our first observations.

Stored sera, collected from May to July 1983 for other epidemiological purposes from 221 healthy soldiers assigned to five different units were screened for immunoelectro-osmophoresis reactivity to B19 antibodies. Polyethylene glycol 6000 was added to the gel to increase the sensitivity of the test. The antigen was detected in two subjects stationed in different units. Serum samples of both subjects had low immunoelectro-osmophoresis reactivity. The serum of one subject with mild respiratory syndrome some days before the sampling (incomplete information), was weakly positive for B19 DHA by molecular hybridisation,³ and viral particles of the B19 size and morphology were seen by electron microscopy. Although specific antibodies had not been added, virions and empty particles appeared as aggregates, and cross linking Ig was visible. Precipitating antibodies to B19

Table Nitrate reductase: results for eight bacterial strains using three different methods

Organism	Nitrate reduction test				
	Cook plate		Commercial strip		Griess-Ilosvay test 24 hour
	24 hour	48 hour	4 hour	24 hour	
Coagulase negative <i>Staphylococcus</i>	—	+	+	+	+
Coagulase negative <i>Staphylococcus</i>	—	+	+	+	+
<i>Acinetobacter lwoffii</i>	—	—	+	+	+
<i>Acinetobacter lwoffii</i>	—	+	—	—	+
<i>Pseudomonas aeruginosa</i> (four strains)	+	+	+	—	+

virus were, indeed, present in the serum, and specific IgM was detected by radio-immunoassay.⁴ The other subject had no record of any recent clinical symptoms. His serum had traces of DNA and was negative for IgM and IgG.

Antibodies shown by immunoelectro-osmophoresis were found in about 25% of the contacts of the two antigen positive subjects. A similar prevalence of human parvovirus infection has been observed in the general population (study in preparation). We must point out, however, that the lack of a follow up on the antibodies in the subjects does not prove that this high incidence of viraemia was related to human parvovirus outbreaks in the two units at that time.

The detection in serum of antigen and antibody at the same time, although uncommon, is of some interest and adds evidence to some previous observations on the persistence of the human parvovirus antigenemia during the initial phase of the humoral response.^{4,5} Immunocomplex deposits cause damage leading to a variety of syndromes and some of them, such as rashes and arthralgia, may follow infection with parvovirus.¹ It would be interesting to find associations between immune complexes and the clinical course of the disease. Assays of circulating human parvovirus immune complexes in acute and early convalescent serum samples may provide useful information.

The DNA and IgM and IgG tests were performed in the department of medical microbiology, University College Hospital, London.

We thank Dr MJ Anderson for providing virus, reference sera, and research facilities.

O BARTOLOMEI CORSI
A AZZI

*Institute of Microbiology,
University of Florence,
Viale Morgagni 48,
50134 Florence, Italy*

References

- Anderson MJ, Pattison JR. The human parvovirus. *Arch Virol* 1984;**82**:137-48.
- Siegl G, Bates RC, Berns KI, et al. Characteristics and taxonomy of parvoviridae. *Inter-virology* 1985;**23**:61-73.
- Anderson MJ, Jones SE, Minson AC. Diagnosis of human parvovirus infection by dot-blot hybridisation using cloned viral DNA. *J Med Virol* 1985;**15**:163-72.
- Cohen BJ, Mortimer PP, Pereira MS. Diagnostic assays with monoclonal antibodies for the human serum parvovirus-like virus (SPLV). *J Hyg* 1983;**91**:113-30.
- Pattison JR, Jones SE, Hodgson J, et al. Parvovirus infections and hypoplastic crisis in sickle-cell anemia. *Lancet* 1981;**i**:664-5.

Survival of *Bordetella pertussis* in transport media

Concern has been repeatedly expressed over the low isolation rates of *B. pertussis* from clinical cases of whooping cough.¹⁻³ Poor recovery from pernasal swabs is thought to be due to previous immunisation,¹ faulty swabbing technique,⁴ or retention of the organism on the swab.⁵ This study was performed to determine what effect the transport media used had on the survival of the organism.

A heavy suspension of the laboratory stock strain of *B. pertussis* was made in saline. Forty two cotton wool pernasal swabs (Medical Wire and Equipment) were then dipped into this suspension. Fourteen swabs were replaced into their plastic tubes (dry swabs). The ends of the remaining swabs were cut off and half of them placed into bijoux bottles of Amies' transport medium (Difco Laboratories); the other half were placed into a similar container of Stuart's transport medium (Oxoid). All the swabs were left on the open bench for one, two, four, eight, twenty four, forty eight, or ninety six hours. At the appropriate time the swabs were transferred to 1 ml of sterile physiological saline in a bijoux bottle and shaken vigorously for thirty seconds on a bench shaker. Miles and Misra counts⁶ were then performed on the resulting suspension on Bordet-Gengou medium and the plates incubated at 37°C for three days before counting.

Table 1 shows the mean viable count of

B. pertussis recovered from the swabs at various times. The counts on the dry swabs were higher for the first four hours than those of the swabs in Stuart's or Amies' media. After four hours the counts fell most rapidly on the swabs in Stuart's medium but less rapidly on the dry swabs. The counts on the swabs in Amies' medium, however, remained high after ninety six hours when the other swabs yielded no growth.

Table 2 shows the decimal reduction times of the viable counts calculated from the linear regression curves over twenty four, forty eight, and ninety six hours. The decimal reduction times were consistently higher in Amies' medium and lower in Stuart's medium.

The results of this study suggest that if a pernasal swab can be transported to the laboratory within four hours transport medium is unnecessary. If the swab is likely to be delayed for more than four hours Amies' transport medium should be used rather than Stuart's transport medium.

PR HUNTER

*Public Health Laboratory,
University Hospital of Wales,
Cardiff CF4 4XW.*

References

- Public Health Laboratory Service Working Party. Diagnosis of whooping cough: comparisons of serological tests with isolation of *Bordetella pertussis*. A combined Scottish study. *Br Med J* 1970;**IV**:637-9.
- Lewis FA, Gust ID, Bennett NMCK. On the aetiology of whooping cough. *J Hyg* 1973;**71**:139-44.

Table 1 Mean viable counts of *B. pertussis* on pernasal swabs at various intervals after inoculation

Time after inoculation (hours)	Transport conditions		
	Dry	Stuart's	Amies'
1	3.5×10^6	1.2×10^6	1.2×10^6
2	2.1×10^6	3.5×10^6	6.5×10^5
4	2.3×10^6	1.3×10^6	1.5×10^6
8	7.3×10^4	3.8×10^4	2.6×10^5
24	3.9×10^4	1×10^2	1.8×10^6
48	4.1×10^2	25	1.8×10^6
96	0 < 25	0 < 25	5.0×10^5

Table 2 Decimal reduction times of viable counts of *B. pertussis*

Calculated over initial time (hours)	Transport conditions		
	Dry	Stuart's	Amies'
24	9.3	5.1	16.5
48	12.0	7.7	23.0
96			37.6