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. 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. The reaction in the plate test is irreversible and is unaffected by any subsequent reduction of the nitrite. The zones of discoloration 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 bacteria by detecting nitrite derived from dietary metabolites (Ames Division, Miles Laboratories). In this test the nitrate 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 bijou 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 10^8 colony forming units/ml. After incubation at 37°C the broth was tested for the presence of nitrate 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% sulphamic acid and 0.5% Cleve’s acid, dimethyl-a-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. 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.

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), Saureus (5), Actinomyces (3), and Branhamella catarrhalis (3); Nitrate negative (1): Listeria (5), Bordetella (2), Flavobacterium (2), Lactobacillus (5), and A. anitratum (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 reaction 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, for supplying the nitrite reagent strips.

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References


Human parvovirus specific IgM in antineutrophic serum

In 1984 a programme of virological studies on infection with the newly described human parvovirus, now designated B19 virus, 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 immuno-electro-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 immuno-electro-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, 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 was visible. Precipitating antibodies to B19...
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,\(^4\) faulty swabbing technique,\(^5\) or retention of the organism on the swab.\(^6\) 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 bijou bottle and shaken vigorously for thirty seconds on a bench shaker. Miles and Misra counts\(^6\) 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.

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References


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

<table>
<thead>
<tr>
<th>Time after inoculation (hours)</th>
<th>Transport conditions</th>
<th>Dry</th>
<th>Stuart's</th>
<th>Amies(^7)</th>
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Table 2  Decimal reduction times of viable counts of B pertussis

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<th>Calculated over initial time (hours)</th>
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