Table Detection by the PCR of *C trachomatis* elementary bodies on slides

No of elementary bodies detected by DFA	No of slides tested	No of slides positive by the PCR
5-10	6	6
4	3	2
3	4	3
2	9	6
1	11	7
0	15	0

containing only one elementary body. All 15 smears in which elementary bodies were not detected when examined by the DFA test were also negative when tested by the PCR, suggesting that they had not been contaminated with DNA.

Discussion

The technique of examining fixed smears on slides by the PCR is valuable in two respects. First, it provides further validation of the ability of the DFA test to detect small numbers of elementary bodies on slides,⁴ and at the same time the results confirm the sensitivity and specificity of the PCR for *C trachomatis*.² We regard it as remarkable that fixed and stained smears of material seen to contain one or two elementary bodies can be removed from a slide and shown almost always to be positive by the PCR. The organisms in these samples or their DNA apparently remained within the smears on the slides and there was nothing to

be gained by also testing the coverslips. However, a smear is sometimes seen to be "floating" when examined by the DFA test. In this specific circumstance material on the underside of the coverslip should be rinsed off and added to the suspension on the slide. The practical value of the procedure we describe lies not only in its ability to confirm or refute, on the same specimen, results obtained previously by using the DFA test, but also in permitting retrospective examination of stored slides such as those, for example, of synovial fluid deposits from patients with sexually acquired reactive arthritis,⁵ for which the use of a confirmatory test would be of particular value.

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Rapid method for differentiating strains of *Branhamella catarrhalis*

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Abstract

The ability of *Branhamella catarrhalis* to cause nosocomial infections is a matter of some controversy. The API ZYM research kit for detecting 89 enzymes was used on 49 isolates of *B catarrhalis* to select enzymes of potential use in differentiating clinical isolates. Twenty nine enzymes were produced by all isolates (13 strongly positive) and many of these were esterases; 16 enzymes were not detected in any isolate (40 if a more stringent criterion was used). Twenty enzymes were selected to form a prototype biotyping panel which allowed 17 different patterns of reactivity to be recognised. Of the 49, 34 isolates were confined to the three commonest

patterns. Only one isolate was untypable using this panel due to lack of reactivity. A kit with these 20 substrates may be sufficiently discriminatory to be useful in the rapid study of outbreaks of infection caused by *B catarrhalis*.

Now widely accepted as a potential pathogen of the upper and lower respiratory tracts, *Branhamella catarrhalis* is also gaining recognition as a cause of conjunctivitis, meningitis, and septicaemia.¹ Although often addressed as *Moraxella* (*Branhamella*) *catarrhalis*, proposals have once again been made to alter the taxonomic status of this organism.² In recent years much attention has been focused on the

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increasing propensity of clinical isolates to produce β -lactamase, while its suspected ability to cause nosocomial infections is currently being debated by microbiologists and clini-

Table 1 Number of isolates positive for each enzyme tested

Enzyme	% Positive	% strongly positive
1 C4 esterase	100	100
2 C5 esterase	100	100
3 C6 esterase	100	100
4 C8 esterase	100	100
5 C9 esterase	100	100
6 C10 esterase	100	100
7 L-aspartate arylamidase	100	100
8 L-alanine arylamidase	100	100
9 L-glutamine arylamidase	100	100
10 α -L-glutamate arylamidase	100	100
11 glycyl-L-alanine arylamidase	100	100
12 L-leucyl-L-alanine arylamidase	100	100
13 L-phenylalanyl-L-proline arylamidase	100	100
14 L-arginine arylamidase	100	98
15 L-lysine arylamidase	100	96
16 L-seryl-L-methionine arylamidase	100	94
17 C18 esterase	100	94
18 α -L-glutamyl-a-L-glutamic arylamidase	100	93
19 α -L-aspartyl-L-alanine arylamidase	100	83
20 L-lysyl-L-alanine arylamidase	100	80
21 L-lysyl-L-lysine arylamidase	100	74
22 glycyl-L-arginine arylamidase	100	59
23 glycyl-proline arylamidase	100	54
24 α -L-aspartyl-L-arginine arylamidase	100	46
25* L-prolyl-L-arginine arylamidase	100	44
26 N-acetyl- β -D-glucosaminidase	100	35
27 glycyl-glycine arylamidase	100	13
28 L-serine arylamidase	100	13
29 L-ornithine arylamidase	100	9
30* L-phenylalanyl-L-prolyl-L-alanine arylamidase	98	96
31 α -L-arabinoside	98	13
32* L-phenylalanine arylamidase	98	6
33* L-lysyl-L-serine-4-methoxy arylamidase	98	2
L-alanyl-L-phenylalanyl-L-prolyl-L-alanine arylamidase		
34* L-alanyl-L-arginine arylamidase	96	94
35* L-alanyl-L-phenylalanyl-L-proline arylamidase	96	43
36* L-tyrosine arylamidase	96	24
37 glycine arylamidase	94	0
38* L-arginyl-L-arginine arylamidase	91	4
39* α -D-xylosidase	87	11
40 L-isoleucine arylamidase	87	0
41* β -D-mannosidase	81	2
42 L-histidyl-L-phenylalanine arylamidase	78	0
43* glycyl-phenylalanine arylamidase	72	2
44* L-phenylalanyl-L-arginine arylamidase	59	9
45* α -L-glutamyl-L-histidine arylamidase	56	6
46* methionine arylamidase	56	4
47* leucyl-glycine arylamidase	43	9
48* L-leucyl-L-leucyl-L-valyl-L-tyrosyl-L-seryl arylamidase	37	6
49* α -maltosidase	37	2
50 -D-galacturonohydrolase	19	0
51 α -L-fucosidase	17	0
52 α -D-galactosidase	17	0
53 β -D-glucuronidase	15	0
54 β -L-fucosidase	15	0
55 β -D-galactosidase	15	0
56 L-proline arylamidase	13	2
57* L-seryl-tyrosine arylamidase	11	2
58 β -D-fucosidase	11	0
59 β -D-lactosidase	11	0
60 N-CBZ-arginine-4-methoxy arylamidase	9	0
61 α -D-glucosidase	9	0
62 β -maltosidase	8	0
63 glycyl-L-tryptophane arylamidase	7	0
64 L-histidyl-L-serine arylamidase	6	2
65 s-benzyl-cysteine arylamidase	6	0
66* β -D-glucosidase	6	0
67 β -D-xylosidase	6	0
68 L-tryptophane arylamidase	6	0
69 L-valyl-L-tyrosyl L-serine arylamidase	4	0
70 L-alanine arylamidase	4	0
71 L-histidine arylamidase	2	2
72* L-threonine arylamidase	2	0
73 N-acetyl-glycyl-L-lysine arylamidase	2	0
74 C12 esterase	0	0
75 C14 esterase	0	0
76 C16 esterase	0	0
77 L-pyrrolidone arylamidase	0	0
78 L-hydroxyproline arylamidase	0	0
79 g-glutamyl transferase	0	0
80 N-benzoyl-leucine arylamidase	0	0
81 N-CBZ-glycyl-glycyl-arginine arylamidase	0	0
82 L-histidyl-L-leucyl-L-histidine arylamidase	0	0
83 N-benzoyl-L-alanine-4-methoxy arylamidase	0	0
84 N-CBZ-arginyl-4-methoxy arylamidase	0	0
85 N-CBZ-glycyl-glycyl-L-arginine arylamidase	0	0
86 α -L-rhamnosidase	0	0
87 α -D-mannosidase	0	0
88 N-acetyl- α -D-glucosaminidase	0	0
89	0	0

* chosen for inclusion in panel of 20

Table 2 Characterisation of enzyme reactivity patterns and distribution of isolates among patterns

Pattern	Enzymes strongly positive*	Numbers of isolates
A	30,34	24
B	30,34,35	6
C	30,34,36	4
D	30,34,35,36	1
E	30,34,35,45	1
F	30,34,46	1
G	30,34,35,39	1
H	30,34,35,36,38	1
I	30,34,35,36,46,72	1
J	30,34,35,44,45,47,48	1
K	30,34,35,36,44,47,48	1
L	30,34,35,36,41,47	1
M	30,32,34,35,36,39,47,57	1
N	30,34,35,36,39,44	1
O	30,32,33,34,35,36,39,43,44,45,66	1
P	30,32,34,35,36,38,39,44,47,48,49	1
Q	25	1
NT	Not typable	1

* from panel of 20 selected enzymes

cians alike.^{3,4} Recent attempts to prove that there are differences among the strains have included analyses of whole cell protein profiles,⁵ lipopolysaccharide serology,⁶ isoenzymes,⁴ resistotyping⁷ and restriction endonuclease typing.^{3,8} Although these have met with some success, no universal typing scheme for the genus has yet been devised.

Methods

Forty nine clinical isolates of *B. catarrhalis*, collected from a range of body sites from patients with a wide variety of diseases, were tested with the API ZYM Research Kit (API-bioMerieux (UK) Ltd, Grafton Way, Basingstoke, England). This strip based test consists of enzymic substrates with a colour development system to detect the presence of 89 enzymes and is based on the commercially available 20 enzyme API ZYM kit.⁹ Cultures were identified as *B. catarrhalis* if they were Gram negative cocci, oxidase, tributyrin, and DNase positive, and failed to produce acid from glucose, maltose, sucrose and lactose. Suspensions of cells to a density of McFarland Standards 5 or 6 were made in sodium phosphate buffer 0.01M (pH 7.5) and then used in the kits according to the instructions. Results were recorded in the standard API ZYM manner with a score of 0 (no reaction) to 5 (maximum colour). Scores of 3 or greater were regarded as strong positive.

Results

The percentage of isolates that produced each enzyme is shown in table 1. Twenty substrates thought to be the most discriminatory tests were selected for inclusion in a prototype panel (table 2). Tests for oxidases were omitted because of the difficulty in assessing colour changes with the para-nitro-phenol indicator system used. The data were reanalysed using this panel and 17 different patterns of strong reactivity (scoring 3 or greater) were identified (table 2). The commonest pattern was shared by 23 (47%) of the clinical isolates and the type strain (NCTC 11020). The three most common patterns contained 34 (69%) of the isolates while one isolate failed to react with the

panel and was therefore regarded as not typable. No association was found between any one pattern and the geographical areas or body sites from which isolates were obtained. Pairs of isolates from different body sites of the same patient produced the same pattern in one case and a very similar one in another. The isolates of the latter pair have exhibited differences using other techniques (data not shown).

Discussion

Branhamella catarrhalis is thought to be relatively inert biochemically because of its inability to utilise the routine carbohydrates used for identifying Gram negative cocci in the laboratory. One approach likely to be profitable in typing this organism is to analyse its enzyme profile rather than concentrating on particular substrates. Tests used in the identification and taxonomy of bacteria are most useful if they are present in either 0% or 100% of strains—that is, those that give consistent results. However, tests that are likely to be useful in typing a species are those that are positive in only some strains—that is, those that are likely to give inconsistent results. If a number of the latter tests are used against a single strain a discriminatory typing scheme is likely to result even if individually they are not thought to be useful. However, not all the potentially useful typing tests are exclusive to this group of inconsistently present enzymes. We found that all our isolates consistently produced most esterases, and analysis of isomers of these is a potentially useful typing method.⁴ Previous work on enzymes in this and other related

organisms has relied on electrophoretic methods.¹⁰ Our study shows that a kit of 20 easily detected enzymes has potential as a method of typing this genus quickly and relatively easily. Its use in a suspected outbreak would enable such a scheme to be epidemiologically validated. The 20 selected substrates are not yet available in a single package but this may be addressed by the manufacturers in the future.

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A viscometric method of measuring plasma fibrinogen concentrations

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Abstract

A technique based on deducing the viscosity of serum from that of plasma was compared with the commonly used Clauss method. The two methods correlated closely ($r = 0.914$). The reproducibility of the viscometric method was slightly poorer than the Clauss technique at low fibrinogen concentrations, equal to that at medium fibrinogen concentrations, and marginally better at high concentrations. Fibrinogen can therefore be measured reasonably accurately with the viscometric method, and can be recommended as an alter-

native for laboratories possessing a viscometer.

The identification of fibrinogen as a major cardiovascular risk factor¹ has increased the demand for a quick, simple, inexpensive and reproducible method to determine its plasma concentration. There are many methods of quantifying plasma fibrinogen; possibly the best² and certainly the most common is the Clauss method.³

Due to its large size and spherical shape, fibrinogen strongly influences plasma viscosity.¹ It is the only constituent of plasma that

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