Technical methods

An evaluation of the API-20 STREP system

GLENN S TILLOTSON *Department of Surgery and Microbiology, University of Manchester School of Medicine, Hope Hospital, Salford M6 8HD*

Members of the Streptococcaceae family have a considerable impact on man and animals, causing a variety of diseases. These conditions include classical group A *Streptococcus pyogenes* infections, group B streptococcal meningitis in neonates[1 2] also pneumonia and endometritis in adults,[3] *Strep dysgalactiae* meningitis in neonates,[4] endocarditis due to group G streptococci[5] and newly recognised human diseases due to group R streptococci[6 7] which are normally pathogenic only to animals, particularly pigs. The viridans streptococci produce infections ranging from septicaemia to deep-seated abscesses.[8]

Microbiologists usually rely upon serological techniques to characterise streptococcal isolates as conventional biochemical tests are slow, tedious and time-consuming. These serological methods are based on Lancefield's type specific polysaccharide antigens,[9] and there are several commercial kits available for these tests.[10–12] These techniques give reliable results only with beta-haemolytic streptococci and variable results with group D organisms, also the maximum number of groups identifiable is six. Thus alpha- and non-haemolytic streptococci require further biochemical tests to avoid being termed viridans or indifferent streptococci.

In an attempt to combine various methods into one compact and rapid system, Appareils et Procèdes d'Identification (API) produced the API-STREP system which used reactive enzymes from the API-ZYM[13 14] and hippurate and aesulin hydrolysis along with the fermentation of mannitol, raffinose, and glycogen. This system was evaluated by Waitkins *et al*[15] who found the method to be easy and reliable but conscious of the limited data base API subsequently have produced API-20 STREP to supersede the original kit.

Material and methods

ORGANISMS

The following type strains were obtained from the National Collection of Type Cultures, Colindale, London. *Strep pyogenes* group A (NCTC 8198); *Strep agalactiae* group B (NCTC 993); *Strep* group C (NCTC 7022); *Strep equisimilis* (NCTC 8543); *Strep* group G (NCTC 9063); *Strep* group H (NCTC 10231); *Strep* group L (NCTC 6406); *Strep lactis* group N (NCTC 6681); *Strep* group Q (NCTC 9938); *Strep* group R (NCTC 10234); *Strep* group S (NCTC 10237); and *Strep* group T (NCTC 10446).

The remaining streptococci studied were from routine clinical specimens: *Strep* group A 12; *Strep* group B 13; *Strep* group C 7; and *Strep* group G 2; Lancefield group D 25, consisting of *Strep faecalis* 15, *Strep durans* 2, *Strep bovis* 2, *Strep faecium* 5, and *Strep equinus* 1. The following viridans streptococci were also tested *Strep milleri* 19, *Strep mitis* 9, *Strep sanguis* 5, and *Strep salivarius* 1.

CULTURAL METHODS

The organisms to be tested were first grown on 5% defibrinated horse blood agar using Columbia agar base (code number LAB 1) London Analytical Bacteriological Media (LAB M), Ford Lane, Salford, England.

CONVENTIONAL IDENTIFICATION METHODS

All test isolates were routinely inoculated on to Columbia blood agar and incubated overnight at 37°C in 90% nitrogen, 5% hydrogen, 5% carbon dioxide in a Raven scientific anaerobic cabinet (Forma Scientific, Marietta, Ohio, USA). Haemolysis was noted, and each organism was serologically grouped using Streptex (Wellcome Ltd, Beckenham, Kent); this is the system routinely used in our laboratory. The viridans and non-haemolytic streptococci were identified using the following tests: aesculin hydrolysis, arginine dehydrolase, Voges-Proskauer, and pyruvate hydrolysis[16] also carbohydrate fermentation in Fastidious Anaerobe Broth (LAB M) containing 0·02% bromocresol purple (adaptation of Waitkins *et al*[16]). The carbohydrates tested were lactose, sucrose, mannitol, salicin, glucose, maltose and arabinose; the solutions were dispensed in bijoux and steamed for 10 min. The results were then compared to the scheme of Colman and Williams.[17]

API-STREP MEDIUM

Cysteine	0·5 g
Tryptone	20·0 g
Sodium chloride	5·0 g
Sodium sulphite	0·5 g
Phenol red	0·17 g
Distilled water	1000 ml
pH	7·8

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API-20 STREP IDENTIFICATION METHOD

After confirmation of genus identification one isolated colony was subcultured onto Columbia blood agar (whole plate) and incubated anaerobically overnight at 37°C. Haemolysis was recorded and the manufacturers' instructions followed which unlike API-STREP no longer requires the broth incubation stage and washing with centrifugation. The new method allows inoculation directly from a purity plate. The tests used in this kit and the principle reaction involved are shown in Table 1.

Table 1 *Tests and reactions used in API-20 STREP*

VP pyruvate	Acetoin production
HIP hippurate	Hydrolysis
ESC aesculin	β glucosidase
PYRA pyrrolidonyl-2-naphthylamide	Pyrrolidonylarylamidase
αGAL 6 Br 2 naphthyl αD galactopyranoside	αgalactosidase
βGUR naphthol ASBI βD glucuronate	βglucuronidase
βGAL 2 naphthyl βD galactopyranoside	βgalactosidase
PAL 2 naphthylphosphate	Alkaline phosphatase
LAP L-leucyl 2 naphthylamide	Leucinearylamidase
ADH arginine	Arginine dehydrolase
RIB ribose	Acidification
ARA L-arabinose	
MAN mannitol	
SOR sorbitol	
LAC lactose	
TRE trehalose	
INU inulin	
RAF raffinose	
AMD starch	
GLYG glycogen	

After four hours incubation reagents ZYM A and ZYM B were added to cupules PYRA to LAP, ninhydrin was added to the HIP cupule, and to the VP cupule was added one drop 40% potassium hydroxide and one drop 6% alpha naphtol in ethyl alcohol. These tests required 10 min to develop and were then compared with the manufacturers' guide sheet for results. The ESC, ADH and carbohydrate reactions can be read at 4 h and 24 h.

Results

The API-20 STREP reactions were scored according to the colour changes in each cupule and the results split into triplets coded according to the octel system and the code number fitted into the API profile index. The results obtained are presented in Tables 2, 3 and 4. One hundred organisms were used in this preliminary evaluation. Initially the majority of these strains were identified using conventional techniques, although the NCTC streptococcal groups H, L, N, R, S and T were not identified by the conventional methods of our laboratory. API-20 STREP results correlated 100% with the NCTC identities.

Discussion

Analysis of the results in Table 2 shows that Lancefield groups A and B give 100% correlation with conventional serological procedures and the API-20 STREP identification method. Two particular code numbers 0463015 and 0463415 occurred with both *Strep equisimilis* group C and some group G streptococci, so confusion may arise in the differentiation of certain group C and group G strains but these can usually be separated by serological tests. Within the API data base there are at least four other codes for group G isolates and 10 codes for group C streptococci including *Strep equi*, *Strep zooepidemicus* and *Strep dysgalactiae*.

The Lancefield group F organisms identified by Streptex are regarded as being synonymous with *Strep milleri* and are thus considered in the viridans streptococci results.

Lancefield group D organisms gave correct

Table 2 *Identification of beta-haemolytic streptococci*

Species	*No tested*	*API profile*	*Correct profile*	*No correctly identified*
Lancefield group A	12	0161414	5	5
	12	0161417	6	6
		0161415	1	1
Lancefield group B	13	3063015	1	1
		3263014	1	1
		3463005	1	1
		3463015	7	7
		3463415	3	3
Lancefield group C	7	0463015*	3	3
		0463415	1	1
		0463411	1	1
		0463607	2	2
Lancefield group G	2	0463015*	2	2

*Same code profile for *Strep equisimilis* group C and *Strep* group G Only serological techniques can separate these biotypes.
Lancefield group F are included as *Strep milleri* on Table 4.

Table 3 *Identification of Lancefield group D streptococci*

Species	*No tested*	*API profile*	*Correct profile*	*No correctly identified*
Strep faecalis	15	7143711	8	8
		7153711	3	3
		7143311	3	3
		7153710	1	1
Strep faecium	5	7157110	3	3
		7157510	2	2
Strep faecium ss durans	2	5143410	1	1
		5353410	1	1
Strep bovis I	2	5240553	2	2
Strep equinis	1	5040100	1	1

Table 4 *Identification of the viridans streptococci*

Species	*No tested*	*API profile*	*Correct profile*	*No correctly identified*
Strep milleri I	16	1061414	8	8
		1061014	6	6
		1061415	2	2
Strep milleri II	3	5061430	3	3
		0041410	3	3
		0050401	2	2
		0040450	1	1
Strep mitis	9	0040401	1	1
		0042040	1	1
		0141010	1	1
Strep sanguis I (NCTC 10231 group H)	3	4060410	2	2
NCTC 10231 group H		4261430	1	1
Strep sanguis II	3	0260440	1	1
		0270440	1	1
		1250440	1	1
Strep salivarius	1	5270450	1	1
Strep group L (NCTC 6406)	1	0463017	1	1
Strep group N (NCTC 6681)	1	4043410	1	1
Strep suis I (NCTC 10237 group S)	2	4751433	1	1
(NCTC 10446 group T)		0641433	1	1
Strep suis II (NCTC 10234 group R)	1	4751473	1	1

species identifications with API-20 STREP, *Strep faecalis*, *Strep faecium*, *Strep faecium ss durans*, *Strep bovis* and *Strep equinus*. Only *Strep faecium ss durans* and *Strep equinus* required 24 hours' incubation to obtain identification. Unfortunately not all the species and subspecies within the Lancefield group D could be examined but those which were omitted tend to be of limited clinical significance.

The performance of this new identification system with the viridans streptococci was excellent. Thirty-five strains were identified by this technique as compared with only thirty by conventional methods; the remaining five were identified by external laboratories. The conventional methods required five days incubation before all the results were available; this compared with 24 hours' incubation as the longest period required to identify some of the test strains by API-20 STREP. Waitkins[15] found that the previous kit was not totally reliable for *Strep milleri* but the new system appears to have overcome this and the other viridans streptococci give 100% correlation with conventional methods. This is in agreement with Waitkins' findings.

Several NCTC strains were examined, all of which were non-identifiable by routine methods, whereas API-20 STREP accurately identified Lancefield groups H, L, N, R, S and T. Particular attention was paid to the group R strain in the light of its recent appearance in the literature as being a clinically significant zoonotic agent. Lancefield group Q streptococci or *Strep avium* can be mistaken morphologically for enterococci and thus it is useful that API-20 STREP can identify these organisms accurately. The Lancefield groups L and N are common isolates in veterinary medicine and may occasionally be implicated in human infections. These are admittedly small numbers but have nevertheless been included in this preliminary evaluation.

One of the clinically most important viridans streptococci is *Strep pneumoniae*. Currently this is

not included in the API profile although its reactions are on the result table in the manufacturers' instructions. The resulting code numbers can be identified using the computer data base. Confirmation of the result by morphology, optochin sensitivity and bile solubility is recommended by the manufacturer.

The LAP test is included in the system as a control test for streptococci which are all LAP-positive unlike *Aerococcus viridans* which is LAP-negative.

A list of the streptococci species covered in the API profile is shown in Table 5. This list is not, however, restrictive as the manufacturers have a telephone computer back-up service.

Most strains tested could be identified within 4 hours: only eight of 100 streptococci required 24 hours' incubation for a full identification. Although limited Lancefield grouping may be available with certain systems after only four hours from primary culture, it is preferable to use growth from an overnight purity plate. From this it is possible to group an organism within one hour with half a plate growth. If the result is inconclusive it is possible to use the API-20 STREP system with the other half plate growth and obtain a full identification. This is usually available on the same day although extended incubation may be required. Even though API-20 STREP had occasional problems in differentiating groups C and G, it proved excellent in identifying viridans and group D streptococci as well as Lancefield groups A and B.

It is the "indifferent" and enterococcal organisms that present problems in busy routine laboratories and the API-20 STREP system proved quick, reliable and easy to perform and at a reasonable cost in comparison to other more limited streptococcal grouping systems. The correlation was so good with conventional methods that the API system is now used routinely in our laboratory to identify non-groupable beta-haemolytic strains, viridans organisms and most group D streptococci.

Table 5 *Data base*

Streptococcus	*Lancefield group reaction*
Strep pyogenes	A
Strep agalactiae	B
Strep zooepidemicus	C
Strep dysgalactiae	C
Strep equisimilis	C
Strep equisimilis or Strep Group G	C or G
Strep faecalis	D
Strep faecium	D
Strep faecium ss casseliflavus	D
Strep faecium ss durans	D
Strep bovis I	D
Strep bovis II	D
Strep equinus	D
Strep avium	D or Q
Strep milleri I	A, C, F, G, or—
Strep milleri II	A, C, F, G or—
Strep sangius I	F, H or—
Strep sangius II	A, H, K, or—
Strep salivarius	A, K or—
Strep mitis	A, K, M, O or—
Strep pneumoniae	—
Strep mutans	E or—
Strep group E or P	E or P
Strep group L	L
Strep lactis	N
Strep suis I	S or T
Strep suis II	R
Strep uberis	C, D, E, P or U
Strep acidominimus	E
Aerococcus viridans	—

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Requests for reprints to: Mr GS Tillotson, Department of Surgery and Microbiology, University of Manchester School of Medicine, Hope Hospital, Salford M6 8HD, England.

Use of FITC-protein A in place of fluorescein-conjugated anti-gammaglobulins for rapid virus diagnosis by immunofluorescence

DJ WOOD, G CORBITT *North Manchester Regional Virus Laboratory, Booth Hall Hospital, Manchester, M9 2AA*

The indirect immunofluorescent antibody technique is widely used in the rapid diagnosis of virus infection by demonstration of virus antigens in both clinical specimens and cultured cells.[1] It requires a specific antiviral antiserum, usually raised by animal immunisation, and an appropriate fluorescein-linked anti-species globulin. Various animals are often used as sources of antiviral antisera and therefore corresponding anti-species conjugates are required. The number of second stage reagents should be kept to a minimum since each needs to be tested against the full range of virus-infected and uninfected cell cultures together with clinical specimens before routine use.[2] Also the wrong anti-species conjugate may inadvertantly be used.

Protein-A binds specifically to the Fc region of IgG (with the exception of sub-class 3) of many animal species[3] and is available commercially as a fluorescein-linked reagent. It has previously been used in veterinary work for identifying virus isolates by immunofluorescence and, when conjugated with peroxidase, for assaying anti-viral antibodies in animal sera from different animal species by means of ELISA techniques.[4] It was therefore of interest to test FITC-protein A for its suitability as a routine reagent.

Material and methods

VIRUS-INFECTED MONOLAYERS

Confluent monolayers of cells, grown in 12-well PTFE-coated slides (CA Handley Ltd, Essex), were infected with virus diluted in maintenance medium. When approximately 10% of the cell sheet showed evidence of virus growth, slides were washed in phosphate-buffered saline (PBS) and fixed in cold, dry acetone for 10 min.

Alternatively, viruses were grown in tube cell cultures. When growth of virus could be seen in some 10% of the cell sheet, cells were removed from the glass with trypsin, washed and resuspended in

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