Streptococcus grouping latex kits: evaluation of five commercially available examples

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ABSTRACT

This study compares a recently introduced latex agglutination test for the serogrouping of β-haemolytic streptococci against four internationally used commercial kits. The new kit is Prolex-Blue (Pro-Lab Diagnostics) and the comparators are Streptex (Murex), PathoDx (DPC), Streptococcus Grouping kit (Oxoid) and Prolex-White (Pro-Lab Diagnostics). A total of 302 consecutive clinical isolates are tested against all five kits, following the individual manufacturer's protocol, for both accuracy and speed. In addition, the data produced permits determination of the strengths or weaknesses of the kits against individual serotypes. Prolex-Blue proved to be both accurate and rapid, with a sensitivity of 99% and a specificity of 100%. Furthermore, average time to agglutination was substantially less than achieved by three of the other four kits evaluated.

KEY WORDS: Agglutination tests. Enterococcus. Streptococcus.

Introduction

Previous studies show that upper respiratory tract carriage rates of β-haemolytic streptococci (BHST) vary considerably.[1-3] Furthermore, it is argued that microbiological verification is of fundamental importance in the diagnosis of streptococcal infection,[4] although the value of taking throat swabs is questioned,[5] and techniques that detect streptococci directly from them have been evaluated with variable success.[6-8]

For BHST, grouping is based on the specific cell-wall carbohydrate C antigen, which nearly all β-haemolytic streptococci possess. This C antigen is composed of chains of N-acetyl glucosamine/rhamnose residues. In 1933, Rebecca Lancefield[9] demonstrated that these antigens could be extracted in a soluble form, and, subsequently, several different extraction procedures have been developed.[10-16]

For colonies grown on agar plates, commercially available streptococcal grouping latex agglutination kits provide a rapid method for both the extraction and detection of streptococcal antigen. This permits the serological identification of the vast majority of haemolytic streptococci of medical importance, namely Lancefield groups A, B, C, F and G. However, several of the kits also extract and detect the group-D antigen.

Group-D antigen is composed of teichoic acid, which is not located on the cell wall. Both enterococcal and streptococcal species may possess group-D antigen; however, these can be differentiated using biochemical reactions such as 40% bile aesculin and the pyrrolidonyl aminopeptidase (PYR) test. Group-D antigen is also found in groups Q, R and S streptococci,[17,18] but these are unlikely isolates from human samples.

Streptococcal grouping is a procedure that the majority of microbiology laboratories perform and it consumes valuable technical time; therefore, any improvement in accuracy and/or speed should always be sought. As Prolex-Blue (Pro-Lab Diagnostics, Neston, Wirral, UK) has been introduced recently, we decided to compare this kit with four other commonly used commercial kits.

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Materials and methods

Prolex-Blue was compared to Streptex (Murex Diagnostics, Dartford, Kent, UK), PathoDx (DPC, Los Angeles, California), Streptococcus Grouping kit (Oxoid, Basingstoke, Hampshire, UK) and Prolex-White (Pro-Lab Diagnostics). Each was used according to the manufacturer's instructions. Prolex-Blue, PathoDx and Prolex-White all use an acid extraction procedure, whereas the Oxoid kit and Streptex use an enzymatic step. PathoDx was the only kit not to claim detection of the group-D antigen.

To reduce the possibility of individual bias, four other experienced biomedical scientists were enlisted to help with the study. A total of 302 different strains of haemolytic streptococci were examined, with the aim of collecting approximately 50 strains of each of the six medically important serogroups.

Initially, all haemolytic streptococci that required routine streptococcal grouping were subcultured for inclusion in the trial. Patients' details were checked to ensure uniqueness of the individual strains. After overnight incubation, the subcultures were batched into groups of 10 and the streptococcal group of each individual strain was determined, as was the time taken to agglutinate. To minimise bias, all 10 strains were tested against the first kit, then the second, then the third, etc.

Results from each kit were recorded separately and were hidden until the final kit had been tested. The order in which the kits were used was rotated after each batch of 10. The identity and time taken were recorded and then transferred onto a Microsoft Excel spreadsheet. This enabled

comparison of the kits and also determination of whether or not a particular kit was weak at detecting individual serogroups.

Results

Overall, we investigated 302 streptococcal strains comprising 64 BHST group A, 66 group B, 44 group C, 55 group D, 56 group G and four group F. Also, 55 enterococci possessing the group-D antigen were tested. Of the haemolytic strains tested, 12 failed to group by any of the methods and these were removed from the study, leaving 290 isolates that possessed detectable streptococcal antigens.

Accuracy of identification

In general, there was consistency between the kits as to which serogroup was identified. One exception was a BHST group A, which was identified as group G with the Prolex-White kit. On further testing, this strain identified as *Streptococcus pyogenes* with API 20 Strep (bioMérieux UK, Basingstoke, UK) and hence the Prolex-White result was proved invalid.

Table 1 shows that, with the exception of Prolex-White, all the kits performed well. Sensitivities were: Prolex-Blue (98.6%), Oxoid (97.9%), Streptex (92.4%) and Prolex-White (70.3%). PathoDx produced a sensitivity of 80.3% for all the groupable isolates found in the study, but was not designed to identify the group-D antigen. When group-D strains were excluded, sensitivity increased to 99.1% (233/235).

Looking at the serogroups individually it was found that, with the exception of Prolex-White, all kits detected group-A antigen accurately; all accurately detected the group-B antigen (one isolate detected only by PathoDx proved to belong to the *Streptococcus milleri* group on further testing); one group-C strain reacted only with the Oxoid kit, and a further strain was detected by all except Streptex; none of the kits extracted and identified all of the 55 group-D isolates (Prolex-Blue performed slightly better than the Oxoid kit, which performed significantly better than the Streptex kit, while Prolex-White performed poorly); all kits performed very well with the 56 BHST group-G strains (Prolex-White successfully identified 53 of the 56 strains, but it misidentified one BHST group A as group G); and only the Streptex kit failed to detect all four strains of the *Streptococcus milleri* group possessing a group-F antigen.

The *Streptococcus milleri* group is renowned for its ability to appear to possess different C antigens.[19,20] This study included 15 strains of this group: 10 possessed a group-C antigen that was detected by all except the Prolex-White kit, which missed one strain; four possessed group-F antigens and one possessed group B.

Speed of agglutination

The time to agglutination was recorded as the interval between the start of the mixing stage and the detection of agglutination. The times achieved were grouped into subdivisions of 10 sec. Overall results for all isolates are shown in Table 2 and Figure 1. Overall, Prolex-Blue and Oxoid gave very similar and the most rapid results, followed by Streptex and PathoDx, with Prolex-White appearing very slow.

When each serogroup was considered individually, however, a different picture emerged (Figs 2–6) as Prolex-Blue outperformed the others with group A; Oxoid and Prolex-Blue outperformed the others with group B; Oxoid performed best, followed by Streptex with group C; Oxoid and Prolex-Blue performed similarly with group D; Oxoid and Prolex-Blue performed best, followed by Streptex with group G; and all kits performed better than Streptex (which failed to identify one of the four strains) with group F.

Table 1. Number of strains correctly identified by the five commercial kits studied

	Prolex-B	Oxoid	Streptex	PathoDx	Prolex-W	Total
Group A	64	64	64	64	41	64
Group B	66	66	66	67	65	67
Group C	43	44	42	43	36	44
Group D	53	50	37	0	5	55
Group G	56	56	56	55	53	56
Group F	4	4	3	4	4	4
Total	286	284	268	233	204	290

Table 2. Speed of agglutination of the five commercial kits studied

Time (sec)	Prolex-Blue	Oxoid	Streptex	PathoDx	Prolex-White
<=10	66	74	11	18	8
<=20	195	197	99	92	54
<=30	242	256	185	141	78
<=40	264	273	217	165	96
<=50	274	279	239	185	117
<=60	283	283	253	213	144
<=90	286	284	264	233	167
<=120	286	284	268	233	204
Total (*n*)	286	284	268	233	204

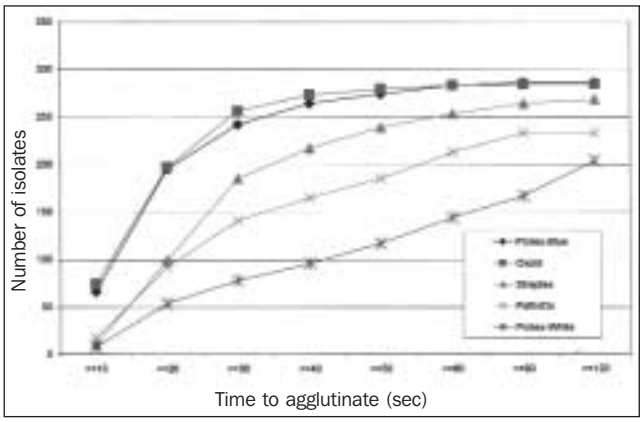

Fig.1. Results for all groupable isolates (n =290).

Kits examined independently

Agglutination time can also be used to determine which serogroups the individual kits identified most efficiently and so heighten the manufacturer's awareness of which of the antibody/latex combinations need improving. This is shown for Prolex-Blue in Table 3 and Figure 7, and for the other kits

Table 3. Efficiency of Prolex-Blue with individual serogroups

	Time to agglutination (sec)							
Group	<=10	11-20	21-30	31-40	41-50	51-60	61-90	Total (*n*)
A	33	26	5	0	0	0	0	64
B	20	33	9	1	2	1	0	66
C	0	14	8	12	5	4	0	43
D	7	23	10	6	1	3	0	53
G	6	32	14	2	1	1	3	56
F	0	1	1	1	1	0	0	4

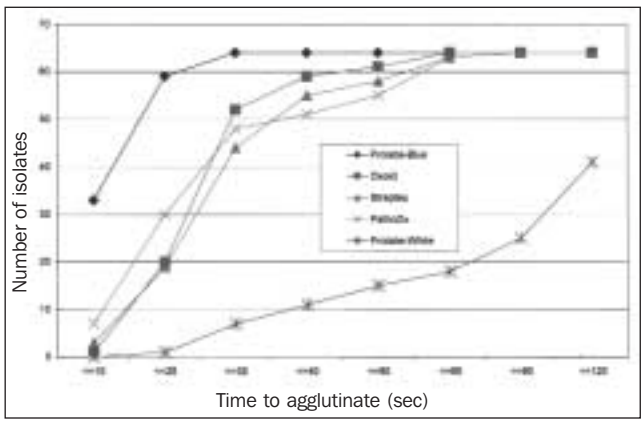

Fig. 2. Haemolytic streptococcus group A (*n* =64).

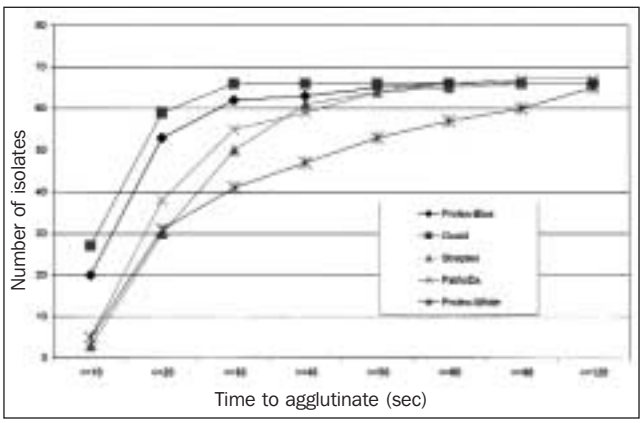

Fig. 3. Haemolytic streptococcus group B (*n* =67).

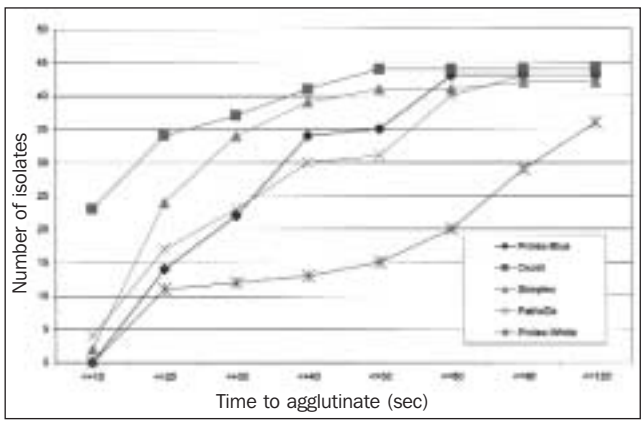

Fig. 4. Haemolytic streptococcus group C (*n* =44).

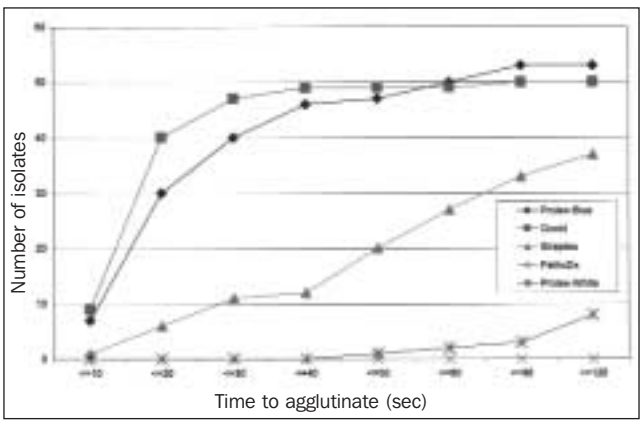

Fig. 5. Enterococci/haemolytic streptococci group D (*n* =55)

in Figures 8–11. In brief, Prolex-Blue proved the most efficient with BHST groups A and B but the weakest at detecting group C; Oxoid rapidly identified BHST groups B and C but was slowest with group A; Streptex performed well with most serogroups but struggled to detect group-D antigen; PathoDx performed well with BHST groups A and B, was slow in reacting to groups C and G, and did not detect group-D antigen; and Prolex-White performed poorly in our hands and only reliably identified BHST groups B and G.

Cross-reactions

Cross-reactions were rare and, if present, were much weaker than true reactions for the individual strains. There were no cross-reactions with the PathoDx kit. With both Prolex-Blue and Oxoid, only one stain of BHST group B cross-reacted with group D. For Streptex, the same BHST group-B strain cross-reacted with the group-D latex and one isolate reacted with both group-D and group-A latex reagents. This was confirmed as an *Enterococcus* species. However, there were 10 strains that cross-reacted with the Prolex-White kit and this was mainly present when the true identity took up to 2 min to become apparent.

Discussion

Four of the five kits performed very well. The one exception was Prolex-White, which performed poorly in our hands and only reliably identified BHST groups B and G. Conversely, the Prolex-Blue kit performed extremely well.

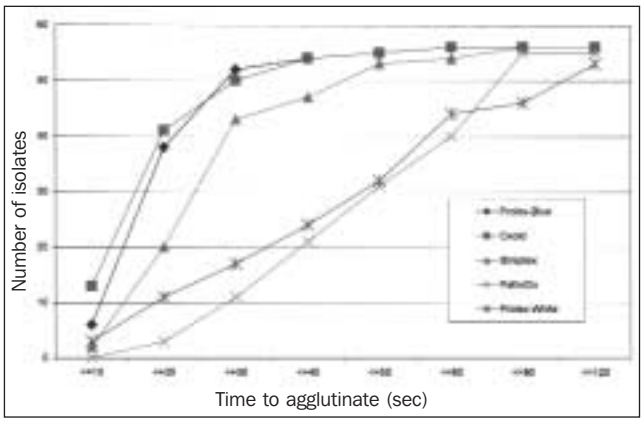

Fig. 6. Haemolytic streptococci group G (n =56)

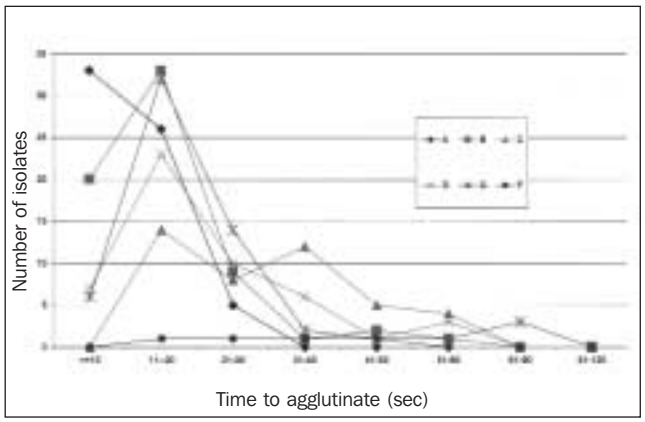

Fig. 7. Prolex-Blue (n =286)

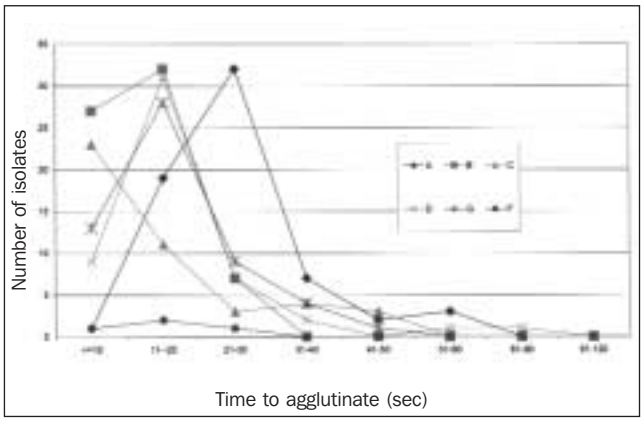

Fig. 8. Oxoid (n =284)

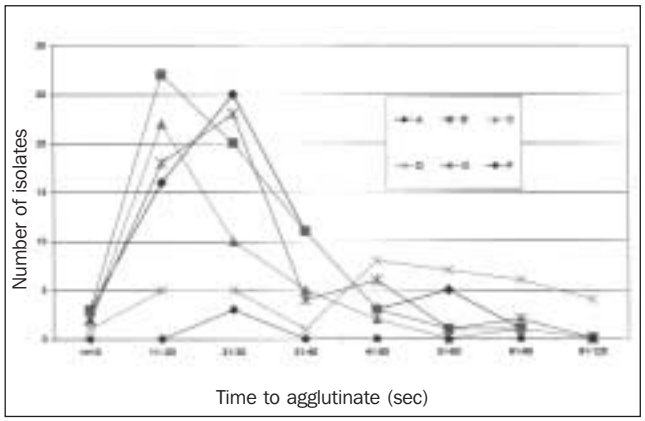

Fig. 9. Streptex (n =268)

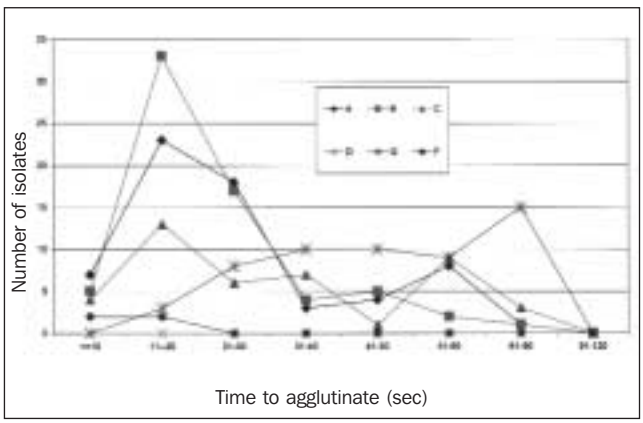

Fig. 10. PathoDx (n =233)

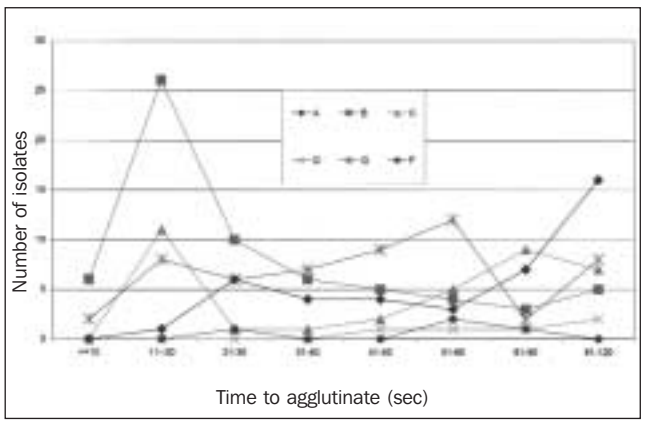

Fig. 11. Prolex-White (n =204)

The speed and accuracy with which it identified BHST group-A isolates was very impressive. These results concur with earlier work performed by Hodgins and Raybould,[21] who demonstrated that the nitrous acid extraction technique produced the greatest amount of rhamnose (group A-specific carbohydrate).

Overall, only the Oxoid kit proved comparable to Prolex-Blue. Oxoid gave similar overall speed and sensitivity, with faster agglutination results than Prolex-Blue achieved against BHST groups B and C, but slower results against BHST group A. Oxoid produces Streptococcal Grouping kits with either enzyme or acid extraction procedures, but its version of acid extraction (nitrous acid) does not extract group-D antigen (the enzyme-based version was evaluated in this study). The choice of acid-based or enzyme-based extraction technique rests with customer preference, but certainly the ability to perform single tests is easier and quicker using acid-based extraction.

Harvey and McIllmurray[22] and Birch, Keaney and Ganguli[23] have reported the isolation of enterococci containing both group-D and group-G antigens. This, however, did not prove a problem during the present study.

In summary, Prolex-Blue performed extremely well in this study, giving rapid and reliable results, and we recommend it for use in routine microbiology laboratories. □

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