Evaluation of new streptococcal latex grouping kit

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Abstract

Aims: To evaluate a new streptococcal latex grouping kit (Shield Diagnostics Ltd) and compare it against an established latex agglutination method (Streptex; Wellcome Diagnostics).

Methods: Two hundred and forty seven strains of streptococci and enterococci were tested with each kit by one operator and according to the manufacturer's instructions. Strains failing to group or giving discordant results were identified to species level.

Results: Two discrepant grouping results were observed and 13 non- β haemolytic streptococci failed to group with either product. The Shield kit successfully identified 232 isolates at 15 minutes of enzyme extraction incubation compared with 224 and 233 on short (15 minutes) and long (1 hour) incubations, respectively, for Streptex ($p > 0.23$ for both comparisons). On short incubation only, the Shield kit detected significantly more strains of *Enterococcus faecium* ($p = 0.007$). The reaction strengths were similar for both kits ($p > 0.16$). No cross-reactions were observed but the Streptex kit produced significantly fewer tests with visible granularity ($p < 0.003$).

Conclusions: Although the Shield product appeared to detect group D antigen more readily, overall no important differences in performance were observed. Prospective users of the new method should first become familiar with its characteristics.

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Since Lancefield first demonstrated the value of streptococcal serological grouping,¹ faster and simpler methods have been sought.²⁻⁵ Pronase-B extraction⁴⁻⁵ and latex agglutination methods⁶⁻⁷ have made streptococcal grouping feasible for most laboratories. This report compares a new latex agglutination kit, the Shield Diagnostics Streptococcal Identification kit (SDSI), (Shield Diagnostics Ltd., Dundee) with the Streptex latex agglutination kit (WS) (Wellcome Diagnostics, Dartford).

Methods

Two hundred and eleven consecutive streptococcal clinical isolates and 12 strains of *Enterococcus faecium* with 24 strains of group G streptococci taken from stored clinical isolates were tested. Organisms were tested directly

from the primary plates or, failing this, after overnight subculture on Columbia horse blood agar at 35°C in air with 5% CO₂. All isolates were tested, by a single operator (AFV), using the manufacturer's recommendations.

A light suspension of the organism was prepared in 400 μ l extraction enzyme solution (McFarland 0.5 to 1 for SDSI and growth equivalent to at least 5 large colonies for WS). This was incubated for 15 minutes at 37°C and shaken after 5 minutes. The latex reagents were shaken and one drop of each of the six groups dispensed onto a separate well of a clean reaction card. Thereafter, one drop of extraction solution (about 20 μ l) was added to each well, the two were mixed, and spread to cover the entire reaction area. Finally, the card was gently rocked for 1 minute and examined, by naked eye, for visible agglutination. Any isolate failing to group by the WS kit was retested after an hour's extraction incubation according to the manufacturer's instructions. Any isolate failing to group with either or both kits, or giving discrepant results, was formally identified by API 20 Strep (BioMérieux, Basingstoke).

Reaction strengths were recorded as strong, weak, trace and no reaction. Results were analysed by Yates' corrected χ^2 tests and the Fisher exact test where appropriate.

Results

The overall results of grouping by each kit are shown in table 1—combined long (1 hour) and short (15 minutes) incubation in the case of WS compared with SDSI (15 minutes) incubation. Two discrepancies were observed: on 1 hour incubation WS identified a strain of *E faecium* (trace group D reaction) and another isolate, subsequently identified as *Streptococcus mitis*, as a trace group F on short incubation, but both failed to group with SDSI.

Thirteen non- β haemolytic streptococci failed to group with either kit. These were identified as *S milleri* (n = 5), *S mitis* (n = 3), *S sanguis* (n = 3), *S bovis* and *S morbillorum* (one each). In total, WS successfully identified 233 isolates but only 224 after short incubation, with one false positive result. SDSI successfully identified 232 isolates after short incubation but missed one strain of *E faecium*. There was no significant difference between the number of strains grouped by SDSI and either the short or combined WS results ($p > 0.23$). Similarly, there were no statistical differences between the grouping results for the following organism subsets: (i) β haemolytic streptococci

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Table 1 Overall grouping results ($n = 247$ isolates)

Streptococcal group	SDSI kit						
	A	B	C	D	F	G	FTG*
WS kit	50	49	24	48	10	51	13
A							
B							
C							
D							
F							
G							
FTG*							

*Failed to group

Table 2 Recorded reaction strengths for correct identification in short incubation tests

Reaction strength	SDSI kit		WS kit	
	No	(%)	No	(%)
Trace	6	2.6	10	4.5
Moderate	46	19.8	50	27.3
Strong	180	77.6	164	73.2
Total	232	100	224	100

Table 3 Occurrence of visible granularity by kit and streptococcal group

Streptococcal group (No)	Reaction showing granularity (No of isolates)	
	WS kit	SDSI kit
A (50)	D (1)	—
B (49)	D (13)	D (34)
C (24)	D (2), A/F (1)	D (1), A/D/F (1)
D (49)	G (2)	G (2)
F (10)	—	D/G (1)
G (51)	D (5)	D (9), C (1)

excluding β haemolytic *S. milleri* ($n = 167$); (ii) non- β haemolytic strains excluding *E. faecium* and non- β haemolytic *S. milleri* ($n = 42$); (iii) *Streptococcus milleri* strains ($n = 17$).

Evaluation of the short incubation reaction strengths, for all the above comparisons, showed no significant differences ($p > 0.16$) (table 2).

The only such difference in kit performance, was found with *E. faecium* where, after short incubation, SDSI grouped 14/15 as group D compared with 7/15 by WS (Fisher exact test; $p = 0.007$). This difference, however, was not maintained after prolonged incubation, when WS correctly grouped 15/15 (Fisher's exact test; $p = 0.5$).

No cross-reactions were detected, but visible granularity was observed significantly more often with SDSI ($p < 0.003$). The single group where this reached significance was with B reactions ($p < 0.00006$) (table 3).

Discussion

The SDSI kit performed as well as the commercially available WS kit in terms of reaction strength, isolate numbers grouped, and result correlation. Although SDSI identified more organisms by short incubation than WS and produced more "strong" reactions, neither difference was significant.

SDSI appeared to detect group D antigen more readily—significantly more strains of *Enterococcus faecium* were identified after short incubation. This may also be reflected in the non-specific granularity seen most commonly in the D well, particularly with the SDSI kit, and B isolates. Granularity did not complicate interpretation as its quality and slowness to develop differentiated it easily from true agglutination. However, the SDSI inoculum is critical and we experienced troublesome cross-reaction agglutination if heavier than recommended suspensions were used.

The trial protocol did not include a comparison of reaction strengths between kits but results with the SDSI kit were generally more pronounced at each grade. This observation does however, need, to be formally evaluated.

The test procedures were very similar and this, taken with the above, means there is little difference between the kits. However, the SDSI kit is shown to have its own characteristics so centres considering its use must not view it as a straight replacement for their current system but should first become familiar with its performance.

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