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Evaluation of Three Commercially Available Kits for Grouping of Streptococci.

P. Martin[1], J. Broestler[1], K. Wrighton[2], W. Phillips[2], A. Hsiung[2], J. Hardy[2]

[1]Microbiology Department, Travis Air Force Base, California;
[2]Hardy Diagnostics, Santa Maria, California

StrepPRO™ Grouping Kit

Abstract

The rapid and accurate identification of the Lancefield group of beta-hemolytic streptococci and enterococci is an important procedure and widely performed in clinical laboratories. Although the latex agglutination method is rapid and often sensitive, reports of cross reactions and weak reactions have been occasionally documented. The methodology used by most currently available kits is based on nitrous acid extraction method.

An alternative newer method, modified nitrous acid, in combination with the use of larger latex particles has recently been made available and considered to be highly reliable as well. The purpose of this study was to compare the Hardy Diagnostics' StrepPRO™ grouping kit, which uses the modified nitrous acid method with larger latex particles, against two widely used kits, Strep Plus™ (Oxoid) and PathoDx™ (DPC), that are based on the nitrous acid method. A total of 95 isolates previously identified with well-defined serotypes belonging to all Lancefield groups were tested against the three kits. Overall, StrepPRO™ and PathoDx™ correctly identified all of the isolates (100%). Strep Plus™ correctly identified 95.6% of the isolates. Four isolates were classified as non-groupable by the Strep Plus kit. Besides the overall high sensitivity of all kits tested, it was observed that the StrepPRO™ kit demonstrated a stronger clumping reaction and faster clumping time.

Based on these findings, the newly developed modified nitrous acid method in combination with larger latex particles is equivalent or superior to the currently available kits. Therefore, StrepPRO™ can be used as reliable tool for Lancefield grouping of Streptococci.

Introduction

Beta-hemolytic streptococci are the most frequently isolated human pathogens among the representatives of the genus Streptococcus. Nearly all the beta-hemolytic streptococci possess specific carbohydrate antigens, known as streptococcal grouping antigens. Depending on the isolation site, correct serotyping of Streptococci is of great importance in aiding the clinician to determine the relevancy of infection and necessity of antimicrobial treatment.

Previously Lancefield showed that these antigens can be extracted in soluble form and identified by precipitation reactions with homologous antisera. Currently different procedures for extraction of streptococcal antigens are in use. The nitrous acid extraction is the most widely used method. Hardy Diagnostics StrepPRO™ Grouping Kit liberates the specific antigen from the bacterial cell wall using a modified nitrous acid extraction. In addition to the modified extraction method, StrepPRO™ also utilizes larger and more sensitized latex particles that agglutinate faster and stronger in the presence of homologous antigen. These newly improved latex particles will also reduce the occurrence of cross-agglutination with other Lancefield groups. The goal of this study is to compare and evaluate the most commonly used agglutination kits against Hardy Diagnostics' newly launched StrepPRO™ kit.

Materials and Methods

- Clinical isolates were collected from the microbiology department of Travis Air Force Base, California, and also retrieved from Hardy Diagnostics' culture collection.
- MicroBioLogics control organisms of each serotype were used as quality control organisms.
- The isolates were subcultured twice on a blood agar plate and isolated colonies that were 18 to 24 hours old were selected for agglutination.
- Extraction and agglutination procedures were performed according to each manufacturer's recommendations.

Results

Table 1. Overall Results per Isolate

Organism	StrepPRO™	StrepPlus™	PathoDx™	Comments
Streptococcus pyogenes (Group A)				
ATCC 19615	+	+	+/-	PathoDx™ weakest rxn
2A-13	+	+	+	
2-A74	+/-	+/-	+/-	weak rxn
atypical A1	+	+	+	
2A-6113	+	+	+	
2-A65	+	+	+	
2A-29	+	+	+/-	StrepPRO™ reaction was strongest, PathoDx gave weak rxn
2A-21	+	+	+	
2A-22	+	+	+	
2A-23	+	+	+	
2A-27	+	+	+	
2A-28	+	+	+	
2A-30	+	+	+	
2A-33	+	+	+	StrepPRO™ strongest reaction
2A-42	+	+	+	
2-A58	+	+	+	
2-A61	+	+	+	
2-A62	+	+	+	
2-A63	+	+	+	
2-A64	+	+	+	
2-A76	+	+	+	
2A-81	+	+	+/-	PathoDx™ weakest rxn
2A-83	+	+	+	
2A-84	+	+	+	
2-A85	+	+	+	
2-A86	+	+	+	
2-A87	+	+	+	
2-A88	+	+	+	
2-A89	+	+	+	
2-A90	+	+	+	
2A-91	+	+	+	
LCom2	+	+	+	
S. pyogenes AM1-510	+	+	+	
S. pyogenes AM1-512	+	+	+	
S. pyogenes AM1-514	+	+	+	
TAF1	+	+	+	
S. anginosus w/ A antigen	+	+	NT	

Organism	StrepPRO™	StrepPlus™	PathoDx™	Comments
Streptococcus agalactiae (Group B)				
ATCC 12386	+	+	+	
1A-MS30	+	+	+	
1-MS10	+	+	+/-	PathoDx™ weakest rxn
1-MS11	+	+	+	
1-MS13	+	+	+	
1-MS14	+	+	+	
1-MS15	+	+	+	
1-MS17	+	+	+	
1-MS2	+	+	+/-	PathoDx™ weakest rxn
1-MS3	+	+	+	
1-MS4	+	+	+	
1-MS5	+	+	+	
1-MS6	+	+	+	
1-MS7	+	+	+	
1-MS8	+	+	+	
1-MS9	+	+	+	
2-B1	+	+	+	StrepPRO™ strongest reaction
2-B2	+	+	+	StrepPRO™ strongest reaction
2-B3	+	+	+	
2-B4	+	+	+	
P003-001MS	+	+	+	Strep Plus™ strongest reaction
QOVH1	+	+	+	
SP-1	+	+	+	
SP-2	+	+	+	
SP-3	+	+	+	
SP-5	+	+	+	
SP-6	+	+	+	
SP-7	+	+	+	
SP-8	+	+	+	
SP-9	+	+	+	
SP-10	+	+	+	
SP-11	+	+	+	
TAF1	+	-	NT	
TAF2	+	-	NT	
TAF3	+	-	NT	
Group C Streptococcus				
ATCC 12388	+	+	+	
5-C1	+	+	+/-	PathoDx™ weakest rxn
5-C4	+	+	+	
MS1	+	+	+	
MS2	+	+	+	
MS3	+	+	+	
Group D Streptococcus				
ATCC 19433	+	N/A	N/A	
5-D4	+	N/A	N/A	
S. bovis with D antigen	+	N/A	N/A	
Group F Streptococcus				
ATCC 12392	+	+	+	
5-F2	+	+	+	
5-F3	+	+	+	
5-F4	+	+	+	
5-F5	+	-	+	
5-F6	+	+	+	
5-F7	+	+	+	
Group G Streptococcus				
ATCC 12394	+	+	+	
5-G1	+	+	+	
5-G2	+	+	+	
5-G3	+	+	+	
5-G4	+	+	+	
5-G5	+	+	+	
5-G6	+	+	+	

For more information on StrepPRO™ contact Andre Hsiung, MS (email: hsiunga@hardydiagnostics.com)

Results

Table 2. Overall Comparison Results per Methodology

	StrepPRO™	Strep Plus™	PathoDx™
	Total correct / Total tested (%)		
S. pyogenes	36/36 (100%)	36/36 (100%)	36/36 (100%)
S. anginosus (with group A antigen)	1/1 (100%)	1/1 (100%)	NT
S. agalactiae	35/35 (100%)	32/35 (91.4%)	32/32 (100%)
Group C beta-hemolytic Streptococci	6/6 (100%)	6/6 (100%)	6/6 (100%)
E. faecalis	2/2 (100%)	N/A	N/A
S. bovis (with group D antigen)	1/1 (100%)	N/A	N/A
Group F beta-hemolytic Streptococci	7/7 (100%)	6/7 (85.7%)	7/7 (100%)
Group G beta-hemolytic Streptococci	7/7 (100%)	7/7 (100%)	7/7 (100%)
Total	95/95 (100%)	88/92 (95.6%)	88/88 (100%)

NT – not tested; N/A – group D latex not available in the kit.

Table 3. Cost Analysis

	Serotypes available	Number of tests per kit	List Price	Price per test
StrepPRO	A, B, C, D, F, G	60	$205.00	$3.41
Strep Plus	A, B, C, F, G	50	$309.38	$6.18
PathoDx	A, B, C, F, G	60	$253.82	$4.23

- Overall StrepPRO™ and PathoDx™ presented the highest accuracy (100%) followed by Strep Plus™ (95.6%).
- Three Group B Streptococci and one Group F Streptococci were not detected by the Strep Plus™ kit. The identification of these isolates was further confirmed by biochemical tests.
- StrepPRO™ showed strongest clumping reaction with 4 isolates.
- Strep Plus™ showed strongest clumping reaction with 1 isolate.
- PathoDx™'s clumping reaction was significantly weaker that StrepPRO™ and Strep Plus™ in 6 cases.
- Overall the StrepPRO™ kit also showed fastest clumping time.
- Besides Enterococci, many species of Streptococci may also present Group D antigen. StrepPRO™ successfully detected this antigen in the strain of *Streptococcus bovis*. PathoDx™ and Strep Plus™ do not offer Group D reagent in their kits.
- StrepPRO™ is also the most cost-effective method, as shown in Table 3.

Discussion/Conclusion

- Hardy Diagnostics' StrepPRO™ kit's high sensitivity is due to the combination of the newly developed method of modified nitrous acid, in combination with the use of larger latex particles for faster clumping reaction.
- Hardy Diagnostics' newly launched StrepPRO™ can be considered equivalent or superior to currently available kits in terms of sensitivity and cost.
- StrepPRO™ can be used as a reliable and cost effective tool for the serotyping of Streptococci.

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