Evaluation of Three Commercial Latex Agglutination Kits for Serogrouping ß-Hemolytic Streptococci

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Introduction

Group A Streptococcus (GAS) is one of the most common pathogens recovered from patients suffering from pharyngitis. Unlike some of the other Beta Hemolytic Streptococci (BHS), GAS possesses a number of virulence factors that allow it to cause a wide variety of sequelae including endocarditis, meningitis, respiratory, skin and soft tissue infections. When left untreated, sequelae such as rheumatic fever, glomerular nephritis and scarlet fever may occur. Early diagnosis and differentiation from other BHS is important in preventing the severe complications that may be caused by prolonged, untreated GAS infection.

The demand for rapid turn around time (TAT) is an ongoing issue in microbiology laboratories using standard culture methodology for GAS. An area in which some time can be saved is in the streptococcal grouping latex kit utilized. Several commercial kits are available and each laboratory must consider factors such as

1. Accuracy
2. Efficiency and rapidity of agglutination
3. Ease of execution
4. Customization of kit component purchases

This study compared 2 coagglutination methods: PathoDx (Oxoid) and Phadebact (Boule) and one acid extraction latex agglutination test kit, Prolex-Blue (Pro-Lab Diagnostics).

Materials and Methods

A total of 279 isolates of BHS were tested. All of these originated from fresh clinical throat swabs initially plated on 5% Sheep Blood Columbia Agar (BA) and incubated 16-18 hours under anaerobic conditions to enhance hemolysis. Subcultures were prepared for each isolate to ensure purity. Any suspicious, minute colony types belonging to the Streptococcus mitis group were ruled out using the Vogues Proskauer (VP) test. The Oxoid Enzyme Extraction kit, considered our reference standard, was used to resolve any discrepant results.

Once the Lancefield group was determined, each of the isolates was tested with each latex group using;

1. Phadebact Direct Colony method
2. PathoDx Direct Colony method
3. Prolex Blue Acid Extraction method

All kits were used according to instructions defined by the manufacturer. Each isolate was tested with each latex group to check for cross-reactivity. (Group D was not considered.)

Times for agglutination to occur as well as time taken to complete the technique per test kit were monitored. To reduce bias, several other experienced technologists were enlisted.

Results

Of the 279 isolates, 191 were Group A, 28 were Group B, 26 were Group C, 26 were Group G and 4 were group F. Four were unknown, not group A, B, C, D, F or G. The 4 unknowns were found to be VP positive and therefore likely members of the S.mitis group. All of the group A and B isolates were detected by each method. Phadebact had one false negative for Group C (3.8%). PathoDx Group G latex cross reacted with 2 group B and 2 group C isolates.

Group	Phadebact		PathoDx		Prolex	
	Positive	Negative	Positive	Negative	Positive	Negative
A 191	191 (100)	0	191 (100)	0	191 (100)	0
B 28	28 (100)	0	28 (100)	0	28 (100)	0
C 26	25	1 (3.8)	26 (100)	0	26 (100)	0
F 4	4 (100)	0	4 (100)	0	4 (100)	0
G 26	26 (100)	0	26 (100)	0	26 (100)	0

Table 1: Number of Strains correctly identified

Phadebact group G latex cross reacted with I group C isolate; group A latex cross reacted with 1 group C isolate. Prolex Group F latex cross reacted with one group A isolate. All cross reactions occurred between 50-60 seconds after latex reagents were mixed.

Group	Phadebact	PathoDx	Prolex
A	0	0	11
B	0	2G	0
C	1C, 1A	2C	0
F	0	0	0
G	0	0	0

Table 2: Cross Reactions

Agglutination time (interval between beginning of mixing and agglutination detection) varied from immediate to 30 seconds and, for the Direct Colony methods, was very dependent on technique. Time was also dependent on amount of organism, but independent of group of testing kit.

Time	Phadebact	PathoDx	Prolex
<10 sec	170	24	161
10-20 sec	106	38	95
20-30 sec	49	10	23
>30 sec	0	0	0

Table 3 Time taken for agglutination

The time taken to perform each test was highest for the Prolex kit as it is an acid extraction method that requires additional manipulations. Phadebact and PathoDx took less time but accuracy was dependent on technique.

	Phadebact	PathoDx	Prolex
Time	33 sec	29 sec	72 sec

Table 4 Average time taken to test all five Lancefield groups per isolate.

Results

	Phadebact	PathoDx	Prolex
Time	33 sec	29 sec	72 sec

Table 5 Average time taken to test one Lancefield group per isolate

Proper Phadebact and PathoDx direct colony preparation technique are essential to achieve accurate results.

Figure 1: Proper position of applicator stick.

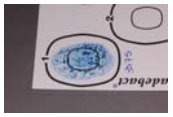

Figure 2: Strong agglutination after proper application of specimen to reaction card.

The method of applying the suspect colonies to the reaction card is extremely important. It is necessary to use the blunt end of a firm applicator stick to smoothly and firmly smear the organisms onto the card. (Figure1) Proper application of the organisms leads to a very clear agglutination reaction. (Figure 2)

Figure 3: Improper position of applicator stick.

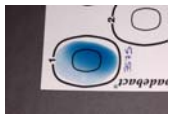

Figure 4: Weak reaction after improper application of specimen to reaction card.

Using the edge of the stick does not allow for even distribution of the organisms onto the reaction card. (Figure 3). This may significantly reduce the strength and visibility of agglutination.

As a separate survey, each kit was tested for its ability to group a single (1) 24 hour old GAS colony.

Note: Phadebact and PathoDx instructions state that 4 colonies are needed to ensure an accurate result.

Time	Phadebact	PathoDx	Prolex
<10 sec	0	0	2
10-20 sec	1	1	14
20-30 sec	1	3	7
30-60 sec	15	13	0
No rxn	1	2	0

Table 6: Time taken for agglutination to become visible testing a single colony.

Discussion

All test kits performed well with respect to accuracy. Only one false negative occurred with Phadebact Group C. Cross reactions were negligible for all kits. The few cross reactions that did occur were detected very close to the one minute time restriction, long after the actual group had reacted. PathoDx had 4 cross reactions (1.4%) whereas Phadebact had 2 (0.7%) and Prolex had 1 (0.3%).

Agglutination time was least for Prolex but all kits gave results within 30 seconds, well within the one minute deadline. No substantial time differences were found in rapidity when performing all 5 latex reactions per isolate. However, there were considerable time differences when testing only one latex group per isolate. Because of the extra manipulation time involved in acid extraction techniques, Prolex required on average twice as long as the direct methods of PathoDx and Phadebact. The latter two rely on proper technique and although product inserts mention that colonies are to be rubbed thoroughly and smoothly onto the test cards, both fail to mention that this step is paramount to obtaining reliable results . Serological grouping of streptococci is technically simple but key components of the test remain vital to obtaining accurate, rapid results.

1. Colony morphology recognition
2. Quality of hemolysis
3. Sub-culture techniques
4. Proper preparation techniques
5. Technologist experience

Conclusion

Rapid direct colony testing methods for grouping hemolytic streptococci, when performed properly, are as accurate as acid extraction methods.

High volume laboratories should consider rapid direct colony co-agglutination kits for faster TAT when performing single latex grouping.

Direct colony methods require proper preparation technique.

PathoDx kits were easier to use in terms of dropper bottle safety and size, reaction cards were larger and offered more tests per slide.

All test kit results were easy to read.

Acknowledgements

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