Thermo Scientific PathoDxtra Strep Grouping Kit Shows Superior Performance Over Prolex Streptococcal Grouping Latex Kit

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Application Note

Key Words
Thermo Scientific PathoDxtra Strep Grouping kit, streptococci, Lancefield grouping.

Goal
The purpose of this study was to evaluate the performance of the Thermo Scientific™ PathoDxtra™ Strep Grouping kit (Thermo Fisher Scientific) alongside the Prolex™ Streptococcal Grouping Latex kit (Pro-Lab Diagnostics) for identification of Lancefield groups.

Abstract
Streptococcal Lancefield group isolates and ungroupable streptococci were tested on both the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping Latex kit using acid extraction. In addition, the PathoDxtra Strep Grouping kit was tested using direct testing and broth enrichment methods. Prolex™ Streptococcal Grouping Latex kit recommends the use of the acid extraction method only.

The PathoDxtra Strep Grouping kit showed superior performance compared to the Prolex™ Streptococcal Grouping Latex kit for the acid extraction method. The PathoDxtra Strep Grouping kit had a higher overall inclusivity for all methods used than the Prolex™ Streptococcal Grouping Latex kit overall inclusivity using acid extraction methods.

Introduction
Streptococci are facultatively anaerobic, Gram-positive, catalase negative organisms that grow as chains or pairs on Columbia Blood Agar (CBA)[1]. Streptococci are classified according to haemolysis on CBA. Further characterisation is performed according to the specific cell wall antigens found on beta-haemolytic streptococci, termed Lancefield grouping[2].

Figure 1. PathoDxtra Strep Grouping kit

The PathoDxtra Strep Grouping kit (figure 1) and the Prolex™ Streptococcal Grouping Latex kit are both latex agglutination methods for the classification of clinically important streptococci. The nitrous acid extraction instantaneously extracts the cell antigens, and works without the need for incubation, allowing accurate differentiation of all the clinically significant Lancefield Groups: A, B, C, D, F and G.

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The Study

A total of 208 streptococcal Lancefield group isolates (51 group A, 50 group B, 50 group C, 10 group F, 47 group G), 57 Enterococci (Lancefield group D) and 30 ungroupable streptococci (including *S. pneumoniae*, *S. anginosus* and *S. oralis*) were tested on both the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping Latex kit using acid extraction. In addition, the PathoDxtra Strep Grouping kit was tested using direct testing and broth enrichment methods. Prolex™ Streptococcal Grouping Latex kit recommends the use of the acid extraction method only.

Culture of clinical isolates

All streptococcal and non-streptococcal clinical isolates were removed from -80°C storage and cultured onto Columbia Blood Agar (CBA) prior to incubation at 36±1°C for 18-24 hr.

Acid extraction method

All group latexes from both the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping Latex kit were tested with the acid-extracted isolates according to manufacturers' instructions for use. One drop of extraction reagent 1 was added to a tube. The inoculum was re-suspended then one drop of extraction reagent 2 was added to the tube. Five drops of extraction reagent 3 was then added to the tube and mixed. Forty to 50 microlitres of the extract were then mixed with one drop of group latex reagent on the test slide and rocked for up to 60 seconds.

Direct colony testing

All group latexes from the PathoDxtra Strep Grouping kit were tested from colonies directly taken from the clinical isolates that were cultured onto CBA. For each group reagent, four isolated colonies were selected from each clinical isolate culture and rubbed thoroughly and smoothly onto the test slide in the centre of a delineated circle. One drop of each group latex was dropped directly onto the card and mixed into the smeared colonies. Each test slide was rocked for up to 60 seconds. The Prolex™ Streptococcal Grouping Latex kit does not provide an option for direct colony testing procedures.

Broth culture

Two colonies approximately 1 mm in size (or more if smaller colonies were observed) were selected from each clinical isolate culture and inoculated into 10 ml Brain Heart Infusion Broth (Thermo Fisher Scientific). All broths were incubated at 36±1°C for at least four hrs (or until turbid). The broth was centrifuged at 1000 × g for 15 minutes then the broth was carefully removed from the bacterial pellet using a pipette. One drop of extraction reagent 1 from the PathoDxtra Strep Grouping kit was added separately to each bacterial pellet to re-suspend the pellet. The remaining acid extraction steps were then followed and 50 µl of the extract was then tested with all group latexes from the PathoDxtra Strep Grouping kit. The Prolex™ Streptococcal Grouping Latex kit does not provide an option for broth culture testing procedures.

Positive and negative controls

Positive and negative controls from each streptococcal grouping kit were performed on a daily basis.

Observation and recording of results

The time (in seconds) at which an agglutination reaction was observed, plus presence and strength of agglutination at 60 seconds from any of the latexes from each of the streptococcal grouping kit tests were recorded.

Confirmation of Streptococcus species and/or Lancefield grouping

When available, MALDI-TOF mass spectrometry was performed to confirm species identification of isolates. The final determination of Streptococcal Lancefield grouping of each isolate was based on a combination of the species identification via MALDI-TOF and the most commonly observed agglutination reactions from each group latex of both kits tested. Determination of the performance of each group latex from the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping Latex kit was made based on results from the acid extraction, direct testing and broth culture methods combined.

Table 1. Inclusivity of the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping Latex kit from Lancefield group A, B, C, D, F and G isolates

Lancefield Group Reagent	Inclusivity (%)					
	PathoDxtra Strep Grouping kit			Prolex™ Streptococcal Grouping Latex kit		
	Acid extraction	Direct testing	Broth culture	Acid extraction	Direct testing	Broth culture
A (n=51)	96.1 (95% CI = 90.8-101.8)	70.6 (95% CI = 58.1-83.1)	82.4 (95% CI = 71.9-92.8)	92.2 (95% CI = 84.8-99.5)	n/a*	n/a*
B (n=50)	94.0 (95% CI = 87.4-100.6)	94.0 (95% CI = 87.4-100.6)	98.0 (95% CI = 94.1-101.9)	72.0 (95% CI = 59.6-84.4)	n/a	n/a
C (n=50)	88.2 (95% CI = 79.3-97.1)	62.8 (95% CI = 49.4-76.2)	94.1 (95% CI = 87.6-100.6)	62.8 (95% CI = 49.4-76.2)	n/a	n/a
D (n=57)	73.7 (95% CI = 62.3-85.1)	98.3 (95% CI = 94.9-101.7)	96.5 (95% CI = 91.7-101.3)	42.1 (95% CI = 29.3-54.9)	n/a	n/a
F (n=10)	90.0 (95% CI = 71.4-108.6)	30.0 (95% CI = 1.6-58.4)	50.0 (95% CI = 19.0-81.0)	30.0 (95% CI = 1.6-58.4)	n/a	n/a
G (n=47)	93.6 (95% CI = 86.6-100.6)	66.0 (95% CI = 52.5-79.5)	83.0 (95% CI = 72.3-93.7)	59.6 (95% CI = 45.6-73.6)	n/a	n/a
Total (n=265)	**88.7** (95% CI = 84.9-92.5)	**77.1** (95% CI = 72.0-82.2)	**89.5** (95% CI = 85.8-93.2)	**63.9** (95% CI = 58.1-69.7)	n/a	n/a

* Direct testing and broth enrichment methods were not used with the Prolex™ Strep Grouping Latex kit due to the method not being supported by the manufacturer.

Results and discussion

Inclusivity

Inclusivity from 265 Lancefield group A, B, C, D, F and G isolates using acid extraction for the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping Latex kit, and the addition of the direct testing method and broth culture method for the PathoDxtra Strep Grouping kit only is shown in table 1.

When the acid extraction method was used, the inclusivity of the PathoDxtra Strep Grouping kit was statistically significantly greater than the inclusivity of the Prolex™ Streptococcal Grouping Latex kit for group B latex (P=0.0026), group C latex (P=0.0036), group D latex (P=<0.00001), group G latex (P=0.0004) and when overall inclusivity of the PathoDxtra Strep Grouping kit was considered (P=<0.00001). When there was no statistically significant difference between the inclusivity of the two kits, the inclusivity of the PathoDxtra Strep Grouping kit was still greater than that of the Prolex™ Strep Grouping Latex kit when using the acid extraction method.

Out of the three methods used for the PathoDxtra Strep Grouping kit, the broth culture method has the highest overall inclusivity (89.5%), closely followed by the acid extraction method (88.7 %). The direct testing method had the lowest overall inclusivity of all methods used (77.1%) for the PathoDxtra Strep Grouping kit.

All methods used with the PathoDxtra Strep Grouping kit gave a higher overall inclusivity than the overall inclusivity for the Prolex™ Streptococcal Grouping Latex kit when the acid extraction method was used.

For Lancefield group A latex, the direct testing and broth enrichment methods gave a lower inclusivity (70.6% and 82.4% respectively) for the PathoDxtra Strep Grouping kit, than the inclusivity for the Prolex™ Strep Grouping Latex kit for the acid extraction method (92.2%). All other individual group latexes for all methods used on the PathoDxtra Strep Grouping kit had a higher or equal inclusivity compared to the Prolex™ Strep Grouping Latex kit.

Table 2. Exclusivity of the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping from Lancefield group A, B, C, D, F and G isolates and ungroupable streptococci

Lancefield Group Reagent	Exclusivity (%)					
	PathoDxtra Strep Grouping kit			Prolex™ Streptococcal Grouping Latex kit		
	Acid extraction	Direct testing	Broth culture	Acid extraction	Direct testing	Broth culture
A	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	n/a*	n/a*
B	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	n/a	n/a
C	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	n/a	n/a
D	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	n/a	n/a
F	100 (95% CI = 100)	100 (95% CI = 100)	99.7 (95% CI = 99.4-100)	100 (95% CI = 100)	n/a	n/a
G	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	100 (95% CI = 100)	n/a	n/a
Total (n=1776)	**100** (95% CI = 100)	**100** (95% CI = 100)	**99.9** (95% CI = 99.8-100)	**100** (95% CI = 100)	n/a	n/a

* Direct testing and broth enrichment methods were not used with the Prolex™ Strep Grouping Latex kit due to the method not being supported by the manufacturer.

Exclusivity

Exclusivity using the acid extraction method for the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping Latex kit is shown in table 2. In addition, the broth culture method and direct testing method were carried out with the PathoDxtra Strep Grouping kit. The Prolex™ Streptococcal Grouping Latex kit recommends use with the acid extraction method only.

Exclusivity has been calculated based on the number of cross reactions (i.e. false positive reactions) seen on the Lancefield grouped streptococci and ungroupable streptococci tested.

There was no difference between the exclusivity of the PathoDxtra Strep Grouping kit and exclusivity of the Prolex™ Streptococcal Grouping Latex kit for each individual group latex *and* when all group latexes were combined when using the acid extraction method (exclusivity was 100% for all kits tested).

Exclusivity of the PathoDxtra Strep Grouping kit was identical for each individual group latex *and* when all group latexes were combined when using the direct testing method (100%).

When using the broth culture method to test the PathoDxtra Strep Grouping kit, the overall exclusivity was slightly reduced (99.9%) due to one false positive result from an ungroupable *S. oralis* on the group F latex (inclusivity 99.7%).

Cross reactions

There were no cross reactions observed on any of the group latexes from the PathoDxtra Strep Grouping kit and the Prolex™ Streptococcal Grouping Latex kit when using the acid extraction method.

The PathoDxtra Strep Grouping kit showed two cross reactions when using the broth culture method. Firstly, the group C latex showed agglutination on one group A isolate (*S. pyogenes*). Secondly, group F latex showed agglutination on one ungroupable, alpha-haemolytic isolate (*S. oralis*). The group F latex from the PathoDxtra Strep Grouping kit also showed agglutination on this same isolate.

Conclusions

- The Prolex™ Streptococcal Grouping Latex kit is used with one method only; acid extraction. Whereas the PathoDxtra Strep Grouping kit offers an additional two methods for use; direct testing method and broth culture method.

- When using the acid extraction method, the PathoDxtra Strep Grouping kit showed a superior inclusivity to that of the Prolex™ Streptococcal Grouping Latex kit.

- The additional broth culture method used with the PathoDxtra Strep Grouping kit gave a comparable performance to the acid extraction method in terms of overall inclusivity.

- The additional direct testing method used with the PathoDxtra Strep Grouping kit gave a slightly reduced overall inclusivity in comparison to the acid extraction method and broth culture method.

- Exclusivity was comparable between the two kits tested when using the acid extraction method.

- The acid extraction and direct testing methods showed comparable performance in terms of exclusivity for the PathoDxtra Strep Grouping kit; the broth culture method showed a slightly reduced exclusivity compared to the other two methods when using the PathoDxtra Strep Grouping kit.

References

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994-064
LT2173A
August 2015

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