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Evaluation of the Oxoid Dryspot Streptococcal Grouping Kit for Grouping Beta-Hemolytic Streptococci

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The latex agglutination kits that are widely used for grouping of beta-hemolytic streptococci in clinical laboratories use liquid latex suspensions. The Oxoid Dryspot kit (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) uses predispensed latex dried onto reaction cards or cardboard strips. All streptococci of groups A (85 strains), B (87 strains), C (30 strains), D (38 strains), F (23 strains), and G (65 strains) were correctly grouped by using these reagents. The Oxoid Dryspot Streptococcal Grouping kit is a reliable method for grouping of the beta-hemolytic streptococci encountered in clinical laboratories.

Latex agglutination or coagglutination methods have largely superseded precipitation methods for the determination of the Lancefield group of beta-hemolytic streptococci (BHS) isolated from clinical material (7). Latex agglutination kits are available for the detection of group A, B, C, D, F, and G streptococcus antigens (2, 5). In these kits, the latex is a liquid suspension. The latex in Oxoid Dryspot Streptococcal Grouping kits (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) has a novel format. It is predried onto reaction cards or strips. This study assessed the ability of the Dryspot reagents to group BHS of groups A, B, C, D, F, and G. The strains of BHS used in the study were also tested with Oxoid streptococcal grouping reagents and Streptex (Murex, Dartford, Kent, United Kingdom), which both use conventional liquid latex suspensions.

Bacterial strains. Altogether, 230 strains were used in the first investigation and 202 were used in the second investigation. A total of 49 strains from the culture collection of Basildon Hospital and the National Collection of Type Cultures (NCTC) were used in both investigations. Strains of BHS isolated and identified in the Department of Microbiology, Basildon Hospital, from clinical material submitted for examination during this study were also used in both investigations. In the first investigation, 181 clinical isolates were tested. In the second investigation, 153 clinical isolates were tested; 60 of these had also been tested in the first investigation. The strains from the culture collections consisted of groups A (three *Streptococcus pyogenes* strains [including NCTC 8198], two *S. anginosus* strains, and one *S. constellatus* strain), B (four *S. agalactiae* strains [including NCTC 8181]), C (two *S. anginosus* strains, one *S. dysgalactiae* strain [NCTC 4335], three *S. equi* strains [including NCTC 9682], two *S. equisimilis* strains, and two *S. zooepidemicus* strains [including NCTC 6176]), D (one *S. bovis* strain and four *Enterococcus faecalis* strains, three of which had both D and G antigens), F (seven *S. constellatus* strains and five *Streptococcus* sp. strains [including NCTC 5389]), and G (two *S. anginosus* strains [including NCTC 10713], three *S. canis* strains [including NCTC 12191], and two large-colony group G *Streptococcus* sp. strains), one *Streptococcus* sp. group L strain, and four strains of *Listeria monocytogenes*. The strains from the culture collection of Basildon Hospital have been previously characterized (4–6). The clinical strains were confirmed as catalase-negative, gram-positive cocci, and their Lancefield groups were determined with Oxoid latex streptococcal grouping reagents, but the results were not revealed to me until the end of the investigation.

Strains from the culture collections were grown on Columbia agar (Oxoid) containing 5% horse blood (CBA). Grouping of the clinical strains was performed directly from the original isolation medium, which was either CBA or a selective medium for streptococci, colistin-oxolinic acid blood agar (4), or from a colistin-oxolinic acid blood agar purity plate. The clinical isolates that were used in both investigations were maintained in Robertson's Cooked Meat Medium (Oxoid) at room temperature and subcultured onto CBA for testing. All culture plates were incubated at 37°C overnight in air enriched with 5% CO_2.

Grouping methods. Two formats of the Oxoid Dryspot latex reagents were evaluated, the card format and the cardboard strip format. In the card format, four spots of streptococcal grouping latex had been dried onto an area of a reaction card, one area each for groups A, B, C, D, F, and G. In the strip format, four spots of latex had been dried onto a 0.5-cm^2 area at the end of a strip (45 by 12 mm) of stiff paper. The strips were identified according to the Lancefield group they were intended to react with, and there were separate strips for each of groups A, B, C, D, F, and G. The cards and strips were stored at room temperature in resealable foil envelopes containing a sachet of silica gel. The strips were packaged as one group per foil envelope. All other reagents were stored at 4°C and brought to room temperature prior to use.

The study was conducted in two parts. In the first investigation, strains were tested with the Dryspot card format. In the second investigation, strains were tested with the Dryspot strip format. In both investigations, enzyme and nitrous acid extracts of the strains were tested. All strains were also tested with the Oxoid Streptococcal Grouping kit and Streptex by using both the enzyme and nitrous acid extracts. The tests were performed in accordance with the manufacturer's instructions outlined below.

Oxoid Dryspot card format. Strains were extracted with Oxoid extraction enzyme or Oxoid nitrous acid reagents as described below. One drop, approximately 50 μl, of extract was added to each of the test areas on the Dryspot test card provided. This was carefully mixed into the dry spots of latex in

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TABLE 1. Performance of the streptococcal grouping reagents used in this study[a]

Lancefield group or species	Oxoid Dryspot				Oxoid liquid latex[b]		Streptex	
	Card format		Stick format		Enzyme extract	Nitrous acid extract	Enzyme extract	Nitrous acid extract
	Enzyme extract	Nitrous acid extract	Enzyme extract	Nitrous acid extract				
A	59/59	59/59	42/42	42/42	85/85	85/85	85/85	85/85
B	54/54	54/54	47/47	47/47	87/87	87/87	87/87	87/87
C	25/25	25/25	25/25	25/25	30/30	30/30	30/30	30/30
D[c]	28/28	NT[d]	25/25	NT	38/38	NT	38/38	NT
F	19/19	19/19	16/16	16/16	23/23	23/23	23/23	23/23
G	40/40	40/40	42/42	42/42	65/65	65/65	65/65	65/65
L	1/0[e]	1/NR[f]	1/0[e]	1/NR	1/0[e]	1/NR	1/0[e]	1/NR
Listeria monocytogenes	4/NR	4/NR	4/NR	4/NR	4/NR	4/NR	4/NR	4/NR

[a] The data are the number of isolates tested followed by the number giving the correct reaction.
[b] Cumulative total of individual strains tested in both sections of the study; the strains tested in both sections were only counted once.
[c] Includes three strains with both D and G antigens.
[d] NT, not tested.
[e] Strain reacted with group A latex.
[f] NR, no reaction with any of the latex suspensions used.

each oval area until the latex was resuspended. A separate mixing stick was used for each latex test spot.

Oxoid Dryspot strip format. Strains were extracted with Oxoid streptococcal grouping enzyme or Oxoid nitrous acid reagents as described below. One drop, approximately 50 μl, of extract was added to each of the test areas on the reaction card provided. The area containing the dried latex was brought into contact with the extract, and the strip was pushed down so that it folded at the embossed hinge. The surface of the strip was then rotated in contact with the streptococcal extract in order to rehydrate and release the latex and mix it with the extract.

Oxoid Streptococcal Grouping kits. (i) Enzyme extraction. A 0.4-ml volume of extraction enzyme was added to a polystyrene tube (75 by 10 mm), and a culture quantity equivalent to two to five large colonies was suspended in the mixture by using a 1-μl disposable loop. The mixture was incubated at 37°C for 10 min.

(ii) Nitrous acid extraction. Three drops of extraction reagent 1 and 3 drops of extraction reagent 2 were added to a polystyrene tube (75 by 10 mm), and a culture quantity equivalent to two to five large colonies was suspended in the mixture by using a 1-μl disposable loop. Three drops of extraction reagent 3 were added to the suspension, and the contents were mixed.

Streptex. (i) Enzyme extraction. A 0.4-ml volume of extraction enzyme was added to a polystyrene tube (75 by 10 mm), and a culture quantity equivalent to two to five large colonies was suspended in the mixture by using a 1-μl disposable loop. The mixture was incubated at 37°C for 10 min.

(ii) Nitrous acid extraction. One volume of extraction reagent 1 was added to the reaction tube provided, and a culture quantity sufficient to cover the blunt end of the wooden stick provided in the kit was added to the tube and mixed with the contents. The stick was left in the reaction tube for 1 min and then 1 volume of extraction reagent 2 was added to the tube.

For each of the Oxoid and Streptex methods, 1 drop of each latex reagent was added to the appropriate area on a reaction card, 1 drop, approximately 50 μl, of the streptococcal extract was added to each area, and the contents were mixed with a separate stick until a smooth suspension was obtained.

To ensure comparability, the same culture plate was used for each of the grouping methods used, as appropriate for each part of the study. The positive control material provided in each kit was used to check the correct functioning of each kit. For all of the methods used in this study, enzyme extracts were reacted with latexes for groups A, B, C, D, F, and G and with nitrous acid extract latexes for groups A, B, C, F, and G. The reaction cards were rocked gently for up to 1 min. The timing of the reactions commenced after the mixing of all of the latexes had been completed, and the time at which a positive reaction occurred was recorded by rounding up to the nearest 5 s. A time of 0 s was recorded if strong agglutination was visible before the reaction card was rocked. Reactions were read without magnification. The strength of the positive reactions was graded as 4+ (complete agglutination of the latex with a clear background), 3+ (extensive agglutination with a mostly clear background), 2+ (moderate agglutination with a visible decrease in background density), or 1+ (fine agglutination with an essentially unchanged background). A negative reaction was taken as one where there was no agglutination of the latex visible to the unaided eye. Strains which produced anomalous reactions were retested with all of the kits from fresh subcultures.

Results and discussion. The results obtained with both of the Dryspot methods were identical to those obtained with the liquid latex methods for the equivalent strains (Table 1). The clinical strains tested in both investigations were placed in the same group in both studies, and the results for the clinical strains were identical to those obtained by the routine diagnostic laboratory. The collection strains were correctly grouped by all of the methods used. In the first investigation, 60 strains were placed in group A when enzyme extracts were tested and 59 were placed in group A when nitrous acid extracts were tested; 53 of these strains were clinical isolates. There were 54 group B (50 clinical), 25 group C (15 clinical), 28 group D (23 clinical), 19 group F (7 clinical), and 40 group G (33 clinical) strains. In the second investigation, 43 strains were identified as members of group A when enzyme extracts were tested and 42 were placed in group A when nitrous acid extracts were tested; 40 of these strains were clinical isolates, including 10 which had been tested in the first study. There were 47 group B (43 clinical, including 10 from the first study), 25 group C (15 clinical, including 10 from the first study), 25 group D (20 clinical, including 10 from the first study), 16 group F (4 clinical), and 42 group G (35 clinical, including 10 from the first study) strains.

Enzyme extracts from the strain of *Streptococcus* sp. group L were identified as members of group A by all of the methods used in both studies; however, nitrous acid extracts from this strain did not react with any of the latexes in any of the meth-

ods. None of the four strains of *L. monocytogenes* reacted with any of the latexes in any of the methods used in either of the studies. The group G antigen carried by the three strains of *E. faecalis* was detected in enzyme extracts with all of the methods used in both studies but not in nitrous acid extracts. Neither format of the Dryspot reagents nor the other methods used produced any nonspecific reactions, i.e., reactions with more than one latex (other than the *E. faecalis* strains noted above). It was noted that some strains of group B streptococci extracted with the Oxoid enzyme gave an initial stringy appearance to all of the latexes among both the Oxoid Dryspot and liquid latexes. However, with a little more mixing, the suspension became smooth and this did not interfere with the agglutination reactions. This did not occur with the Streptex reagents. The observed grouping of *Streptococcus* sp. group L as group A has been previously described, and the antigenic determinants for both of the groups are very similar (1).

There were differences between the methods in reaction speed and the size and visibility of the agglutinates obtained. With enzyme extracts, 95% of the strains tested with the Dryspot card format were positive by 5 s (mode, 0 s; range, 0 to 15 s), 97% were positive with the Dryspot stick format (mode, 0 s; range, 0 to 10 s), and 94% were positive with the Oxoid liquid latex method (mode, 5 s; range, 0 to 20 s), but only 33% were positive with Streptex (mode, 10 s; range, 0 to 30 s). With nitrous acid extracts, the corresponding values were 76% (5, 0 to 15), 99% (0, 0 to 10), 89% (5, 0 to 20), and 21% (10, 5 to 25). For both formats, the dried latexes were easy to resuspend and produced smooth suspensions. The latex used in all of the Oxoid products was blue and gave good contrast with the white reaction card, making the reactions easy to see. The Streptex latex was white or pale cream and did not give such strong contrast on the black reaction cards. The size of the agglutination reaction with the Oxoid methods generally appeared larger than that produced with Streptex. However, with all of the methods used, all but one or two strains gave 4+ reactions and the remaining positive strains gave 3+ reactions. The results obtained in this study show that the Oxoid Dryspot streptococcal grouping reagents allow rapid grouping of BHS. The novel predispensed dried format has the added advantage of a shelf life of 2 years when stored at room temperature.

Since the completion of this study, the manufacturers have decided to market the strip format of the kit. With this format, strips can be used individually or in various combinations.

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