STREPPRO™ GROUPING KIT

Cat. no. PL030HD	StrepPRO™ Grouping Kit	60 tests/kit
	Each kit contains:	
	* PL031HD Blue Latex Suspension Group A, 3ml	1 vial/kit
	* PL032HD Blue Latex Suspension Group B, 3ml	1 vial/kit
	* PL033HD Blue Latex Suspension Group C, 3ml	1 vial/kit
	* PL034HD Blue Latex Suspension Group D, 3ml	1 vial/kit
	* PL035HD Blue Latex Suspension Group F, 3ml	1 vial/kit
	* PL036HD Blue Latex Suspension Group G, 3ml	1 vial/kit
	* PL037HD Extraction Reagent 1, 3.5ml	1 vial/kit
	* PL038HD Extraction Reagent 2, 3.5ml	1 vial/kit
	* PL039HD Extraction Reagent 3, 8.5ml	2 vials/kit
	* PL040HD Polyvalent Positive Control, 3.5ml	1 vial/kit
	* PL092HD Latex Test Cards	48 cards/kit
	* Mixing Sticks	300 sticks/kit
	* Components also available separately.	

INTENDED USE

Hardy Diagnostics StrepPRO™ Grouping Kit provides a rapid latex agglutination method for the serological identification of Lancefield's groups A, B, C, D, F, and G from isolated colonies of beta-hemolytic *Streptococcus* spp. and *Enterococcus faecalis*.

SUMMARY

Clinical, epidemiological, and microbiological studies have conclusively shown that the diagnosis of streptococcal infections based on clinical symptoms always require microbiological verification.[4] 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. 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.[1,2,6,7]

Hardy Diagnostics StrepPRO™ Grouping Kit liberates the specific antigen from the bacterial cell wall using a

modified nitrous acid extraction. The extracted antigen, in conjunction with latex agglutination, offers a rapid, sensitive, and specific method for identification of streptococcal groups A, B, C, D, F, and G from primary culture plates. Extraction Reagents 1 and 2 contain a chemical substance able to extract the streptococcal group specific antigens at room temperature. Extraction Reagent 3 contains a neutralizing solution. The neutralized extracts can then be easily identified using blue latex particles sensitized with purified group specific rabbit immunoglobulins. These blue latex particles agglutinate strongly in the presence of homologous antigen and will not agglutinate when homologous antigen is absent.

MATERIALS SUPPLIED

Blue Latex Suspension Group A	Blue latex particles coated with purified rabbit antibodies to group A streptococci, suspended in phosphate buffer with 0.098% sodium azide
Blue Latex Suspension Group B	Blue latex particles coated with purified rabbit antibodies to group B streptococci, suspended in phosphate buffer with 0.098% sodium azide
Blue Latex Suspension Group C	Blue latex particles coated with purified rabbit antibodies to group C streptococci, suspended in phosphate buffer with 0.098% sodium azide
Blue Latex Suspension Group D	Blue latex particles coated with purified rabbit antibodies to group D streptococci, suspended in phosphate buffer with 0.098% sodium azide
Blue Latex Suspension Group F	Blue latex particles coated with purified rabbit antibodies to group F streptococci, suspended in phosphate buffer with 0.098% sodium azide
Blue Latex Suspension Group G	Blue latex particles coated with purified rabbit antibodies to group G streptococci, suspended in phosphate buffer with 0.098% sodium azide
Extraction Reagent 1	Extraction Reagent 1 with 0.098% sodium azide
Extraction Reagent 2	Extraction Reagent 2
Extraction Reagent 3	Extraction Reagent 3 with 0.098% sodium azide
Polyvalent Positive Control	Polyvalent Extract with antigens from inactivated streptococcal groups A, B, C, D, F, and G
Latex Test Cards	Disposable white cards with ten raised reaction circles
Mixing Sticks	Disposable 4 inch wooden sticks

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, pasteur pipettes, 12x75mm test tube, timers, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C away from direct light. **Do not freeze.** This kit, or any of its reagents, should not be used if there are any signs of discoloration, contamination, or if the expiration date has passed.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The

product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

The Blue Latex Reagents, Extraction Reagent 1, and Extraction Reagent 3 contain sodium azide as a preservative. Sodium azide can react explosively with copper or lead if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal, large quantities of water should be used when flushing these reagents down the sink.

Extraction Reagents 1, 2, and 3 contain a caustic agent. In case of skin contact, immediately wash with soap and copious amounts of water. In case of eye contact, flush for at least 15 minutes with water.

PROCEDURE

Specimen Collection:
For specific procedures regarding specimen collection and preparation of primary cultures consult standard microbiological references.[4,10-12]

In general, an overnight, gram-positive, beta-hemolytic isolate of streptococcal isolate is required for use in this assay. Colonies should be chosen from an area demonstrating obvious isolation. One to four isolated colonies are recommended for grouping testing, however, if colonies are pinpoint, an increased number of colonies, approximately a loopful, should be used.

Test Protocol:

1. Allow all reagents to acclimatize to room temperature for 10 minutes prior to use.

2. Label one 12x75mm test tube for each specimen tested.

3. Add one drop of Extraction Reagent 1 to each tube.

4. Using a loop, select 1 to 4 isolated beta-hemolytic colonies and suspend them in Extraction Reagent 1. If colonies are minute, pick a loopful of colonies, such that the Extraction Reagent 1 becomes turbid.

5. Add one drop of Extraction Reagent 2 to each tube.

6. Mix the reaction by tapping the tube with a finger for 10 seconds.

7. Add five drops of Extraction Reagent 3 to each tube. Mix the reaction as in step 6.

8. Prior to use, resuspend the Blue Latex Reagents, by inverting the tubes. Dispense one drop of each blue latex suspension onto separate circles on the test card.

9. Using a pasteur pipette, place one drop of the extract suspension next to the drop of latex suspension in each circle. Ensure that the pipette tip does not touch the latex suspension.

10. Mix the blue latex and the extract with the wooden sticks provided, using the complete area of the circle. A new stick should be used for each reagent.

11. Gently hand rock the entire card, allowing the mixture to flow slowly within the ring area.

12. At one minute, under normal lighting conditions, observe for agglutination, or strong clumping, of the blue latex particles. See Interpretation of Results for more information.

INTERPRETATION OF RESULTS

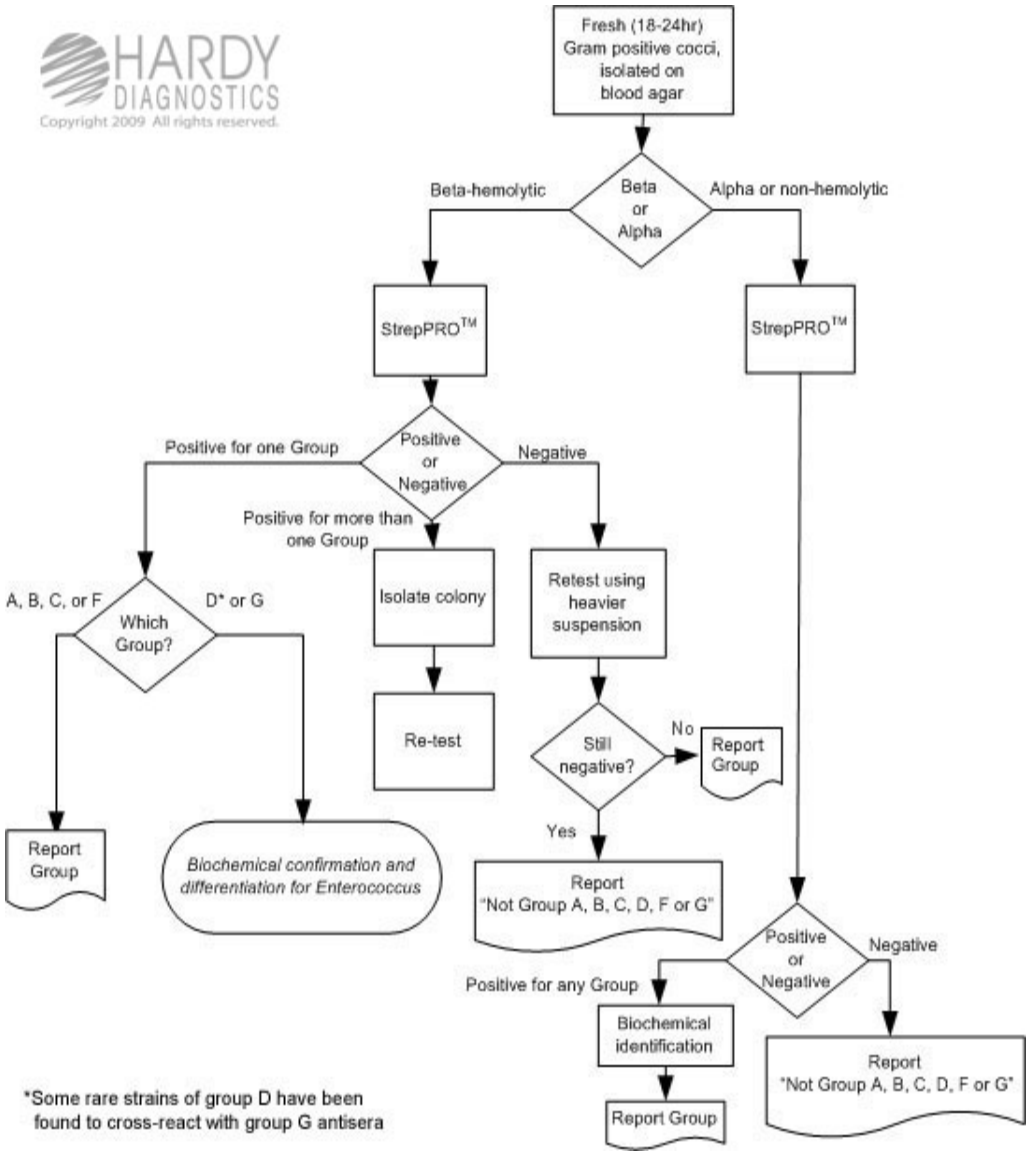
Positive Results:
A rapid and significantly strong clumping of the blue latex particles, to form an agglutination pattern, with only one of the latex reagents indicates an identification of the streptococcal isolate for the Lancefield group.

A weak reaction with a single blue latex reagent should be repeated using a heavier inoculum. The repeated test is considered positive if a visible agglutination occurs with only one of the blue latex reagents.

Negative Results:
No visible agglutination of the latex particles indicates a negative reaction for the particular Lancefield group.

FLOW CHART

Figure 1: Suggested Scheme for Streptococcal Grouping:



LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

This kit is intended for use in the identification of beta-hemolytic streptococci. If alpha or gamma streptococci are identified, the identification should be confirmed by biochemical tests. See flow chart for the recommended procedure for grouping streptococci.[5,9]

Do not perform the procedure using a broth culture as false reactions can occur.

False-negative or false-positive results can occur if inadequate amounts of culture or Extraction Reagents are used.

False-positive reactions have been known to occur with organisms from unrelated genera, including gram-negatives such as *Escherichia coli*, *Klebsiella*, and *Pseudomonas* species. These are likely to non-specifically agglutinate all latex reagents. This kit is recommended only for testing of gram-positive organisms.

Listeria monocytogenes may cross react with the Streptococcal Latex Reagents Group B and/or Group G, since *L. monocytogenes* exhibits similar antigenicity to group B and G streptococci. The catalase test may be performed to distinguish between *Listeria*, which are catalase-positive, and streptococci, which are catalase-negative. Gram staining and motility testing may be performed as further aids in differentiation.

Some rare strains of group D streptococci have been found to cross react with group G antisera. These strains may be confirmed as group D by a positive Bile Esculin (Cat. no. G12 or L10) test.

Refer to the document "Limitations of Procedures and Warranty" for more information.

QUALITY CONTROL

The following organisms are routinely tested on each lot of StrepPRO™ Grouping Kit by the manufacturer:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Streptococcus pyogenes ATCC® 19615	*	1 min.	35°C	Aerobic or CO_2**	Agglutination observed only with Blue Latex Suspension Group A
Streptococcus agalactiae (Group B) ATCC® 12386	*	1 min.	35°C	Aerobic or CO_2**	Agglutination observed only with Blue Latex Suspension Group B
Streptococcus spp. (Group C) ATCC® 12388	*	1 min.	35°C	Aerobic or CO_2**	Agglutination observed only with Blue Latex Suspension Group C
Enterococcus faecalis ATCC® 19433	*	1 min.	35°C	Aerobic or CO_2**	Agglutination observed only with Blue Latex Suspension Group D
Streptococcus spp. (Group F) ATCC® 12392	*	1 min.	35°C	Aerobic	Agglutination observed only with Blue Latex Suspension Group F
Streptococcus spp. (Group G) ATCC® 12394	*	1 min.	35°C	Aerobic	Agglutination observed only with Blue Latex Suspension Group G

In addition, each Blue Latex Suspension is tested for the absence of cross-reactions against extracts of the following additional quality control organisms:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli				Aerobic or	No agglutination

ATCC® 25922	*	1 min.	35°C	CO_2**	observed with any Blue Latex Suspensions
Klebsiella pneumoniae ATCC® 13883	*	1 min.	35°C	Aerobic or CO_2**	No agglutination observed with any Blue Latex Suspensions
Staphylococcus aureus ATCC® 25923	*	1 min.	35°C	Aerobic or CO_2**	No agglutination observed with any Blue Latex Suspensions
Haemophilus influenza Type B ATCC® 10211	*	1 min.	35°C	Aerobic or CO_2**	No agglutination observed with any Blue Latex Suspensions

* Refer to the section entitled Procedure for a detailed description of the inoculation protocol.

** Atmosphere of incubation is enriched with 5-10% CO_2.

StrepPRO™ Grouping Kit. Showing agglutination (LEFT) and no agglutination (RIGHT).

END USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

Latex Suspension
Verify the performance of each Latex Suspension by agglutinating with the Polyvalent Positive Control (included in the kit).
Expected Results: positive agglutination.

Extraction Reagents
Verify the performance of Extraction Reagents 1, 2, and 3 with *S. pyogenes* ATCC® 19615. Agglutinate the extract

with the Latex Suspension Group A that was previously approved in the step above.
Expected Results: positive agglutination.

Absence of Autoagglutination
Verify the lack of autoagglutination by performing the test procedure on one of the Latex Reagents without adding bacteria or the Polyvalent Positive Control to the Extraction Reagents.
Expected Results: negative agglutination.

PERFORMANCE CHARACTERISTICS

A. Cross Reactivity Studies:

Hardy Diagnostics StrepPRO™ Grouping Kit was tested for cross-reactivity using 33 ATCC® reference strains. The kit successfully grouped all streptococci containing Lancefield groups A, B, C, D, F, and G (N=16). No cross-reactivity was observed during the testing of other streptococcal strains (N=7) nor of other non-streptococcal organisms (N=10).

B. Clinical Performance Studies:

Hardy Diagnostics StrepPRO™ Grouping Kit was evaluated at a Microbiological Center in Oxford, England. In this study 468 primary culture were tested by the StrepPRO™ Grouping Kit and an alternative grouping kit. Overall agreement between the two kits upon first time testing occurred with 452 of 468 isolates tested (96.6%). Anomalous results (N=16; all minute colonies) were repeated using a heavier inoculum. 13 of the 16 anomalous results agreed after retest which included 1 group A, 2 group B, 3 group D, 1 group F, 5 group G, and 1 non-groupable strains. Two of the 3 discrepant strains were further identified as non-beta-hemolytic strains, while the third isolate grouped as group D by StrepPRO™ Grouping Kit, while determined non-groupable by the alternative kit. This isolate gave a positive group D isolate result with the alternative kit following subculture. Overall agreement between the StrepPRO™ Grouping Kit and the alternative grouping kit after retest of anomalous results occurred with 463 of 468 isolates tested (99.4%). The 468 Streptococci isolates used in this study included 127 group A, 93 group B, 30 group C, 28 group D, 8 group F, 107 group G, and 75 non-groupable strains.

A second performance study was carried out a Health Center in Ontario, Canada. In this study, 111 primary cultures were included (110 tested, 1 inadequate). All the strains were originally grouped by Lancefield precipitation reactions. All group D were further biochemically confirmed using Bile Esculin and PYR assay protocol. The primary cultures were tested in parallel using the StrepPRO™ Grouping Kit and an alternative grouping kit. In this study, the overall agreement between StrepPRO™ Grouping Kit and the Lancefield results occurred with 109 of the 110 isolates tested (99%), while overall agreement between the alternative kit and Lancefield results occurred with 106 of 110 isolates tested (96.3%). The 110 Streptococci primary isolates used in this study included 15 group A, 40 group B, 13 group C, 4 group D, 11 group F, 12 group G, and 15 non-groupable strains.

REFERENCES

1. Edereer, G.M., et al. 1972. Rapid Extraction Methods with Pronase B for Grouping Beta-Hemolytic Streptococci. *Appl. Microbiol.*; 23, 285.

2. E.L. Kholy, et al. 1974. Simplified Extraction Procedure for Serological Grouping of Beta-Hemoltyic Streptococci. *Appl. Microbiol.*; 28, 836.

3. Elliot, S.D., et al. 1978. The Type-Specific Polysaccharides of Streptococcus suis. *J. Exp. Med.*; 148, 1699.

4. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

5. Facklam, R.R. 1977. Physiological Differentiation of Viridans Streptococci. *J. Clin. Microbiol.*; 5, 184.

6. Fuller, A.T. 1938. The Formamide Method for the Extraction of Polysaccharides from Hemolytic Streptococci.

Brit. J. Exp. Path.; 19, 130.

7. Maxted, W.R. 1948. *Preparation of Streptococcal Extracts for Lancefield Grouping*. Lancet, ii, 255.

8. Nowlan, S.S., and Deibel, R.H. 1967. Group Q Streptococci. I. Ecology, Serology, Physiology and Relationships to Established Enterococci. *J. Bact.*; 94, 291.

9. Petts, D.N. 1984. Early Detection of Streptococci in Swabs by Latex Agglutination Before Culture. *J. Clin. Microbiol.*; 19, 432.

10. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

11. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

12. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*. J.B. Lippincott Company, Philadelphia, PA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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