LK06 – HiStrep™ Latex Test Kit Product Information

Product Code	Reagents provided**	LK06	
		25 Nos.	50 Nos.
LK06a	Strep group A Latex Reagent	1.25 ml	2.5 ml
LK06b	Strep group B Latex Reagent	1.25 ml	2.5 ml
LK06c	Strep group C Latex Reagent	1.25 ml	2.5 ml
LK06d	Strep group D Latex Reagent	1.25 ml	2.5 ml
LK06e	Strep group F Latex Reagent	1.25 ml	2.5 ml
LK06f	Strep group G Latex Reagent	1.25 ml	2.5 ml
LK06g	Strep groups A to G Positive Control	0.5 ml	1.0 ml
LK06h	Strep group Enzyme Extraction Buffer	10 ml	2 x 10 ml

**** Agglutination slides and mixing sticks are provided in the kit.**

Intended Use

HiStrep™ Latex Test Kit is a rapid latex agglutination slide test for grouping Streptococci of Lancefield groups A, B, C, D, F and G from culture plates. Most strains of Streptococci, which have been isolated from human infections, possess serological group specific antigens. Identification of the organisms includes extraction and characterization of these antigens from organisms grown in culture. The Streptococcal grouping system provides an enzyme reagent for rapid extraction of the carbohydrate antigens and a series of latex agglutination reagents, specific for groups A, B, C, D, F and G, for rapid detection and identification of the extracted antigens. This product is intended *in vitro* diagnostic use only. Not for Medicinal Use.

Principle of the Test

Latex particles in the HiStrep™ Latex Test Kit are individually sensitized with rabbit antibodies specific to one of the Streptococcal carbohydrate antigens of groups A, B,C, D, F or G. Streptococcal colonies from culture plates are incubated in an enzyme solution to extract the antigen. The extract/antigen preparation is tested on a reaction card against six suspensions of antibody coated latex particles each specific for one of the groups A,B,C,D,F and G. In the presence of homologous antigen, particles in one of the suspensions will aggregate to give visible agglutination in contrast to the other suspensions which will remain unagglutinated.

Kit Contents

Each kit contains sufficient reagents for 50 tests. The date of expiry of each reagent is indicated on the vial labels.

1. **LK06a Strep group A Latex Reagent** Contains rabbit Strep Group A antibody sensitized latex particles in buffer with stabiliser and sodium azide 0.099% as preservative.

ISO 9001-2008 CERTIFIED WHO GMP CERTIFIED CE ISO 13485-2003 CERTIFIED

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2. **LK06b Strep group B Latex Reagent** Contains rabbit Strep Group B antibody sensitized latex particles in buffer with stabiliser and sodium azide 0.099% as preservative.

3. **LK06c Strep group C Latex Reagent** Contains rabbit Strep Group C antibody sensitized latex particles in buffer with stabiliser and sodium azide 0.099% as preservative.

4. **LK06d Strep group D Latex Reagent** Contains rabbit Strep Group D antibody sensitized latex particles in buffer with stabilizer and sodium azide 0.099% as preservative.

5. **LK06e Strep group F Latex Reagent** Contains rabbit Strep group F antibody sensitized latex particles in buffer with stabilizer and sodium azide 0.099% as preservative.

6. **LK06f Strep group G Latex Reagent** Contains rabbit Strep Group G antibody sensitized latex particles in buffer with stabilizer and sodium azide 0.099% as preservative.

7. **LK06g Strep groups A to G Positive Control** Positive Control contains inactivated polyvalent antigenic extracts to groups A,B,C,D,F and G preserved with 0.099% sodium azide.

8. **LK06h Strep group Enzyme Extraction** Lyophilized extraction enzyme

Instructions for Use

- Disposable agglutination slides
- Disposable mixing sticks

Additional Requirement

- Bacteriological loops (PW012 Hi-FlexiLoop 2)
- Pasteur pipettes
- Micropipettes and tips
- Glass or Plastic test tubes
- Pipette to dispense 0.4ml volumes
- Water bath set at 37°C.
- Laboratory timer

Warnings

Safety:

1. HiStrep™ Latex Test Kit is for *in vitro* diagnostic use only. Not for Medicinal Use.
2. The reagents in this kit contain 0.099% sodium azide as a preservative which should be handled with care. Sodium azide can react with lead and copper plumbing to form explosive azides. Upon disposal of reagents, flush with copious quantities of water to prevent azide build up.
3. Do not use reagents after the expiry date stated on the kit carton label.
4. Do not cross contaminate reagents or samples.
5. The test should only be performed in accordance with the instructions supplied with the kit.
6. Do not pipette specimens or reagents by mouth.

Specimen Collection and Handling

This test is designed for the testing of colonies which have the appearance and growth characteristics of Streptococci, after overnight growth on routine laboratory culture media. For details concerning collection and handling of specimens and the choice of, inoculation and incubation of culture media, a standard text book should be consulted.

Colonies may be taken from primary culture plates, or from pure subcultures, for testing on the day following inoculation of the medium.

Stored cultures should not be used. The haemolytic properties of the cultures are important to its identification and it should be determined whether or not the growth taken for testing originates from blood based medium.

Indications of Deterioration

Deterioration of reagents should be suspected if,

· Clumping of the latex reagent is evident and cannot be removed by shaking vigorously for a few seconds.

· The positive control of extraction of enzyme becomes cloudy and forms sediment.

· The positive control fails to cause agglutination of one or more latex reagents within the recommended reaction time.

· Uninoculated Extraction Enzyme causes agglutination of the any of the latex reagents.

Reagents showing signs of deterioration should not be used.

Test Procedure

Allow HiStrep™ Latex Test Kit reagents to reach room temperature prior to use.

Proceed as follows for each organism to be grouped

1. Allow the Latex reagents and positive control to reach room temperature.

2. Just prior to use, reconstitute a bottle of enzyme by adding 10ml distilled water. Mix gently to ensure complete reconstitution. Dispense 0.4ml Extraction Enzyme into a test tube.

3. Pick Streptococcal colonies from the surface of the agar using a bacteriological loop and emulsify thoroughly in the Extraction enzyme. To obtain best results, Pick atleast 4 or 5 average sized colonies or their equivalent for extraction. Excessive inoculation of extraction enzyme may cause nonspecific agglutination. For minute colony strains, a sweep of growth will be necessary.

4. Incubate the tube for 10 to 15 minutes in a 37°C water bath. Shake the tube after the first 5 minutes incubation and shake vigorously prior to testing to obtain even suspension of antigen

5. Vigorously shake Latex reagents for a few seconds to obtain even suspension. Dispense 20µl of each Latex reagent separately into six circles on a reaction card.

6. Transfer 20µl of well mixed extract (or Positive Control) into the six separate circles next to the drop of Latex reagent.

7. Mix the contents of each circle using separate mixing sticks and spread the liquid to cover the area of the circle. Do not use the same mixing stick for more than one circle.

8. Slowly and gently, rock and rotate the reaction card to mix the reagents for a maximum of one minute.

9. Inspect the card for agglutination. If present, agglutination should be clearly visible with the naked eye.

Storage and Shelf Life

Store all reagents at 2-8°C. Do not freeze. Under these conditions the reagents will be usable until the date printed on the outer carton label. Extraction Enzyme is stable for 3 months after reconstitution if stored at 2-8°C. To prolong the life of the enzyme, it may be dispensed into suitable test tubes in 0.4ml volumes and stored frozen, at -20°C or below when it will be stable for 6 months. Enzyme should not be frozen and thawed more than once.

Quality Control:

Organism	**Agglutination with latex Reagent**
Streptococci	+
Key : + is agglutination , - is no agglutination	

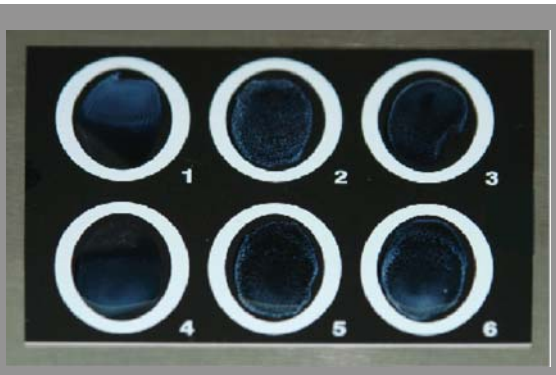
Performance & Evaluation

The following controls should be performed each time the kit is used:

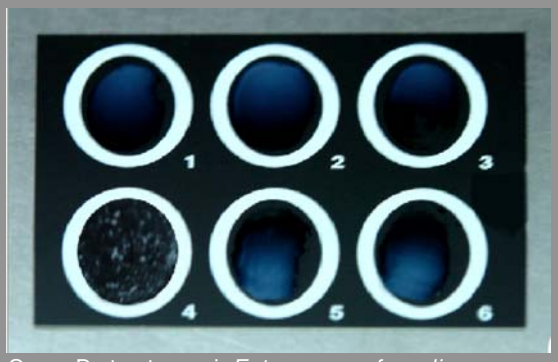
The positive control should be tested regularly to ensure that the reagents are functioning correctly. The control is supplied ready for use and should be tested in place of the culture extract in the test procedure. The positive control should give agglutination with all the test latex reagents. Failure of the positive control to give an agglutination pattern may be evidence of latex reagent deterioration. If a negative control is desired, uninoculated extraction enzyme should be tested in place of the culture extract in the test procedure.

When, during the one minute reaction time, the latex particles aggregate into visible clumps, the result is positive for that suspension. If extract contains high quantity of antigen, agglutination may be very

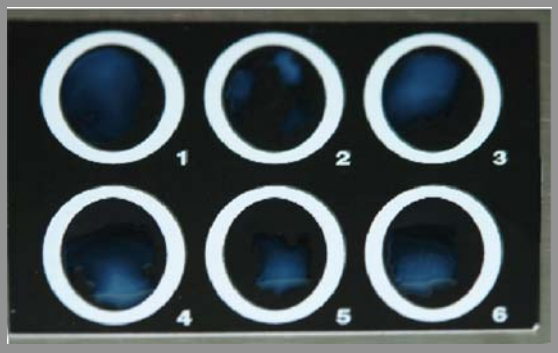
rapid giving large clumps. With weaker extracts agglutination may take longer to appear and give smaller clumps but there should be no difficulty distinguishing positive and negative reactions.



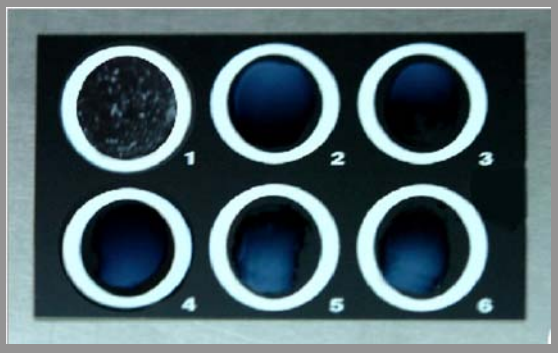
Positive control showing agglutination reactions with
1. Reagent A 2.Reagent B 3. Reagent C
4. Reagent D 5. Reagent F 6. Reagent G



Group D streptococci, *Enterococcus faecalis* (ATCC 29212), showing positive reaction with Reagent D (well 4)



Streptococcus pneumoniae (ATCC 6305) showing no reaction with any reagents.



Group A streptococci, *Streptococcus pyogenes* (ATCC 19615), showing positive reaction with Reagent A (well 1)

When the Latex particles retain their original milky appearance, without any significant aggregation, the result is negative for that suspension. Traces of indistinct aggregation should be ignored and considered negative.

EXPECTED RESULTS

Colonies associated with beta-haemolysis:

1. Agglutination of a single latex reagent indicates the group identity of the strain. Complimentary tests should be considered to confirm the results, in particular:

(i) For group D strains, biochemical tests to differentiate Enterococcus species (the former has relatively high antibiotic resistance).

(ii) For group A,C or G strains with minute colony morphology,biochemical tests to confirm *S. milleri* / *S. anginosus* identification.

2. Agglutination of more than one latex reagent indicates the possibility of mixed growth of organisms from different groups or presence of strains with more than one group antigen. (for example some group D streptococci also possess group G antigen)

Further procedures to be considered:

(i) Subculture to obtain pure isolates for retesting.

(ii) For strains with group D and group G antigen, biochemical tests to differentiate Enterococcus species from group D streptococcus species (Enterococcus strains with both these antigens may be more antibiotic resistant than those with only group D antigen).

3. Agglutination of all the latex reagents may indicate excessive inoculation of culture to the extraction enzyme or contamination of the test culture with organisms which cause non-specific agglutination of latex particles (these are normally simple to recognize from growth characteristics). Further procedures to be considered:

(a) Boiling the remaining extract for two or three minutes, cooling and retesting may lead to clear results.
(b) Repeating the test using a smaller inoculation of the Extraction Enzyme. Subculture to obtain pure isolates which may be retested.

4. No significant agglutination in any of the latex reagents indicates either that, no group A,B,C,D,F or G streptococci were present in the test sample, or that they were present in numbers below the threshold of sensitivity of the test. Further procedures to be considered:

(i) retest using a higher inoculum, particularly if group D or group F streptococci are suspected.
(ii) b-haemolytic streptococci which do not group may be identified using biochemical test procedures if necessary.

Colonies not associated with b- haemolysis:

Agglutination of a single latex reagent showing a result of group B or group D gives a reliable identification of the strain. If the result is group A, C, F, or G it may not be relevant to the identification of the strain and other identification methods are necessary.

Further procedures to be considered:

If the result is group D, biochemical differentiation between Enterococci and group D streptococci (see above). Any other combination of results should be interpreted using the information provided above.

Limitations of the procedure:

Results must be evaluated in the light of other available clinical and laboratory information. Accurate results depend on testing an appropriate amount of growth. This is not usually a problem; however

some strains of streptococci belonging to group D possess lower or negligible quantities of group antigen and some strains of group F may be difficult to remove from the surface of agar plates. Antigen production in group D strains may be improved by culturing them on agar supplemented with 0.5 to 1.0% glucose. This supplement does obscure demonstration of haemolysis but it may be considered in situations where it is important to resolve identification. Growth of minute-colony strains may be improved by culture in a carbon dioxide enriched atmosphere. Streptococci from groups Q, R and S may also possess detectable levels of group D antigen. Antigens common to the Streptococcal group antigens have been described in a number of unrelated species. For example false positive reactions can occur with Escherichia, Klebsiella or Pseudomonas. These are normally easily differentiated by cultural characteristics and cause no confusion in Streptococcal identification.

Performance characteristics:

The HiStrep™ Latex Test kit has been evaluated against a leading commercial latex kit as a reference for grouping Streptococci, using clinical samples at a number of independent sites. Overall results are shown in Table 1.

Table 1. Comparison of HiStrep™ Latex Test Kit and a Commercial Latex test for grouping of Streptococci.

	HiStrep™ Latex Test Kit		
		+ve	-ve
	+ve	607	55
Leading Commercial Test	-ve	0	24

Sensitivity 607/ 662 = 92%

Specificity 24/24 =100%

Reproducibility

Intra Batch reproducibility was evaluated by testing sensitivity of one batch of each of the test latexes on ten separate occasions with three different operators against serial dilutions of reference antigens. End point titers varied by a maximum of one doubling dilution from assay to assay.

Inter Batch reproducibility was examined by testing sensitivity and specificity of 10 batches of product against serial dilutions of reference antigens. Between batches variation in titers was a maximum of one doubling dilution of antigen and qualitative results correlated 100%.

Disposal

Appropriate precautions should be taken when handling or disposing of potential pathogens. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30 minutes. Liquid waste containing acid must be neutralized before treatment.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com.

IVD In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

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