LK01 – HiClostridium difficile Latex Test Kit

Product Code	Reagents provided**	LK01	
		25 Nos.	50 Nos.
LK01a	C. difficile Latex Reagent	1.5 ml	3.0 ml
LK01b	Positive Control	0.25 ml	0.5 ml
LK01c	Sample Diluent	2.5 ml	5 ml

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

Intended Use

HiClostridium difficile Latex Kit is a rapid latex agglutination test intended for confirmatory identification of *Clostridium difficile* cultured on selective solid media from faecal samples from patients with suspected pseudomembranous colitis, antibiotic-associated diarrhoea and postoperative diarrhoea. The kit is intended for *in vitro* diagnostic use only. Not for Medicinal Use.

Principle of the Test

Latex particles are coated with rabbit IgG antibodies specific for C. difficile cell wall antigens. When the sensitized latex particles are mixed with a suspension of C. difficile colonies, a sensitive and specific immunochemical reaction takes place causing the finely dispersed latex particles to agglutinate rapidly into aggregates that are easily visible to the unaided eye.

Kit Contents

1. **LK01a C.difficile Latex Reagent**
   Latex particles coated with rabbit antibodies to C. difficile antigens, preserved with 0.099% sodium azide.

2. **LK01b Positive Control**
   Suspension of inactivated C. difficile antigens reactive with Test Latex Reagent, preserved with 0.099% sodium azide.

3. **LK01c Sample diluent (0.85% Isotonic saline)**
   Preserved with 0.099% Sodium azide

Instructions for Use

- Disposable agglutination slides
- Disposable mixing sticks

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

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Additional Requirement

Bacteriological loops (PW012 Hi-FlexiLoop 2)

- Micropipettes and tips
- Clostridium difficile agar base (M836) plates with FD010*
- Bacteriological loops (PW012 Hi-FlexiLoop 2)

***Note** - FD010- Clostridium difficile supplement. For more details refer HiMedia Product Manual.

Warnings

Safety :

1. The reagents supplied in this kit are for *in vitro* diagnostic use only.

2. Sodium azide which is used as a preservative in the kit reagents can react with lead or copper plumbing to form potentially explosive metal azides. Dispose by flushing with a large volume of water to prevent azide build-up.

3. 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.

4. The positive control has been inactivated during the manufacturing process. However, it should be handled as though potentially infectious.

Specimen Collection and Handling

Faecal samples should be cultured in enrichment broth at 37°C for 18-24 hours and then sub-cultured on to Clostridium difficile agar base (M836) plates (FD010). Plates should be incubated anaerobically at 37°C for 24-48 hours. Colonies with morphology resembling C. difficile are removed for testing with HiClostridium difficile latex kit.

Procedure

1. HiClostridium difficile Latex Test Kit should be used according to the kit instructions.
2. Allow all reagents to reach room temperature before use.
3. Do not dilute any of the kit reagents.
4. Do not intermix reagents from different batches of kits.
5. Do not freeze any of the kit reagents.
6. Be careful only to record agglutination. Reactions that are “curdy” or “stringy” may not be true agglutination.
7. Ensure the agglutination slide is clean and dry prior to use.

8. The kit should not be used if the latex reagent fails to agglutinate with the positive control, or if the latex reagent agglutinates in isotonic saline only.

Storage and Shelf Life

HiClostridium difficile Latex Test Kit should be stored at 2-8°C when not in use. The kit should not be used after the expiry date printed on the carton label.

Quality Control

Organism	**Agglutination with latex Reagent**
C.difficile	+
Key : + is agglutination , - is no agglutination	

Performance & Evaluation

The following checks should be performed each time the kit is used to confirm that the reagents are functioning correctly:

1. Reagent Control: Add 20µl of C.difficile Latex Reagent to 20µl of isotonic saline in the same circle on an agglutination slide. Mix with a mixing stick and observe for agglutination. No agglutination should be seen. If this control shows agglutination, at least one of the reagents is contaminated and they should be discarded.

2. Positive Control: Gently mix the positive control by inverting several times. Place 20µl on a circle of an agglutination slide. Add 20µl of C.difficile Latex Reagent to the same circle and mix. Agglutination should be visible within 2 minutes. If no agglutination is seen the reagents should be discarded.

Test Procedure

1. Dispense 20µl of isotonic saline on to 1 circle of a clean, dry HiClostridium difficile Latex agglutination slide.

2. Using an inoculating loop, remove a suspected C. difficile colony from the selective agar plate. Only select colonies whose morphology resembles that of C. difficile. Emulsify the colony in the 20 µl of saline on the test card to produce a heavy, smooth suspension.

3. Observe the suspension for any agglutination or clumping which would indicate auto-agglutination. If the suspension remains smooth, proceed to the next step. If auto agglutination is seen, the organism cannot be tested using HiClostridium difficile Latex Kit. Alternative test methods should be used.

4. Gently mix the C.difficile Latex Reagent by inverting the vial several times. Add 20µl to the colony suspension on the slide. Do not allow the tip to touch the organism suspension.

5. Mix the latex reagent and organism suspension together with a clean mixing stick for 30 seconds. Continue mixing by rocking the slide.

6. Examine for agglutination after 2 minutes from initial mixing of latex and sample.

7. After reading, discard the used slides and mixing sticks into suitable disinfectant.

Interpretation

Agglutination within 2 minutes is a positive result and indicates the presence of C. difficile.
No agglutination within 2 minutes is a negative result.

Limitation of Use

1. Results should be interpreted by the clinician in the context of all available clinical and laboratory information. The isolation of C. difficile does not constitute a diagnosis of pseudomembranous colitis or antibiotic-associated diarrhoea.

2. Identification of C. difficile using HiClostridium difficile Latex Test Kit should be performed on selective cultures as this increases the isolation rate.

3. Culture-derived suspensions which auto-agglutinate cannot be tested by HiClostridium difficile Latex Test Kit. Alternative methods should be used.

Precautions

Read the procedure carefully before starting the experiment.

Performance Characteristics

HiClostridium difficile Latex Kit has been evaluated as a culture confirmation test both, at an independent microbiology laboratory and in-house. In total, 137 bacterial isolates were cultured on selective agar plates and colonies tested by HiClostridium difficile Latex Test Kit and a well-established commercially available test.

	HiClostridium difficile Latex Test Kit			
		+ve	-ve	Total
Commercial Test	+ve	85*	0	85
	-ve	0	52**	52*
	Total	85	52	137

Sensitivity: 85/85 = 100%

Specificity: 52/52 = 100%

Concordance: 137/137=100%

*Of the 85 isolates in this group, 18 were cross-reactants in both tests. However, 16 of these either will not grow on C. difficile selective medium or the colonies do not resemble C. difficile. The remaining two isolates (both C. glycolicum) will grow slightly but do not exhibit colony fluorescence which is a characteristic of the majority of C. difficile strains.

** 2 of these isolates were classified as C. difficile (serogroups A9, A10). One of these (serogroup A10) exhibited slightly irregular colony morphology. The remaining 50 isolates comprised a wide variety of bacterial species including 5 Clostridium spp. Most of these isolates either do not grow on C. difficile selective agar or exhibit typical colony morphology.

Overall, the results obtained with HiClostridium difficile Latex Kit correlate closely with those obtained using the established commercial product. Although a number of organisms have the potential to cause false positive reactions in both tests, they either do not grow in C.difficile selective culture media or their colony morphologies are not typical of C.difficile.

Reproducibility

Intra-batch reproducibility was established by testing one batch of product on three separate occasions using a different operator for each occasion. Sensitivity was tested using serial dilutions of reference and kit control antigens and specificity was confirmed using a Q.C. organism panel. No substantial differences were seen between the results obtained by the three operators.

Inter-batch reproducibility was examined by testing the sensitivity and specificity of three batches of product using reference and kit control antigens and the Q.C. organism panel. No differences in sensitivity or specificity were seen between the three batches of product.

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.

In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

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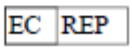

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