LK08– HiRotavirus Latex Test Kit

Product Information

Product Code	Reagents provided	LK08		
		10 Nos.	25 Nos.	50 Nos.
LK08a	Rotavirus Latex Reagent	0.5 ml	1.25 ml	2.5 ml
LK08b	Rotavirus Control Reagent	0.5 ml	1.25 ml	2.5 ml
LK08c	Positive Control	0.2 ml	0.5 ml	1.0 ml
LK08d	Extraction buffer	10 ml	25 ml	50 ml

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

Intended Use

HiRotavirus Latex Test Kit is a rapid and simple latex agglutination test for the detection of Rotavirus antigen in fecal specimens. The kit is intended for *in vitro* diagnostic use only. Not for Medicinal Use.

Principle of the Test

Latex particles in the HiRotavirus Latex Test Kit, Test Reagent are coated with rabbit antibodies raised against a pool of different Rotavirus isolates, including human. When a fecal extract is mixed with the Test Reagent any Rotavirus antigens present will react with the sensitizing antibodies, resulting in visible agglutination of the latex particles.

A Control Reagent, latex particles coated with normal rabbit globulin, is included to identify non-specific reactions which may occur with some fecal specimens.

Kit Contents

1. **LK08a Rotavirus Latex Reagent**
   Rotavirus Latex reagent-Contains (rabbit) Rotavirus antibody-sensitized latex particles in buffer, with stabilizer, and 0.099% sodium azide as preservative.

2. **LK08b Rotavirus Control Reagent**
   Contains (rabbit) normal globulin-sensitized latex particles in buffer, with stabilizer, and 0.099% sodium azide as preservative.

3. **LK08c Positive Control**
   Contains inactivated bovine Rotavirus antigens in buffered cell culture medium, with antibiotics, and 0.099% sodium azide as preservative.

4. **LK08d Extraction buffer**
   Contains working strength buffer, pH 7.2, and 0.099% sodium azide as preservative.

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Instructions for Use

- Disposable agglutination slides
- Disposable mixing sticks

Additional Requirement

- Laboratory Centrifuge
- Stoppered or screw-capped tubes
- Bacteriological loops (PW012 Hi-FlexiLoop 2)
- Micropipettes and tips

Warnings

1. HiRotavirus Latex Test Kit is for *in vitro* diagnostic use only.

2. Do not use reagents after the kit expiration date.

3. Do not interchange reagents from different kit lots.

4. The test should only be performed in accordance with the instructions supplied with the kit.

5. Do not pipette specimens or reagents by mouth.

6. All clinical specimens should be considered infectious and handled and disposed of according to accepted practices. 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.

7. The Positive Control is prepared from inactivated Rotavirus. However, it should be considered as potentially infectious and handled in the same way as a clinical specimen.

8. The reagents in this kit contain sodium azide as preservative which may react with lead or copper plumbing to form highly explosive azides. Upon disposal, flush with a large volume of water to prevent azide build-up.

Specimen Collection and Handling

Fecal specimens should be tested as soon after collection as possible but, if necessary, they may be stored overnight at 2-8°C or for longer periods at -20°C or below. Contaminated samples may give erroneous results.

Prepare an approximate 10% suspension of the fecal specimen by transferring 0.1mL (0.1g) of sample into 1.0mL of Extraction Buffer in a stoppered or screw-capped tube. Mix the contents well. Allow to stand at room temperature for 1-2 minutes before proceeding with the test.

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.

Indications of Deterioration

Deterioration should be suspected if:-

- Clumping of the Rotavirus Latex Reagent (LK08a) or Rotavirus Control Reagent (LK08b) is evident and cannot be removed by gentle mixing.
- The Positive Control (LK08c) or extraction Buffer (LK08d) becomes cloudy or forms sediment.
- The Positive Control (LK08c) fails to cause agglutination of the Test Reagent within the recommended reaction time. Reagents showing signs of deterioration should not be used.

Quality Control

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

Use of Positive Control

Add 20µl of well-mixed Rotavirus Latex Reagent (LK08a) to one well and 20µl of well-mixed Rotavirus Control Reagent (LK08b) to the other. Gently mix the positive control by inverting several times. Place 20µl each on a both wells of an agglutination slide, mix and check for agglutination. The Positive control should give agglutination of the Test Reagent (LK08a) with no agglutination of the Control Reagent (LK08b). Failure to give this agglutination pattern is evidence of reagent deterioration. The Positive control should be tested regularly to ensure that the reagents are functioning correctly. The control is ready for use and should be tested in place of the specimen extract in the Test Procedure.

Test Procedure – Standard Method using Centrifuge

1. Process the specimen as mentioned in specimen storage and preparation.

2. Allow the reagents to reach room temperature.

3. Centrifuge specimen extract at approximately 1000g for 10 mins.

4. Pipette 50µl of clear supernatant onto each of two wells on the reaction card.

5. Add 20µl of well-mixed Test Reagent (LK08a) to one well and 20µl of well-mixed Control Reagent (LK08b) to the other.

6. Mix the contents of each well using a separate mixing stick for each sample, covering the entire area of the well.

7. Gently rock the card and observe the agglutination for up to two minutes.

Interpretation of Results

a) A positive result is indicated by agglutination of the Rotavirus Latex Reagent (LK08a) with no agglutination of the Rotavirus Control Reagent (LK08b).

b) The result is negative if no agglutination of either the Rotavirus Latex Reagent (LK08a) or the Rotavirus Control Reagent (LK08b) is observed within the 2 minute test period.

Note: Agglutination of the Rotavirus Control Reagent (LK08b) is evidence of a non-specific reaction and means that the specimen is unsuitable for testing by this method.

Limitations of the Procedure

HiRotavirus Latex Test Kit results should be evaluated in the light of all other available clinical and laboratory information.

A positive HiRotavirus Latex Test Kit test does not preclude the possibility of other microbial co-infections.

HiRotavirus Latex Test Kit is intended as an acute-phase test. Fecal samples collected after the acute phase may contain antigen concentrations below the threshold of reagent sensitivity.

Precautions

Read the procedure carefully before starting the experiment.

Performance Characteristics

HiRotavirus Latex Test Kit has been independently evaluated in clinical use at three leading microbiology laboratories. Their results can be summarized as follows:

<u>Laboratory 1</u>

69 stool specimens were tested by HiRotavirus Latex Test Kit, in parallel with a respected commercial enzyme immunoassay (EIA) system.

Table 1: Comparison of HiRotavirus Latex Test Kit and a commercial EIA system.

	HiRotavirus Latex Test Kit +	HiRotavirus Latex Test Kit -	Total
EIA+	36	1*	37
EIA -	0	32	32
Total	36	33	69

*EM negative

Sensitivity 97%

Specificity 100%

Predictive value positive 100%

Predictive value negative 97%

Laboratory 2

398 stool specimens were tested for Rotavirus by HiRotavirus Latex Test Kit, electron microscopy, polyacrylamide gel electrophoresis (PAGE) RNA banding, and the WHO reference EIA system.

Table 2: Comparison of HiRotavirus Latex Test Kit, EIA, Electron Microscopy and PAGE electrophoresis

HiRotavirus Latex Test Kit	EIA	EIA	PAGE	No. of samples	% of total samples
+	+	+	+	161	40.25
+	+	+	-	18	4.5
-	+	+	+	8	2.0
+	+	-	+	22	5.5
-	+	-	+	3	0.75
-	+	+	-	7	1.75
+	+	-	-	62	15.5
-	+	-	-	22	5.5
-	-	-	-	95	23.75

If a true Rotavirus positive is defined as a specimen which is positive by two or more tests, performance of the different assays can be summarized as follows:

Table 3: Summary of performance of HiRotavirus Latex Test Kit, in comparison with EIA, EM and PAGE

	HiRotavirus Latex Test Kit	EIA	EM	PAGE

Sensitivity	93%	100%	69%	69%
Specificity	100%	81%	100%	100%
Predictive value positive	100%	93%	100%	100%
Predictive Value negative	87%	100%	57%	57%

Laboratory 3

278 stool specimens were characterized by electron microscopy, EIA and PAGE RNA banding. True positives were considered to be those specimens positive by one or more of these tests. True negatives were those which were negative by all tests. The characterized sera were then tested by HiRotavirus Latex Test Kit in parallel with two other commercially available slide latex tests. Results can be summarized as follows:

Table 4: Comparison of HiRotavirus Latex Test Kit and two commercially available slide latex tests

	HiRotavirus Latex Test Kit	**Latex 1**	**Latex 2**
Sensitivity	91%	87%	76%
Specificity % specimens which agglutinated both test and control latex reagent	1%	2%	14%
Predictive value positive	98%	97%	99%
Predictive Value negative	95%	90%	92%

Note: Specimens giving agglutination of both Test and Control latex reagents were excluded from the calculation of predictive values.

Reproducibility

Intra Batch Reproducibility

Intra batch reproducibility has been determined by running a panel of 5 positive and 5 negative samples on 10 separate occasions using four different operators. No difference in interpretation was noted on any of the test dates or across any of the operators.

Inter batch sensitivity and specificity is maintained by monitoring each batch against reference panels of samples and serial dilutions of a sensitivity standard (Rota positive sample).

For further confirmation the above mentioned test can be performed.

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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